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Cation Distributions and The Energy Status of Cells

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Abstract

Further evidence is presented as to the distribution of the four major cations in biological systems: sodium, potassium, calcium, and magnesium. A relationship between ATPase activity and the cation balances is examined, but as the ATPase activity is itself dependent upon ion gradients the steady state of a cell must be dependent upon the ATPase enzyme concentration, the ion concentrations which it helps to maintain, and the availability of ATP. The effect of the product of these three terms upon the stability of ribosomes and other structures is pursued and its ramifications in cell growth (protein synthesis) and electrical activity discussed. Consequential effects, which could be linked with differentiation and retention of information in such structures as the brain, are also considered.

Introduction

In two previous articles[1,2] we have drawn attention to a correlation between the accumulation of potassium, magnesium, and phosphorus and the rejection of sodium and calcium by a great variety of living cells. Here we shall give further evidence of this partitioning of the inorganic elements in biological systems but we shall be concerned to show also that the ion separation is intimately connected with the level of activity of the cells and may well play a leading role in its control. As in the previous articles we start with an examination of single-cell systems and then deal with cell organizations of greater complexity.

Bacteria

New work on bacteria[3] has confirmed the earlier finding[2,4] that there is a stoichiometric relationship between the internal concentrations of potassium, magnesium, and phosphorus. However, it is now known that the amounts of these elements which are associated with the outer membranes can be considerable.[3] The ratios of in-cell concentrations of Mg/K/P remain close to 1:2:10 when these external amounts of the elements are taken into account.

An important feature of the work on bacteria is that cells moving out of stationary phase pick up K, Mg, and P, before RNA formation and, in turn, protein synthesis.[4] This is in keeping with the observation that *in vitro* K and Mg are required to stabilize RNA. We shall look at the importance of these observations later when considering the activities of more complex cell systems. The different final steady states of growth which are achieved, under different specified conditions, not only have similar ratios of the inorganic elements but have amounts of them which are related to the growth rate. The limiting conditions can be the supply of the inorganic elements or the

supply of organic compounds needed, in part, to accumulate these elements.[3,4] No matter what the rate-limiting factors, the inorganic content of the cell is a reflection of its ability to use energy in growth. It could be then that limitation of ion uptake controls growth.

As bacterial cells accumulate the inorganic elements, rather than reaching a simple equilibrium with their external concentrations, the steady states achieved are functions of membrane permeability as well as of the available energy for accumulation. Two factors affect the permeability: the passive penetrability of the membrane by ions, and the concentration of the various ion-pump systems in the membranes. Bacterial membranes have a low passive permeability for ions. It is now known that the ion-pumping systems of the bacterial cells are similar to those of other cells. In particular, they are closely connected to special ATPases located in, or in close association with, the membranes. It appears that there is a different ATPase for handling Na/K and for handling Mg/Ca. Genetic faults can be produced in bacteria such that they lose their ability to accumulate K or Mg without the other elements suffering equally.[4,5] The study of erythrocytes reveals a very similar picture.

Erythrocytes

Erythrocytes are single cells, but, unlike bacteria, they exist in a constant ionic medium. Many striking differences between the erythrocytes of different species have been described in our previous paper,[1] but the most important facts for the purposes of the present discussion are that (1) those erythrocytes with high K, Mg, and P contents, such as avian erythrocytes, maintain protein synthesis (they have RNA and a nucleus) and expend fairly considerable amounts of energy, while the erythrocytes of cattle which have very low K, Mg, and P contents have virtually no RNA and little metabolic activity; (2) the ratios of Mg/K/P are different from those in bacteria, for although the ratio Mg/P remains close to 1:10, the cell content of potassium is very much higher that that of the other elements; and (3) there are a few species, cat and dog, and some genetic variants, e.g., amongst sheep, in which the K content is quite unrelated to the Mg and P contents (see Fig. 1 of ref. 1). The first fact shows that, much as for bacteria, metabolic activity, here ion pumping to a much larger degree, correlates with the inorganic content of erythrocyte cells, but facts (2) and (3) show, much more strongly than was apparent with bacteria, that the accumulation of Mg is independent of the accumulation of K, though it may well be linked with accumulation of P.

