the prolonged retention of radioactivity in the lymphocytes might not indicate cells with a very long life-span but might be due to a continuous re-utilization of large fragments of nucleic acid or nucleoprotein during the formation of new lymphocytes. The biochemical evidence did not enable a choice to be made between these alternatives, but Trowell suggested that the presence of cell debris in the reticulum cells in lymph nodes could provide cytological support for the idea of re-utilization.

Ottesen described the incorporation of phosphorus-32 into the deoxyribonucleic acid of lymphocytes in the blood of normal human subjects. An analysis of the specific activity curves again suggested the existence of two populations of cells but with shorter life-spans than those calculated by Hamilton for leukæmic lymphocytes: about 20 per cent had a mean age of 2–3 days and 80 per cent a mean age of 100–200 days. There was evidence, both in man and in animals, that lymphocytes may remain in lymph nodes for a considerable time after their formation. Thus, autoradiographs of mouse lymph nodes showed that some lymphocytes labelled with carbon-14 were present in the nodes more than two months after the administration of the isotope.

These experiments with radioactive labels suggested that the life-span of at least some lymphocytes may be considerably longer than was at one time supposed. There was, however, some prejudice against the idea that lymphocytes can live for as long as a year, and, in discussion, this appeared to be a reason for inclining towards the hypothesis of re-utilization.

The problem of the rapid turnover of the lymphocytes in the blood was discussed by J. L. Gowans (Oxford). An apparatus was described for the continuous intravenous infusion of lymph and lymphocytes into unanæsthetized rats with thoracic duct fistulæ. It was shown that there is a direct relationship between the number of lymphocytes entering the blood and the number emerging from the thoracic duct. Thus, the fall in the cell output that occurred on the prolonged drainage of lymph from the thoracic duct could be prevented by the intravenous replacement of all the lymph and cells collected from the fistula. Similarly, after prolonged drainage of lymph, the low output of cells from the thoracic duct could be restored to normal by the transfusion of lymphocytes from other rats of the same inbred strain. A vigorous transfusion of lymphocytes almost doubled the normal cell output of a freshly cannulated rat. The number of lymphocytes transfused could be roughly accounted for by the extra lymphocytes appearing in the thoracic duct lymph of the recipient rat. These effects were most probably mediated by living lymphocytes since the administration of cell-free lymph or of lymphocytes killed by a variety of means had no effect on the cell output. It was felt that the results could not be accounted for by a burst of new cell formation initiated by the transfused cells; they strongly suggested that the apparently high turnover of the lymphocytes in the blood of the intact animal is due to a continuous recirculation of lymphocytes from blood to lymph to blood. This idea was further strengthened by an experiment in which the transfusion of radioactively labelled lymphocytes resulted in their rapid appearance in the thoracic duct lymph.

J. M. Yoffey (Bristol) said that lymphocytes probably recirculated on a small scale via the afferent (peripheral) lymphatics, but that there were a number of difficulties in accepting the hypothesis that recirculation occurred on the scale envisaged by Gowans. Detailed histological observations on the regenerating bone marrow of irradiated guinea pigs tends to suggest that lymphocytes provide a reservoir of stem cells which contribute to the regeneration of marrow.

In the experiments described at the meeting and in discussion it was again made plain that there is, as yet, no real clue to the physiological function of the lymphocyte. Prof. Medawar, in his concluding remarks, expressed the appreciation of those present for the hospitality given by the Ciba Foundation.

ELECTROLYTE PHYSIOLOGY

At the British Association meeting in Dublin, the session held by Section I (Physiology and Biochemistry) on September 9, on "Electrolyte Physiology", opened with a paper by Prof. E. J. Conway (University College, Dublin) on "Energy and Form in the Living Cell". In a paper on so wide a subject, the treatment was necessarily limited to certain aspects, but it includes some basic considerations of ionic permeability of the animal-cell membrane.

