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Molecular Vital Statistics: The Significance of Shape

A REVIEW ARTICLE

BY

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The living cell depends on its enzymes[1] to carry out the chemical reactions necessary for its survival. Enzymes are catalysts; they increase the speed of chemical reactions without altering the nature or result of the reaction. As an example, consider lactase, the trivial name for an enzyme which is found in the lining of the intestine. (Its official name and number are β-D-galactoside galactohydrolase, E.C. 3.2.1.23.[2]) It splits milk sugar (lactose) into two smaller sugars (glucose and galactose). This change occurs spontaneously, but only at an extremely slow rate. The enzyme is essential to allow this chemical reaction to occur sufficiently quickly for the sugar to be absorbed from the intestine. Enzymes, such as lactase, must first combine with the chemical (e.g., lactose) that enters the reaction. This chemical is known as the substrate for the enzyme; whilst it is attached to the enzyme, its shape may be altered or a second substrate may be fixed on to it or, as for lactose, it may be broken into two parts. When this change has occurred, the new chemical substances that have been formed from the substrate are released back into the cell. These new chemicals, e.g., glucose and galactose, are called the products of the enzyme reaction. The same enzyme can be used repeatedly, but unlike many other catalysts, each enzyme has a limited life and must be replaced continuously if the cell is to survive.

Enzymes are proteins; they are made from polypeptides which are chains of amino-acids. Although only 23 amino-acids commonly occur in living tissue, the possible variations in their arrangement in polypeptides are almost infinite; there is virtually no end to the number of proteins that can be formed from the same 23 units. The amino-acids that are used to make the polypeptides and proteins ultimately come from the proteins in the food which are split into their constituent amino-acids before absorption from the intestine. These amino-acids are recombined to make polypeptides in the ribosomes, the small solid particles suspended in the liquid cytoplasm of the cell.

Polypeptide formation in the ribosomes is controlled by the nulcleic acids.[3, 4, 5, 6] Each nucleic acid has a skeleton formed from alternate sugar and phosphoric acid molecules; attached to this skeleton are four kinds of base. A group of three adjacent bases (i.e., side by side on the skeleton) forms a code representing one of the amino-acids. There are three main kinds of nucleic acid: information, messenger and transfer acids. The information acids are confined to the nucleus of the cell; they represent the information that the cell inherits for its development and characteristics. The special chemical peculiarity of the nucleic acids that makes them so valuable as information carriers is their ability to make copies of themselves. To be exact, a perfect copy is not formed; instead, a strand of nucleic acid is produced in which each base in the original acid is replaced by another base in the new acid. The information is not distorted since one particular base (e.g., C) on the original acid is always copied in the new acid by the same base (e.g., G). These pairs of bases (e.g., C and G) are said to be complementary. Consequently, the arrangement of the bases along the skeleton of the new acid is strictly determined by the order of the bases in the original acid. When the new strand of acid copies itself, each base (C) will introduce the original base (G) into the next generation of nucleic acid. Clearly the third acid