The independence of K and Mg accumulations in the erythrocytes has been shown to be due to two different ATPase systems.[6] The Na/K ATPase, which is connected with sodium extrusion and potassium uptake, has been known for a long time and is detected by its ouabain sensitivity.[7] The second ATPase, which we shall refer to as Ca-ATPase, is associated with the pumping of calcium from the cell. In erythrocytes neither of these ATPases is active at anything but a very low level compared with their activity in other cells (see later). In the case of the different cells of the cat the erythrocyte is the cell with by far the lowest Na/K ATPase content[8] (see Table I). The high K content of some erythrocytes is, then, more a reflection of the very low permeability of outer membranes of these cells than of a highly active ATPase. The low magnesium and phosphorus content must indicate the virtual absence of a mechanism for the uptake of one or both of these elements.

TABLE I. ATPase activity of different cell systems of the cat[8]

Origin of cell	ATPase activity (mm/g/h)
Brain grey matter	1·52
Brain white matter	0·34
Nerve	0·1
Kidney	0·2
Liver	0·08
Muscles	
Aorta	0·087
Striated	0·028
Heart	0·030
Stomach	0·005
Erythrocyte	0·00027
(Erythrocyte man	0·004)

Note: The erythrocyte of man, which has a high K/Na ratio (unlike that of the cat), is included so as to compare the same type of cell from different origins.

Leucocytes

The study of leucocyte ionic balances by Woodin and co-workers[9] escaped our notice in earlier papers. It has been shown that the leucocyte accumulates phosphorus and magnesium much more strongly than the erythrocyte, although its Na/K ratio is higher than for human erythrocytes and it appears to be associated with a larger amount of calcium. The high magnesium and phosphorus contents are in keeping with the much higher metabolic rate and the ability to produce proteins of the leucocyte. Interestingly, the cell has a vesicular system from which proteins can be ejected into the external solution but the reaction requires the presence of calcium to trigger it (cf. muscle, nerve, and hormone triggering). It could be that the high calcium, and perhaps the sodium, are artefacts of the content of the vesicles, for on treatment with the protein (leucithin), leucocytes take up both sodium and calcium and simultaneously reject proteins. It is known that the effect of the leucithin is to increase the cell wall permeability, and thus the inward flow of sodium and calcium can be likened to the current pulse on depolarization of the nerve membrane. Recovery, however, is a complex affair in which sodium and potassium movements are unrelated. The vesicles, like those of the muscle, are able to accumulate Ca^{2+}, which indicates that they, like other vesicle membranes, are effectively a closed off inverted protion of endoplasmic membranes. (Presumably the low internal Na^+ inactivates the Na^+/K^+ ATPase.)

Survey of Single-Cell Systems

In the light of the evidence on single-cell systems we feel justified in putting forward for test the following hypothesis. *The metabolic activity of a cell is directly related to ATPase activity and inversely related to membrane permeability.* We include in the ATPase activity those ATP systems which are utilized in protein synthesis and in the maintenance of ion-gradients, so that the hypothesis applies to cells which have a primarily synthetic

function and those which have little or no function except to maintain ionic gradients (see Table I). ATPase activity itself is the product of internal potassium concentration, internal magnesium concentration, internal ATP concentration, and the concentration of the ATPase proteins themselves. We shall maintain that it is no more possible to carry out protein synthesis that it is to carry on any other metabolic activity in the absence of adequate concentration of these four species, and that two of these species, K and Mg, can often be antagonized by related elements, Na and Ca. For this reason it is essential to keep Na and Ca at a low level in active cells. However, as the ATPase has as a primary function, the expulsion of Na and Ca, there is no need to take Na and Ca concentrations explicitly into account when considering the cell metabolic rate, except in those cases (see below), where these ions trigger ATPase. When we deal specifically with maintained,