The energy requirements of the living cell could be listed under the following general headings: (*a*) maintenance of size and composition; (*b*) special functions such as contraction, conduction and secretion; and (*c*) cell growth and division. The treatment was limited to the first heading, the fully developed cell being considered in general, apart from special functions.

Energy required for maintenance of size with respect to water-content. It is reasonable to suppose that the primitive cell was surrounded by a membrane, necessary to retain substances required for its activities. This membrane is considered to be freely distensible, the comparatively rigid plant-cell membrane developing later as an adaptation to sites with variable mixing of sea and fresh water.

As the membrane may be assumed to exist primarily for conserving essential cell substances, it should have some limiting permeability. If it were quite impermeable to solutes, necessary material would be excluded, without some special expenditure of energy. Passing from such permeability to one in which sodium and potassium ions as well as small anions could enter with equal facility per unit concentration, then, from considerations of osmotic, electrical and Donnan equilibria, the following equation can be written[1] for the equilibrium condition

$$\eta = C - (\varepsilon^2 + 4\Sigma bd)^{1/2}$$

Here η represents moles of non-diffusible material within the cell (using the term non-diffusible with reference to the membrane), ε is the arithmetical sum

of the electrical charges thereon (expressed as m.equiv.), and b and d are external concentrations of a diffusible cation and anion. Assuming the external conditions to resemble that of the internal medium of higher animals, then the value for η or for the essential substances within the cell would be very small, approaching zero, even with zero ε value. But if the permeability were reduced so that while the hydrated potassium ions were allowed to pass the larger hydrated sodium ions were excluded, then with the same conditions the η value would be considerable, even with ε about the same as η. It is to be noted that with such an arrangement there would be high internal concentration of potassium ions and a low or vanishing concentration of sodium ions, and such a relation would continue to exist without any energy being specially directed thereto.

However, it may be supposed that a membrane allowing potassium ions to pass freely will not indefinitely exclude sodium ions, and these will enter slowly along with chloride ions, the cell swelling indefinitely. It would appear necessary, then, for the cell to possess some mechanism to counteract this process, either by the active excretion of water, or of chloride ions, or of sodium ions.

What in fact has occurred is that the cell became endowed with the property of excreting sodium ions, the mechanism being referred to as the sodium pump. As the sodium pump operates in the yeast cell as well as in muscle and nerve fibres of higher organisms, it may be assumed to be a general cellular endowment proceeding from the earliest development of the cell in a marine environment.

Position with respect to plant cells. The argument based on a ready distensibility of the cell membrane does not apply to the comparatively rigid membrane of the plant cell. However, the following considerations may be advanced: (*a*) the kind of cell permeability once established in association with the sodium pump in a fully marine environment and presumably over hundreds of millions of years was retained by plant cells as a fixed endowment; (*b*) the long conditioning to relatively high concentrations of potassium within the cell may have had a secondary effect on enzyme activity; (*c*) the long adaptation to the high concentration of potassium may have required—dependent on the conditions—the active transport of potassium into the cell.

Energy required for maintenance of organic composition. It would appear that the organic and essential non-diffusible constituents of the cell are labile and constantly breaking down, and are then necessarily being rebuilt when the cell is in its resting form or respiring condition.

This continuous process of decay and necessary renewal may well constitute a major fraction of the requirement of resting energy, in unicellular organisms or in the tissue cells of poikilothermal organisms. At the same time, in the unicellular organism, it must be taken into account that surplus metabolism is required for growth and cell division, this being essential for the survival of the species.

In certain cell states, as in spores and seeds, associated with a comparatively high degree of desiccation, the breakdown process may be indefinitely suspended, and there are authenticated instances of seeds germinating in suitable environments after lying dormant for centuries.

From a quantitative point of view the non-diffusible and labile components of the cell in general consist very largely of protein and various phosphate esters, much the greater fraction being protein. Here the principle may be entertained that no non-diffusible constituent of the cell is present without functional significance, which would imply that if all the functional non-diffusible material were removed from the cell, nothing is left, except water and salts. But what functional significance is to be attached to cellular protein, ignoring the specialized function of certain fibres? The answer that naturally arises is that, in general, the total protein of the cell is nothing more than the sum of its enzymes; an answer that must have occurred to many workers independently.