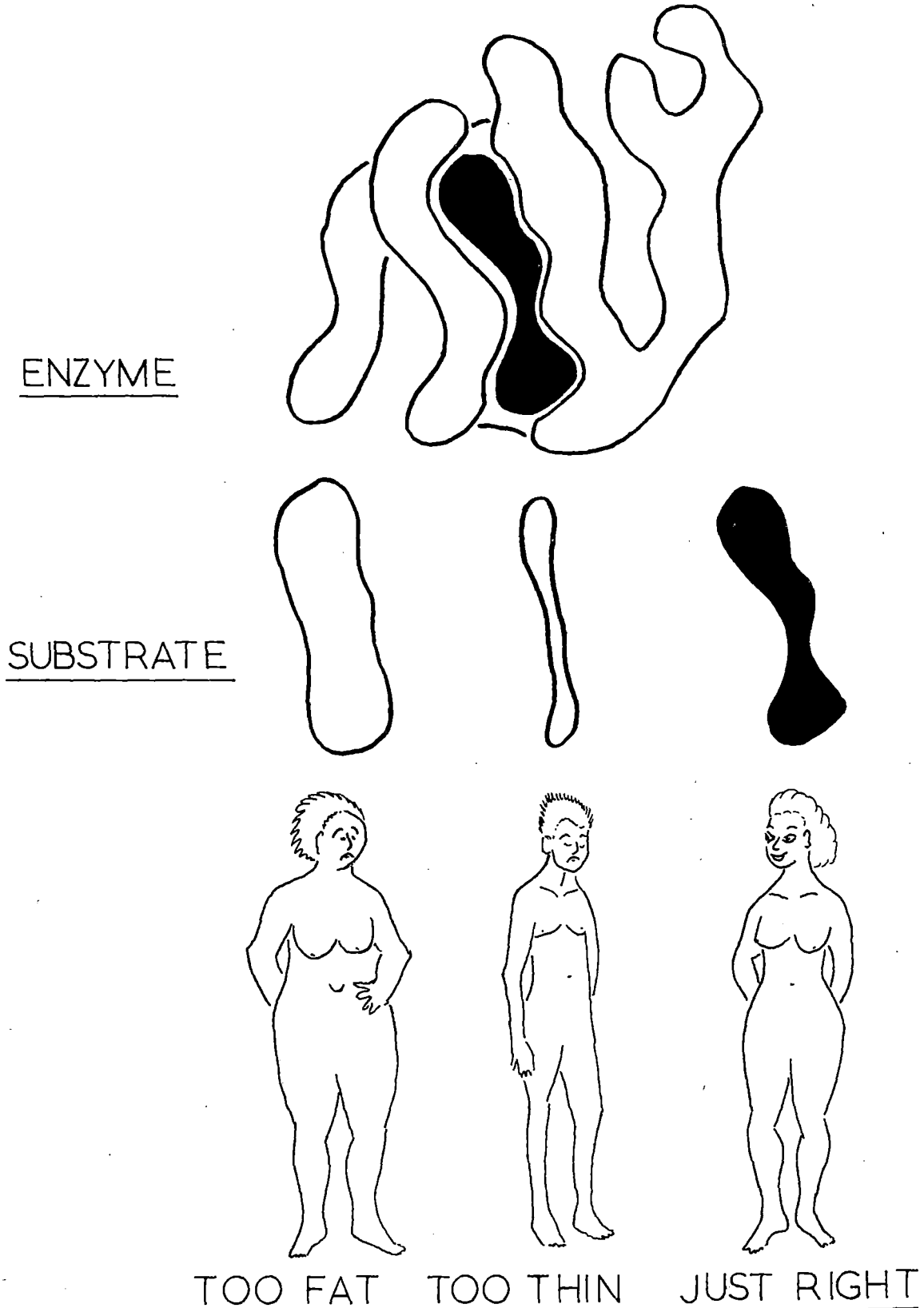

Fig. 1—A short section from a double strand of information acid showing the skeleton of alternate sugars (S) and phosphates (P) with the attached bases (C and G). Note how the complementary bases fit together. The sequence C G C is the code for the amino acid arginine.

will be identical to the original acid and all its information will have been accurately copied and preserved.

The chemical explanation for this pairing of the complementary bases depends on their *shape*. The new strand of acid, as it develops, is wound in a spiral around the original acid and it is only possible for the two acids to fit together when the pairs of complementary bases face one another (Fig. 1). All the nucleic acids in the body are copies of the nucleic acids that were obtained from the parents, half coming from the father and half from the mother. Each time a cell divides, the information acids are copied so that all the original inherited information can reach every cell in the body. Apart from copying the information acid to transfer inherited information to new cells, the order of the bases on the information acid can also be copied on to the second type of nucleic acid called the messenger acid.[7, 8] A number of compounds called nucleotides are joined together to make a messenger acid. Each nucleotide consists of one base joined to a sugar and a phosphoric acid molecule. The nucleotides are attached to one another through the action of an enzyme called a polymerase, which appears to travel along the length of the information acid; as it comes to each base on the information acid, it adds the complementary base, with its nucleotide, to the growing messenger acid. When the messenger is complete it passes from the nucleus to the cytoplasm of the cell and enters the ribosomes.[9, 10]