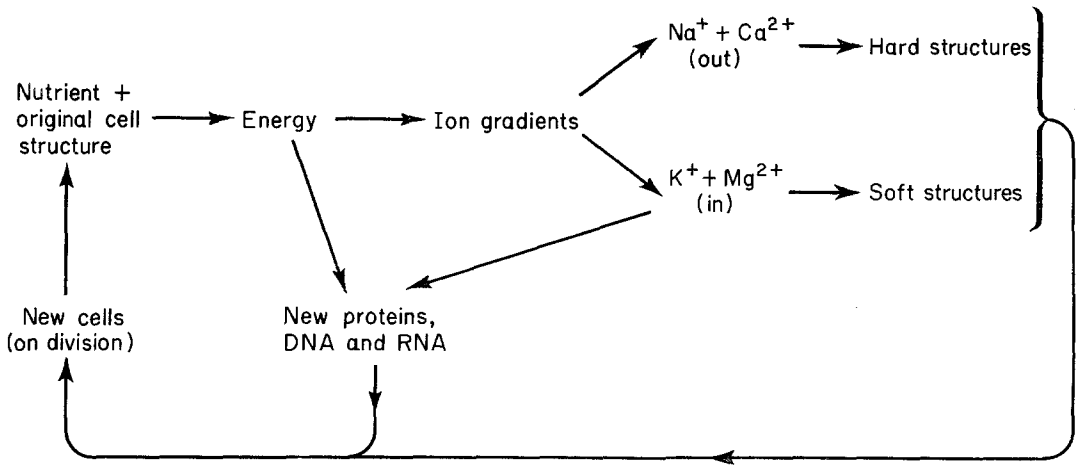

Figure 1. The schematic relationship between the concentration gradients of cations and other cell activities. It is important to notice the control features of the ionic concentrations upon the utilization of energy in such activities as stabilization of RNA and protein synthesis.

steady state, *internal* ionic concentrations we do not need to consider the permeability of the cell either, for this has already been taken into account, implicitly. It follows that amongst a wide variety of cells there should be a relationship between the activity of the cells and these four *internal* concentration terms. In the next sections we shall seek to establish the validity of the hypothesis as expressed in the form

$$\text{Cell activity} \propto [K]\,[Mg]\,[ATP]\,[ATPase]$$

The next most simple systems to which we can apply these criteria are cell particles—mitochondria and chloroplasts. After discussing the "cell activity" of these systems we shall go on to a consider a variety of muscle cells. Here we shall need to remember that ATPase may be triggered by calcium and sodium (cf. leucocyte), so that the cell activity takes on a form which can be curiously time-dependent (pulsed) for the ATPases are revealed by the calcium but they are also responsible for its elimination.

In Fig.1 we set out a scheme which puts together many of the features of ion-pumping in bacteria while also allowing a description of the advanced cells present in mammals. In the diagram we indicate that the ion gradients are as essential for protein synthesis as

they are for all other cell activities, for on the extreme right-hand side of the diagram we divide the rejected cations from those which are accumulated, and indicate the types of centre with which they then associate. Sodium and calcium go to form the external structures, and the stores in vesicles or in the blood solution, which ultimately provide internal or external stimulants depending only on whether the calcium is pulsed into the cell or the contents of the cell are pulsed outwards. Two examples of these cases are hormone release and the release and activation of digestive proteins. Potassium and magnesium, meanwhile, form the soft structures inside the cell—typically they stabilize RNA but they also provide a cofactor for energy utilization so that their cell activity is strongly auto-catalytic. As we shall see, the twin gradients of monovalent and divalent cations also generate electrical activity. Before turning to such problems it will be useful to look at one or two other systems, so as to confirm the picture which is outlined in Fig. 1.

Particle Systems

Mitochondria and chloroplasts can be looked upon as separate energy-developing systems independent of the cell, and they are often studied as such. Their "metabolism" can be thought of as the generation of ATP. For them, too, there is a rather rigid inter-relationship of the energy-producing ability and the internal ionic conditions, as has been shown in a number of ways. For example, mitochondrial function, i.e. generation of ATP, is grossly impaired by the action of carbon tetrachloride, which is known to affect mitochondrial membranes.[10] The change in the membrane occurs together with a large swing in the ionic content of the mitochondria. They accumulate more sodium and calcium and lose both magnesium and potassium.[10] Now, *in vitro* mitochondria readily accumulate calcium from quite low external calcium solutions at the expense of oxidative phosphorylation, i.e. high calcium mitochondria do not generate ATP. It would appear that the uncoupling effect of calcium could be closely connected with its influence upon the membrane, for ATP generation occurs in the membrane. This influence cannot be exerted *in vivo*, for calcium is excluded from the solutions bathing the membrane of the mitochondria by the pumping of the protoplasmic membrane. In all these types of experiments the build up of available energy, ATP, in the mitochondria is related to the Mg^{2+} and K^{+} content.