Cellular size levels and energy exchanges. Here it is initially helpful if only in a tentative way to consider the advantages and disadvantages that may arise from a diminution of cellular size proceeding from a given level. The following advantages may be mentioned: diffusion of substances necessary for metabolism throughout the cell becomes more rapid; interaction of rapidly acting respiratory redox systems is facilitated; and the entrance of necessary material and excretion of waste products are promoted. At the same time disadvantages of the following kind arise: other things being equal, the rate of entrance of sodium into the cell is increased and so is the work for the sodium pump; while below a certain critical size it may be supposed that each single cell can no longer have the totality of enzymes necessary for its full metabolic life.

Considering the more important aspects of these, it may be noted that, for the functioning of the rapidly acting respiratory enzymes, it would seem that the smaller the cell the better, down even to bacterial size; but for minimizing the work of the sodium pump, and what is probably more important, for achieving the necessary complement of enzyme molecules, a certain critical size, greater than that of bacteria, may be expected.

What in fact happens is of great interest in the light of such considerations. The respiratory enzymes inside the cell are almost entirely incorporated in bacteria-like particles termed mitochondria, and are surrounded by a more extensive cytoplasm. Thus the maximum advantage would appear to be obtained, but not by the apparently obvious device of a critical size governed by a balance of advantages with disadvantages.

Cellular size relative to total energy exchanges. This can be well examined by comparing the sizes of cells of the same tissue-type throughout the great range of adult body-weight in the mammalia. Years ago, I assembled data[2] from the literature relative to the size of the nephron cells and other associated data for several species with a total range in body-weight of a factor of 20,000 (mouse to horse or cow). The surprising fact emerged that the size of the nephron cells was quite independent of the body-weight, but it could be shown that the total effective work done by the single cell is approximately the same. An interesting series of relations was established, and which within the sampling error could be written

$$V = \alpha \times W^{0.666} \qquad g = \delta \times W^{0.111}$$
$$n = \beta \times W^{0.444} \qquad d = \varepsilon \times W^{0.000}$$
$$l = \gamma \times W^{0.222}$$

where W is the body-weight, V the average volume of urine, n the number of glomeruli, l the length of the

first convoluted tubule, g the diameter of the glomerulus and d the diameter of the cell, with α, β, γ, δ and ε constant relative to W. An explanation of these relations was then essayed, which with some modifications would still appear valid.

I also included a brief account of free-energy exchanges within the cell, including the formation and significance of energy-rich phosphate bonds and the thermodynamic theory of the redox pump, which has been incorrectly treated in some recent publications.

Mitochondria and ion transport. The question of ion transport in mitochondria was taken up by Dr. M. G. Harrington (University College, Dublin). The name mitochondria, signifying 'thread' and 'granule', was given by Benda[3], who observed them as filaments or as small rods or granules both in the living cell and in fixed sections.

Mitochondria in the living cell appear to be in a state of constant movement, either a wriggling motion or a transposition from one part of the cell to another, and from the nucleus, for example to the cell membrane and back again[4].

In the isolation of mitochondria from homogenized tissue it is important to observe certain conventional conditions for their separation by centrifugation[4]. Bensley and Hoerr[5] and Claude[6] were the first to stress this point, which was developed very fully by de Duve and Berthet[7]. Concerning the functions of mitochondria there were some early pointers strongly suggesting their connexion with cellular respiration, and at present it would appear from the work of Schneider and Hoogeboom[8] that they are the site of the electron-transporting system and in particular of cytochrome oxidase. From the work of Green[9], Harman[10], de Duve and Berthet[7] and others it appears that they are also the site of oxidative phosphorylation and the enzymes of the Krebs cycle.

While the major significance of mitochondrial study lies with the location of enzymes within the cell, their linkage with respiration and their movements have induced several workers to examine their possible role in active transport.