At this stage the third kind of nucleic acid, the transfer acid, becomes important.[11] One part of each transfer acid carries a group of three bases which form a code[12, 13] for one of the amino-acids. The transfer acid picks up its amino-acid in the cytoplasm of the cell and carries it into the ribosome. Each messenger acid passes through a series of ribosomes[14] which are strung along its length to form a polysome, rather like a necklace of beads on a thread. As each group of three bases on the messenger acid enters a ribosome, it becomes attached to the complementary three bases on a transfer acid. The amino-acid that is carried by the transfer acid is joined to the previous amino-acid that corresponds to the previous three bases on the messenger acid. In this way a chain of amino-acids is produced in which the order of the amino-acids corresponds to the order of the bases on the messenger acid, and ultimately on the order of the bases on the information acid in the nucleus of the cell. A single messenger acid can, at the same time, direct the synthesis of a number of strands of one particular polypeptide; each ribosome that is strung along the messenger acid produces its own chain of amino-acids. The messenger acids are rapidly destroyed[7] and polypeptide formation must stop unless more messengers are released from the nucleus. This is undoubtedly the reason for the limited life of an erythrocyte; it has lost its nucleus and, with it, the power of polypeptide formation.

A fourth type of nucleic acid has recently been described;[15] it is the informational acid, a copy of the information acid which passes from the nucleus to the cytoplasm, where it produces messenger acids that enter the ribosomes. It forms yet another stage in the carriage of information from the nucleus to control polypeptide formation in the ribosomes.

When the polypeptide chain is complete it is released by the ribosome. The end of the chain is marked by a particular group of three bases on the messenger acid that form a "full stop."[16, 17, 18] Beyond the full stop, synthesis starts again to produce another kind of polypeptide. When the polypeptides become separated from the ribosome they coil around one another to make a protein. It is only possible for the amino-acids on the various polypeptide chains to fit together and form a protein when they have the correct *shape*.[19] Consequently each protein has a characteristic shape which, in the final analysis, is determined by the order of the bases on the parent information acid.

An important characteristic of most enzymes is their specificity: they can react only with a very limited range of substrates that have the correct *shape* to fit inside the enzyme molecule (Fig. 2). Lactase, for example, can only combine with a β-D-galactoside; a quite minor change in the shape of the sugar, such as the position of any of the hydrogen and hydroxyl radicles attached to the carbon atoms, prevents its reaction with the enzyme. These transpositions produce an α-D or a β-L-galactoside or a β-D-glucoside; each one of them would require its own specific enzyme. When a suitable β-D-galactoside fits into the lactase molecule, a tension or distortion develops in the link between the sugars; like the medieval instrument of torture, the rack, the enzyme stretches the bond until it breaks.[20, 21]

The portion of an information acid carrying the thousand or so bases that control the production of one type of polypeptide is called a gene; it is the smallest functional unit of genetic material. Since each protein is made from a number of polypeptides, a series of genes, rather than a single gene, is required to direct its for-