The chloroplast membrane would appear to act more like a vesicle of the endoplasmic reticulum membrane or of the mitochondrial membrane, i.e. submitochondrial particles, than like a normal membrane. Thus, when the chloroplast is energized by light, and the ATP level thereby raised, it pumps out potassium and magnesium and absorbs sodium and calcium.[11] As is general, uncoupling agents which effectively allow the ATP level to run down by revealing the ATPase (which is normally running in the direction of ATP synthesis) cause the ion movements to be reversed. Thus, in chloroplasts, as in many *vesicles*, the energy status of the unit is shown by the size of the ion-concentration gradients, but these are inverted with respect to those found in whole cells.

Complex Systems of Cells

We now turn from isolated to complex interacting systems. We shall maintain that the cell activity of complex systems, e.g. nerve/muscle, is a function of the whole system and that the division of parts of that system, into muscle and nerve for example, neglects

the fact that there is a constant metabolic interaction between them. This interaction is necessary for a maintenance of the cell in a functional condition. Thus, for example, a muscle cell maintains its "tone" only in a given nerve environment. We shall show that "tone" is a measure of cell activity and is closely dependent upon the product $[K^+][Mg^{2+}][ATP][ATPase]$. Three new features appear in these cell systems. In the first place the ATP-ases are often latent, for it is required that the muscle-cell contraction should be triggered, and cell activity is maximal only after triggering. Thus the maximum activity of a muscle cell is revealed by the speed of contraction and relaxation, these properties being equivalent to the optimal rate of protein synthesis, after lag phase, in, say, bacterial cells. Secondly, this type of cell, which is part of a higher organism, does not remain constant with time. Development, related to differentiation, of muscle cells occurs, and is seen as a modification and growth of the membrane structures of the muscle (cells coalesce and develop a strong internal system of membranes)—the sarcoplasmic reticulum. The sarcoplasmic reticulum contains a high concentration of ATPase, and so the cell activity is enhanced with its growth. The development of a muscle cell is reflected in its ionic composition changes, which closely parallel the ion changes in other cells, including those of the brain.[2] Later in this article, "cell-activity" in brain will be considered, for it must be remembered that memory (storage of information) is an explicit use of energy, and on the basis of our hypothesis we must consider that memory is related to ion concentrations and ATPases. The third new factor in these organized cells is the much higher ion permeability of their membranes. Thus much of the work they expend, and they are cells of high metabolic activity, is devoted to the maintenance of ion gradients, see Table I.

Muscle Cells

There are two extreme types of muscle cells, classified by their speed as fast and slow muscles, although all types of intermediate behaviour are known. The fast muscles can not be distinguished from the slow muscles on the basis of their metabolism in the resting state. Thus, there is little distinction between the "rest" metabolic rate of fast and slow muscles, although the nature of this metabolism may be very different. For example, many slow muscles are "red" and have a high rate of aerobic metabolism, while most fast muscles are "white" and have a low aerobic, though a relatively high anaerobic, metabolic rate. The suggestion that red muscles are slow and white muscles are fast is notoriously unreliable, as very fast insect muscles are red—i.e. they have a high aerobic rate. Fast muscles differ from slow muscles in structure, however, for the fast muscle has an extensively developed sarcoplasmic reticulum. Moreover, the enervation of the fast muscle is located in a small region of the surface of the muscle, while that of the slow muscle is much more extensive. The last difference appears to be intimately connected with the differences in activation. The triggering of fast muscle contraction is an internal affair concerning the sarcoplasmic reticulum, while that of the slow muscle is external—at the outer membrane. The difference, then, extends to the type of ion currents, which seem to be almost entirely Na/K currents for the fast muscle outer membrane but are also Ca currents for the outer membrane of the slow muscle. In all cases it is the invasion of the calcium,[12] from the sarcoplasmic reticulum, in the case of the fast muscle, but through the outer membrane in the case of the slow muscle, which activates the muscle.