The kind of active transport contemplated for animal-tissue cells is that of the sodium ion, since for muscle, gland and nerve the evidence is very strong that the fluxes of potassium are of a passive kind. Apart from questions of active transport it is also of importance to ascertain exactly how much potassium and sodium are associated with the mitochondria.

An essential prerequisite to any such study would be the determination of the extraparticulate fluid volume. Theoretically this could be expected to have a maximum value of 26 per cent, corresponding to the value for rigid spheres[11]. Using inulin, data in the literature show values as high as 66 per cent[12], which apparently can be interpreted only on the basis of an entrance of the inulin into the intramitochondrial water, presumably in varying amounts depending on the experimental conditions. In this department, inulin, sucrose and thiocyanate were used to study the extraparticulate volume, all three methods giving extremely high values. A closer study of the permeability of the mitochondria to thiocyanate yielded anomalous results which were therefore disregarded. When the mitochondria were rendered sucrose-free, by washing with saline prior to the estimations, serial values were simultaneously obtained, the thiocyanate value being of the order of 70 per cent, that for sucrose 50 per cent while that for inulin was only 20 per cent. These figures may well point to a compartmented structure for mitochondria as visualized by Werkheiser and Bartley[13], and as might be expected from the many electron micrographs of mitochondria clearly showing the existence of internal membranous structures. The significance of the effect of washing in saline is not clear, but it may point to the need for further experiments using solutions closely approximating to cell-fluid itself as the ideal preparative medium for the isolation of the mitochondria.

Hormonal control of electrolyte metabolism. Finally, Dr. D. Hingerty read a paper on the control of electrolyte metabolism by hormones. The secretion of the posterior lobe of the pituitary and that of the adrenal cortex appear to exert a direct control over water and electrolyte exchanges in the body. In recent years much new information on the functioning of these glands has been obtained. The active principles of the posterior lobe have been isolated and their structure established[14]. They are two octapeptides, which have now been synthesized, and are closely related structurally. One is responsible for the antidiuretic and pressor activities, whereas the other has oxytocic functions.

Originating in the hypothalamus, these factors are stored in the posterior lobe and liberated as required in response to changes in plasma volume, electrolyte concentrations in plasma and other stimuli.

The processes whereby the adrenocortical principles are synthesized and released into the circulation have now been deduced in some detail, the final products (mainly corticosterone and 17-hydroxycorticosterone) being produced after condensation of 2-carbon fragments into squalene and cholesterol, successive alterations of the cholesterol molecule being brought about by enzymes located in various components of the adrenocortical cells[15]. These reactions are greatly accelerated by adrenocorticotrophic hormone, but the mode of action is obscure.

In recent years much work has been done on the isolation and investigation of aldosterone[16], a remarkably potent hormone with respect to electrolyte exchanges. It has some unique properties in structure and mode of secretion, its release into the circulation being determined more by levels of circulating electrolytes[17] (especially potassium) than by adrenocorticotrophic hormone.

The antidiuretic and adrenocortical hormones would appear to act (with respect to water and electrolyte exchanges) mainly on the kidney tubule, but a more general action on sodium and potassium exchanges exists as judged from effects on the isolated frog skin[18] and even on fermenting yeast cells[19].

If, in the mammalian nephron, the most prominent action of the adrenocortical hormones is an inhibition of potassium absorption (as happens in fermenting yeast) then, after adrenalectomy, more potassium would be absorbed with a rise in the content of potassium in the plasma. The hypothesis may then be entertained that the increased uptake of potassium ions has an inhibiting effect on the re-absorption of sodium ions, by displacing sodium from the carrier complex. As a result the plasma sodium would tend to fall. With a high concentration of sodium down the nephron lumen such effects may be expected to be minimized. In this way some of the major facts on the inorganic electrolyte distribution of adrenal

insufficiency and high intake of sodium may be interpreted[20].