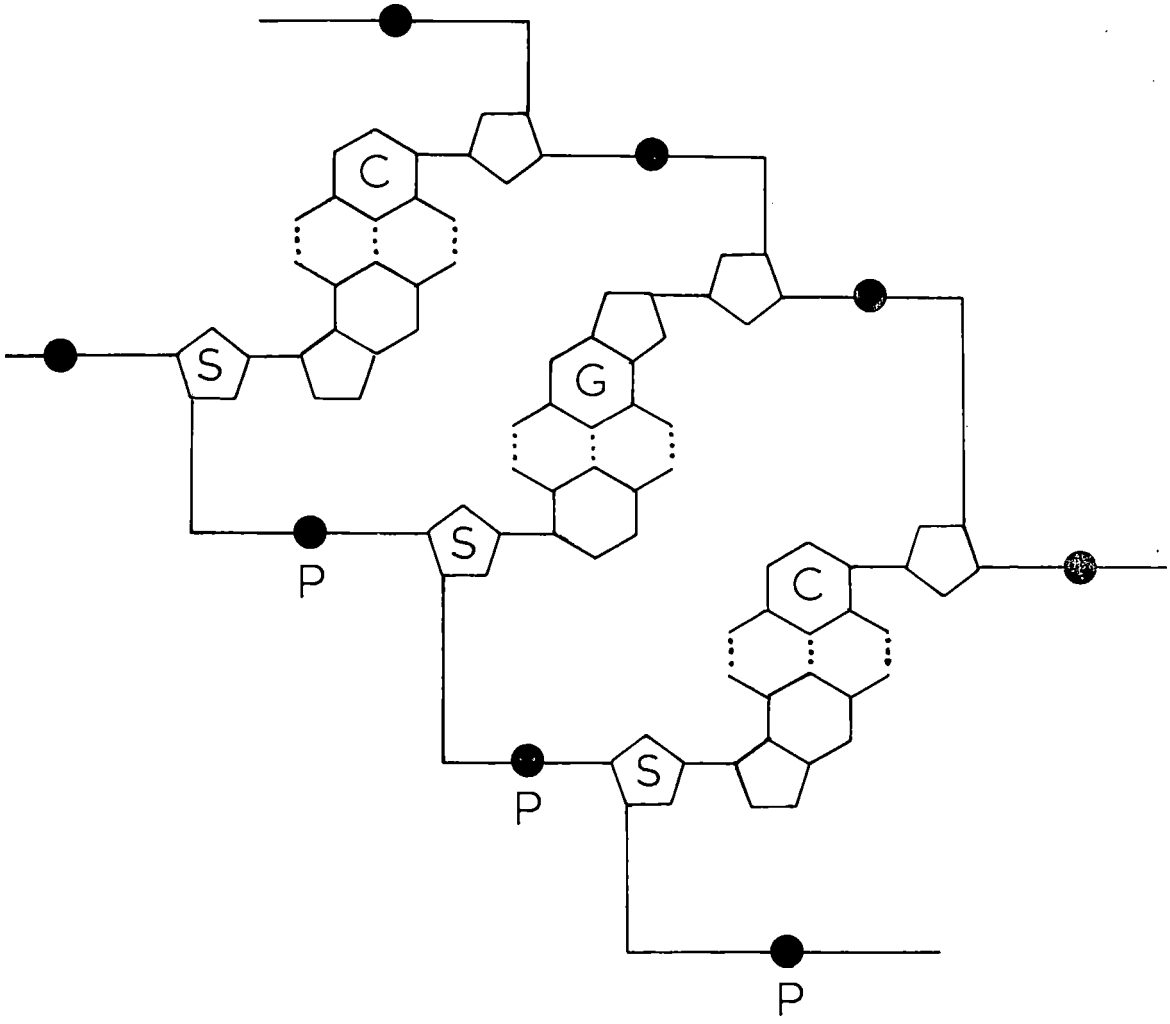

Fig. 2—The shape of the enzyme lysozyme, showing the fissure that receives the substrate. The substrate must be the correct shape to combine with the enzyme and undergo the catalytic reaction.[54]

mation.[22] These genes are placed in order along the information acid in the nucleus, with the code for a full-stop between each of them. When a messenger acid is produced it carries the information to the ribosome to make the complete collection of polypeptides for the whole protein.