In a comparative study of a series of muscles, Close[13] has shown that the speed of shortening of a muscle, i.e. the development of tension with time, is linearly related to the length of duration of the contracted state as measured from the time between half maximal tension on stimulation and half maximal tension on relaxation. This is shown in Fig. 2 and helps to indicate the vast variety of muscle speeds. Now, if it is conceded that the general mechanism of contraction is the same in all types of muscle, and there is a large body of evidence to support such a statement, then both stimulation and recovery rates reflect the extent to which metabolic activity can be turned on to either the development of tension or relaxation—the removal of calcium. The very presence of the extensive sarcoplasmic reticulum in the fast muscles, and the known association of ATPase with this reticulum, as well as with the outer membranes of all cells, is sufficient evidence to show that fast muscles have a higher ATPase concentration than slow muscles (see also Table I). Roughly, we might suppose that the ratio

$$\frac{\text{Surface area of outer and sarcoplasmic membranes}}{\text{Volume}}$$

would give an estimate of the changing amounts of ATPase. In itself, however, this difference is not sufficient to account for the differences between muscles, for the ATPases require a supply of ATP, K, and Mg to be functional. As the metabolic rates of slow and fast muscles at rest are very similar, it is unlikely that their ATP levels will be very different. We have therefore looked at the ionic compositions. It is clearly true from Table II that the faster the muscle the higher the concentration of K, Mg, and P, and the lower the concentration of Na and Cl. Moreover, this ionic composition change correlates only with the speed of the muscle, and not with the distinction between red and white muscles—see, for example, the fast "red" muscle of insects. The assertion that cell activity is related to [ATPase][ATP][K][Mg] appears to be confirmed. Now we must ask further how this difference in ionic composition arises. Although no general answer can be formulated as yet, we wish to put forward an intriguing possibility, which is partly based on the picture of growth outlined for bacteria.

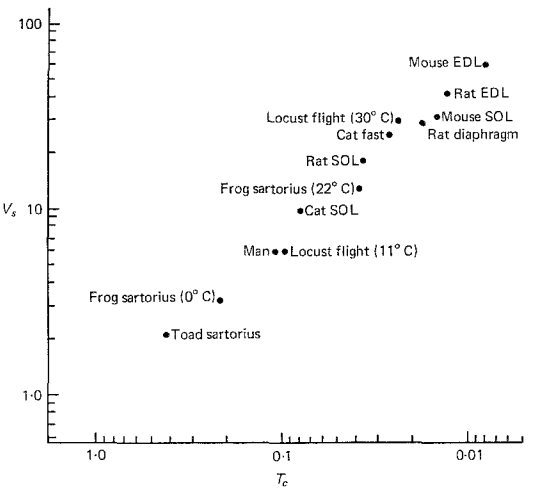

Figure 2. The relationship between the development of tension or speed of shortening, V_s, and the length of duration of the active state of the muscle, T_c, is as described by Close.[13] The duration of the active state is related to the time taken to relax the tension. In the figure, SOL stands for soleus, and EDL for extensor digitorum longus muscles. (This figure is reproduced with the permission of Dr. Close.)