With regard to the rather arbitrary division of the cortical hormones into 'mineralocorticoids' and 'glucocorticoids', it is to be noted that even the very potent 'mineralocorticoid' aldosterone has considerable 'glucocorticoid' activity, the difference between the two groups of hormones being quantitative rather than qualitative. The changes in inorganic content of the cells may modify certain enzymatic actions connected with normal organic metabolism, and in this way the increases of magnesium in plasma and cells which accompany the increased level of potassium after adrenalectomy[21] are of much interest. The increase in magnesium content of muscle fibres is associated with an increase of adenosine triphosphate and phosphocreatine, presumably by inhibition by magnesium ions of adenosine triphosphatase and of phosphokinases. Here one may well have a link with the depression in muscular activity, and of growth and heat production seen in adrenal insufficiency.

E. J. Conway

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SOME ASPECTS OF MICRONUTRIENT ELEMENT METABOLISM IN PLANTS*

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Experimental methods and problems. At present ten micronutrient elements, iron, manganese, copper, zinc, boron, sodium, molybdenum, chlorine, vanadium and cobalt, and six macronutrients satisfy criteria of essentiality for some plant organisms. Quite recently, recognition of molybdenum, chlorine[1] and vanadium[2] resulted either from the use of pure salts initially, regardless of later treatments, or from observation of growth increments due to vanadium in unpurified ferric chloride or to chloride provided by cobalt chloride. Further knowledge may depend on avoiding contamination in containers, water, reagents[3], seeds and the atmosphere[1]. Levels of deficiency shown recently for chloride[1], sodium, and vanadium[2] appear to exceed greatly those of 5×10^{-6} p.p.m. or less necessary to show a deficiency of molybdenum[4,5]. Since there may be about 10^3 atoms of molybdenum in deficient cells[6] it may be necessary further to decrease the supply of some elements by a factor of 10^3 before judging requirements, though negative results cannot be decisive. Total or partial replacement between elements, for example, calcium/strontium, molybdenum/tungsten, molybdenum/vanadium, potassium/rubidium/sodium, or of function, as in the case of molybdenum in nitrate reduction[7], which can be bypassed[8], may modify absolute, as well as quantitative, requirements.

Effects of micronutrients on growth. Mineral deficiencies and excesses have reproducible effects of diagnostic importance in many plants, and reflect reproducible metabolic states. Diverse symptoms may appear in different species, although the roles of many elements are probably general. The diversity may result from quantitative differences between plants in the distribution and activity of particular systems during growth, especially where two or more related enzymes depend on the same metal, or in qualitative differences in metabolism. Thus, the lesions in barley deficient in potassium are due to accumulation of putrescine, which is not observed in flax. Large changes may occur in the levels of free amino-acids and amides[9–12], especially with deficiency of zinc[11]. The pattern produced may vary with both deficiency and plant, and L+isoleucine and L hydroxyproline may induce severe abnormalities.

Indoleacetic acid 'oxidase'. A mechanism for the oxidation of indoleacetic acid occurs widely. Early experiments suggested that a hæm enzyme, possibly peroxidase, was involved and that some interaction occurred with manganese. Galston and others[13,14] suggested that the 'oxidase' system in pea epicotyl comprised a flavoprotein and a peroxidase that mediated a peroxidative reaction between the product of the initial oxidation by the flavoprotein, and hydrogen peroxide produced in this first step, which was regarded as photosensitive; the crude action-spectrum suggesting that of riboflavin. Kenten[15] and Stutz[16] showed that purified peroxidases and a monophenol co-factor comprised 'oxidase' systems without any flavoprotein component. Waygood and associates[17,18] have elucidated several points. The manganic ion, when stabilized as manganiversene, initiates non-enzymic decarboxylation and is reduced to the manganous form. The liberation of a hydrogen ion and a skatole free radical (I) is inferred. This is assumed to take up oxygen spontaneously to form a free radical of a skatole peroxide (II). In the presence of a peroxidase this oxidizes a monophenol co-factor which regenerates a manganous ion by the Kenten and Mann system[19], and yields a stable yellow product (III). The autocatalytic reaction is initiated by traces of manganic ion generated by various means

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