Quite commonly a group of proteins are produced in a cell simultaneously. This is particularly true for the enzymes, for example, when a bacterium makes the enzyme lactase two other enzymes, that are needed to allow lactose to enter the bacterial cell, are synthetised by the ribosomes.[7] The genes controlling the formation of this group of enzymes are all found along the same length of information acid; they can all be copied on to the same messenger acid so that it can produce the complete set of enzymes as it passes through the ribosome.[7]

The formation of an enzyme in a cell is controlled by the information acids in its nucleus; this acid is a copy of the information acid obtained from the parents and is identical in every cell in the body. This implies that all the cells in the body contain the essential information for making every kind of enzyme. It is quite clear that, in fact, this does not occur; the same enzymes are not found in every cell of the body; many enzymes are restricted, often to only one type of cell. A good example is lactase; it is an enzyme that is only found in the cells lining the

small intestine. The explanation must be that although the nuclei of the other cells in the body contain the appropriate base code or genes for making lactase, this particular part of their information acid is not working.

It has been suggested by Monod and Jacob[7, 23] from their work on bacteria that there are special kinds of gene on the information acid that control and regulate the other genes. In addition to the structural genes that contain information for making enzymes and other proteins, three more types of genetic information can be found on the information acid. Like the structural genes, these genes form a code depending on the order of the bases along the information acid; this code controls the amount of information acid carrying about 400 bases. The third kind of controlling gene is the promoter;[26] like the operator, it contains about 400 bases; it is the site on the information acid where the enzyme polymerase becomes attached before it passes over and along the structural genes to copy the arrangement of their bases on to a messenger acid. When the repressor is attached to the operator gene, polymerase cannot combine with the promoter and information from the structural genes can no longer be carried to the ribosomes. In this way a single repressor can control a whole group of structural genes, each one of which directs the synthesis of a particular type of polypeptide chain. This group of genes can only become active again if the repressor

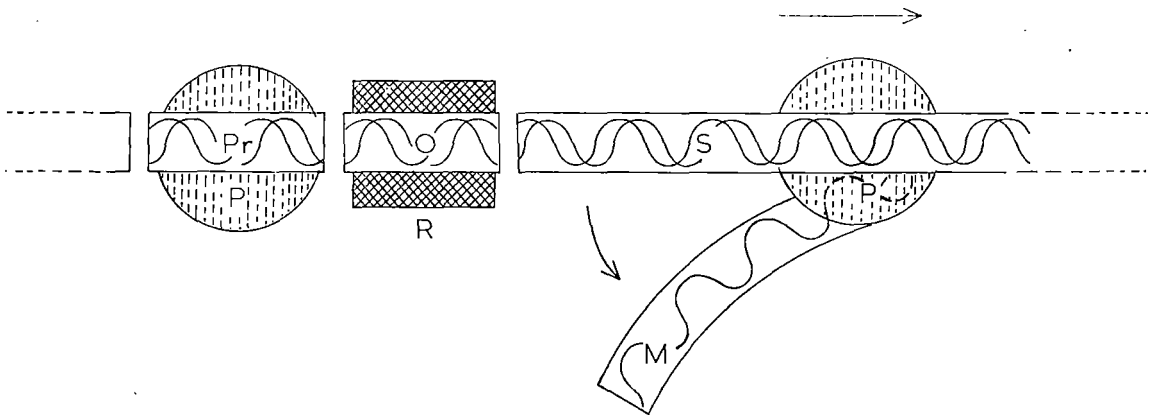

Fig. 3—PrOS represents a double strand of information acid showing the promoter (Pr) gene where the polymerase (P) becomes attached, followed by the operator (O) gene and the structural (S) genes. A polymerase (P') is shown copying the structural genes on to a messenger (M) acid. The repressor (R) can combine with the operator gene so as to block the passage of the polymerase. The repressor in itself may be the wrong *shape* (apo-repressor) and require a co-repressor for its blocking action. The co-repressor is commonly the product of an enzyme reaction that exerts a negative feedback on the synthesis of its enzyme. The repressor itself may be inactivated by an inducer which is commonly the substrate (e.g., lactose) of the enzyme (lactase) that it induces.