The differences between muscles is a slow process to develop in mammals. Table III shows that initially there is little or no distinction between them on the basis of ionic composition—it appears that only with activity does differentiation develop. Of course, it is well known that activity improves muscle tone, i.e. speed of response, and that inactivity leads to atrophy. Atrophy also results in a fall in all the ionic balances.[14] Now, the activity of muscles is controlled by nerve impulses, so that it is the impact of

TABLE II. Ion contents of some muscles (mequiv./kg)

Muscle	Na	K	Mg	Ca	P	Cl	Property	Refs.
Man								
skeletal	27	92	22	3	80	20	Fast	(*a*)
(dystrophic)	54	111	20	6				
heart	45	78	17	4	63	38	Medium	
myometrium	88	66	13	17			Slow	
Frog								
skeletal	10	124	14·5	4	60	30	Fast	
stomach	32	83	10·7	2	44	50	Slow	
Pecten adductor (a)	43	160			66		Fast	(*b*)
(b)	81	163					Slow	
Mytilus adductor (a)	79	152	34	7	39	94	Fast	
(b)	90	148			33	115	Slow	
byssus retractor	95	153			34·6	152	Very slow	
Anodonta adductor (a)	5·3	21·3	4·5	12	26	2·4	Fast	
(b)	7·2	16·9			14	6	Slow	

Refs: (*a*) E. M. Widdowson and J. W. T. Dickerson, in: *Mineral Metabolism*, C. L. Comar and F. Bronner (eds.), Part IIA, Academic Press, New York, 1964, p. 2, (*b*) W. T. W. Potts, *J. Expt. Biol.*, **35** (1958) 749.

TABLE III. The ionic composition of developing human muscles (mequiv./kg)

Ion	13–14 weeks	20–22 weeks	Newborn	4–7 months	Adult
Na	101	91(46)	60(64)	50(60)	36(58)
K	56	58(81)	58(54)	90(50)	92(66)
Mg	12	11	14(11)	20(11)	17(13)
Ca	6	7	4(7)	3(8)	3
Cl	76	66(41)	43(45)	35(50)	22(47)
P	37	40(50)	47(47)	64(50)	59(49)

Note: The figures in brackets refer to the heart muscle, while the others refer to the skeletal muscle. Heart muscle matures early, and in keeping with being a rather slow muscle has low ionic ratios K/Na, Mg/Ca, and P/Cl.

nerve impulses which must generate the tone. As stated above, fast and slow muscles are differently enervated, and a critical experiment of Eccles and his co-workers[15] showed that a switch of a slow motor-neurone from a fast to a slow muscle caused the state of the slow muscle to move in the direction of a fast muscle. Now, the nature of the "message" which is conveyed from the nerve to the muscle is unknown, and therefore we can only consider a series of postulated events, as follows.

(1) Nerve impulses generate improved ionic gradients in the muscle cell—Mg, K, P up; Na, Ca, Cl down.
(2) The improved ionic gradients generate or stabilize RNA.
(3) New proteins, especially those of the membrane and the ATPases in the membranes, are produced.

(4) The new membranes further assist in the differentiation in terms of further improvement in the ionic gradients.
(5) The removal of sodium and calcium allows the stabilization of internal membranes, as is usual with vesicular systems, and so the sarcoplasmic reticulum develops.

This series of events is exactly that seen in the bacteria.[4] Slow and fast muscles are really just differentiated on the basis of the different types of nerve pulses which reach them in the resting state, and this is equivalent to a different level of nutrient supply to bacteria. Such a system of differentiation will develop to a certain degree only, and then, given that oxidative processes will be continuously damaging the organic membrane material,* decay will set in.

Despite the great differences between single bacterial cells and the cells of muscle, the control of their activities could rest in very parallel controls of ionic concentrations.

The Brain

The brain is an organ which undergoes development[16] much as does muscle. The pattern of changes as the brain develops is sometimes given in stages. At first there is little activity in the brain cells, and the cells themselves (neurones and glial cells) multiply slowly. This is clearly shown by the rise in the DNA content of the brain. In the second stage, cell division has ceased and is replaced by a very considerable cell growth. The glial cells grow, as do the axons and dendrites of the neurone. There is little change in DNA in this period but the amount of RNA increases perhaps as much as ten-fold. During the growth of the cells the RNA is seen to clump into Nissl granules. Now, different parts of the brain develop at different times, and it has been observed that development is related to an increased RNA content concomitant with an increase in axon and dendrite growth. It is also observed that when a system of neurones is stimulated many times the dendrites and axons increase. One theory of memory is based on the appearance of "new" RNA in a brain cell. At the end of this period of very rapid growth electrical activity is observed in the brain, and this is usually labelled stage III. The cells continue to grow and become myelinated in this stage. Examination of the ion content of the cells shows that during stage II, and into stage III, a considerable change in ion balance has occurred. The brain cells have a greatly increased K/Na, Mg/Ca ratio and are richer in phosphorus and free amino-acids and poorer in Cl^- (see Table IV). The appearance of electrical activity is associated with a higher metabolic rate. Once again, during the development the nature of metabolism in the cell has been changing, so that glycolysis is replaced by oxygen utilization. The changes occur differentially, so that different regions of the brain are at quite different stages of advancement at any one time. Naturally, the changes are accompanied by marked changes in enzyme composition of the cells. An enzyme of particular interest here, ATPase, increases strongly in amount during the second and third period. Overall, it seems clear that the brain is in working order in stage III.

* All cells which have a long life and which produce proteins slowly are open to modification by oxidative events. In particular, such oxidation—which will generate anions in polysaccharides and proteins—will increase the binding of cations, especially calcium, and in a membrane would be expected to alter the permeability properties of the membrane. In the absence of a "cleaning-up" of such degenerative processes such oxidation could lead to serious malfunction and, finally, failure of part of an organism.

TABLE IV. Composition of cerebral hemispheres of human foetal brain

	Months of gestation								
	3	4	5	6	7	8	9	10	Adult
Na mg%	237	220	220	220	209	197	183	168	140
K mg%	134	141	145	152	162	165	167	186	210

From W. A. Himwich, D. K. Pennelle, and B. E. Tucker, in: *Recent Advances in Biological Psychiatry*, J. Wortis (ed.), Plenum Press, New York, Vol. V, 1963, p. 263, and see Table 4 of ref. 2. F. E. Samson, H. C. Dick, and W. M. Balfour, *Life Sciences*, 3 (1964) 511, describe the corresponding increase in ATPases.

From the point of view of this article the process of brain development is an up-grading of the potential activity of the cells. They contain more K, Mg, and P and less Na, Ca, and Cl. Like bacterial cells they therefore have a high stabilized RNA and a high metabolic rate at optimal development. Like muscle cells constant stimulation is required, and use clearly promotes a higher activity.

Organization of Nerve Cells

We now wish to examine features of ionic gradients, in order to see what it could imply in the system of nerve cells, rather than, say, in a combination of a nerve cell and a muscle cell. Let us follow the conjecture that it is the below-threshold pulses of the nerves which act as a stimulation on a neighbouring cell, so adjusting its membrane permeability that the neighbour cell itself is altered in cation balance. In this way we could well imagine the general rhythm of electrical pulses which sweep the brain as the input which keeps the brain in an active steady state. Now, if we superimpose upon this rhythm a strong activity—i.e. an above-threshold pulse—in a particular nerve and repeat this many times, then we shall establish in the region where the nerve terminates in the system of nerve cells altered levels of ion concentrations. In turn these new ion concentrations will stimulate RNA, and subsequently protein synthesis, much as in bacteria and, as we have supposed, in muscle. Those cells in direct contact with the cells carrying the pulses will grow. Thus new spatial connections are made which reflect the region from which the original message had come. In other words, RNA—and then protein structures based on ionic gradients—are built up and the original message is now represented in the nerve cell structure by patterns of ions, RNA, protein structure, and, finally, by geometric patterns—all of which are mutually dependent. Now, apart from the spatial pattern which is generated, there are temporal limitations to its ability to function. The new protein may be relatively permanent but the RNA and the ionic gradients can die away, so that although the imprint of the original message is left it could become beyond recall. Failure to repeat a message can cause it to be lost. In an independent study of the mathematical patterns of brain activity Grossberg[18] has arrived at a somewhat similar model and has succeeded in giving such notions as are expressed here a more or less quantitative interpretation.

Finally, if we are correct in associating altered levels of activity with altered ionic

levels then this should be seen in a great variety of gross experimental conditions. It is thus worth noting that the change in ionic balances featured in Table III are seen in moulting[19] and hibernation,[20] for example, leading one to suppose that the postulated connection is indeed present, whether or not it is the immediate cause.

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