messenger acid that can be copied from the structural genes. The first of the controlling genes is called the regulator gene; it can be copied on to a kind of messenger acid which behaves as a repressor and prevents information from the structural genes reaching the ribosomes to control protein synthesis. It may well be that the repressor is not, in fact, a nucleic acid, but a small protein,[24] probably a histone,[25] that is made in the ribosomes according to the instructions coded in the repressor gene. The repressor does not directly affect the structural genes, but combines with a second type of controlling gene, the operator gene,[7] which is a short portion of the can be separated from the information acid. According to Monod and Jacob,[7, 27] a further chemical substance called the inducer has this effect. It combines with the repressor so that the bases on the information acid can be copied on to the messenger acid and restart polypeptide and protein formation. The piece of information acid that controls the formation of lactase and its two associated enzymes, together with their operator and promoter genes, has very recently been isolated from a bacterial cell[28] (Fig. 3).

During the development of the embryo the cells become specialised to produce the various adult organs and tissues. This specialisation is

undoubtedly due to part of the genetic information in any one cell being repressed,[29, 30, 31, 32] so that only a small proportion of its information acids are controlling its structural development and chemical reactions. Certain parts of the embryo called organisers make nucleic acids,[33] histones[34, 35] or small proteins called evocators[36] which control the development of the neighbouring cells. One of the best known organisers is the eyeball; as it grows out as an extension of the brain, it releases chemicals which stimulate the overlying skin cells to form the cornea and lens of the eye. In all probability the whole development of the embryo is controlled in this way; each cell, as it is formed by the division of the preceding cell, comes under the influence of the already existing organisers;[37] only a part of its information acid can be used to control its development. In its turn each new cell produces its own chemical evocators to control and influence the next generation of cells as they appear.

These changes that occur in the developing embryo can best be explained through the action of repressors and inducers.[38, 56] The first cells to be formed in the developing embryo have most of their genes inactivated by repressors. As more cells are produced they will be influenced by the repressors and inducers released by the older cells; although they all contain the same genetic material, some of their repressed genes will be freed by inducers and some of the previously active genes will be blocked by repressors. Evocators are probably nothing more than inducers or repressors. An embryo does not grow at the same rate in all directions, consequently the rapidly growing areas, where there is a more rapid production of new cells, will be more influenced by the repressors and inducers from the more slowly growing parts of the embryo containing earlier generations of cells. In this way the different parts of the embryo can become specialised and develop into different types of tissue. Such a mechanism requires a large surplus of genes, since many of them will operate for only a short period of embryonic development and comparatively few will remain effective in the adult.[39, 40] This certainly seems to be the case in man: there are one thousand million base pairs in each adult's nucleus,[14] but only one-tenth of them appear to be controlling the activities of the adult cell.

The action of these inducing chemicals explains why the ability to produce the enzyme lactase is restricted to the cells lining the small intestine. In all the other cells in the body the information acid that controls lactase formation has been inactivated or repressed. Although a cell in a fully developed individual may have retained its capacity to make a particular enzyme throughout the whole of its development, it does not necessarily follow that it will, in fact, produce the enzyme, except in minute quantities. The amount of an enzyme made in a cell is also determined by the availability of its substrate; the substrate is necessary to act as an inducer;[27, 42] it combines with the repressor to free the gene for the enzyme's formation. Lactase is an excellent example: although it is almost always present in a young child's intestine, it often disappears in the adult. I first became aware of this problem when working in Persia. The American Red Cross sent a large quantity of dried milk powder to parts of the country where the protein in the diet was inadequate. I was very surprised to find that the Persian people refused to use the milk powder and complained that it was poisoned and caused diarrhoea. The milk powder was used in the children's hospital without ill effects and it seemed to me, at the time, that an intolerance to milk was unlikely since milk was a common part of the Persian diet. I now realise that the explanation is the disappearance of lactase in the adult Persian, possibly because there is no lactose in the diet. The universal practice in Persia is to turn all their milk into a form of yoghourt called mosst, a process that converts the lactose to lactic acid. Virtually all European and North American adults have fresh milk containing lactose in their diet; the lactose is an inducer for lactase and prevents the repression of its genes in the intestinal cells. Adults in many other parts of the world, particularly the tropics, e.g., Uganda,[43] are unable to use fresh milk and their lactase becomes repressed. A recent study by Clain[44] shows that lactase is deficient in all adults of Mashona origin, although symptoms of intolerance very rarely occur. The disappearance of lactase in the adult does not only depend on the absence of lactose from the diet; it is also an inherited characteristic. There are two distinct sets of genes controlling lactose formation; they produce two separate but closely related enzymes. Such pairs of enzymes with the same action, but a slightly different structure, are called isoenzymes. It has been found that the isoenzyme, lactase 2, is always present in the adult and that a deficiency is confined to the lactase 1 isoenzyme.[45]

Even though a cell contains an enzyme, the enzyme may not exist in its active form; it may be inhibited and unable to enter into the catalytic reactions, presumably because it is unable to combine with its substrate. Again, the best ideas in this field come from Monod and Jacob;[46] they

suggest that enzyme molecules can exist in two shapes:[47] one is active and the other inhibited. Activation can be brought about by small molecules and ions, particularly Ca^{++} and Mg^{++}, which convert the enzyme to its active form.[48] Other compounds known as inhibitors react with the enzyme and alter its shape so that it cannot combine with its substrate.[49] Inhibition is commonly brought about by the products of the enzyme reaction, or even by the products of later stages in a metabolic pathway. This type of inhibition provides a "negative feedback" to prevent an excessive accumulation of the products of enzymatic activity, an accumulation which outstrips the cell's requirements and the capacity of the subsequent enzyme systems.

The first example of this type of negative feedback was found in the erythrocyte;[50] glucose is converted to its phosphate by an enzyme called hexokinase and the glucose phosphate is eventually split into two molecules of DPG (2, 3, diphosphoglycerate). DPG inhibits hexokinase so that a constant level of DPG is maintained within the erythrocyte; should the level tend to fall, the hexokinase becomes active and more glucose can be phosphorylated and split to restore the DPG concentration. When this feedback system for stabilising DPG was discovered it had no apparent purpose or significance; it is now known that DPG is of prime importance in regulating the transport of oxygen by the blood. DPG combines with the respiratory pigment in the erythrocyte and changes the *shape* of the molecule so that it releases oxygen which can pass from the blood to the tissue cells.[51] The greater the amount of DPG in the erythrocyte, the more oxygen that can be released.

Unlike the other glucose metabolites, such as lactic and carbonic acids, which also regulate oxygen transport, DPG displaces oxygen from haemoglobin only when the amount of oxygen in the blood is low; it has no effect on the oxygenation of haemoglobin when there is a plentiful supply as in the capillaries of the lungs.

A common misconception in respiratory physiology is to believe that the four oxygen molecules attached to each haemoglobin molecule are detached one by one, so that at half saturation, when the blood has released half of its oxygen content, each haemoglobin will have lost two oxygens and two will remain combined with each haemoglobin molecule. In fact, once the first oxygen has been detached, the change in the *shape* of the haemoglobin molecule[52] causes the other three to separate very quickly. It would be nearer the truth to say that at half saturation, half the haemoglobin is completely deoxygenated (N.B.: not reduced) and half the molecules retain their full complement of four oxygens. Since the combination of DPG with haemoglobin does not alter its saturation with oxygen in the lungs,[51] it cannot affect its affinity or binding power for the fourth oxygen molecule; it causes a more rapid dissociation of the remaining three as soon as the fourth has been detached.

Although the concentration of DPG in a given cell is stabilised by the hexokinase negative feedback, it is possible for the stabilised level to be altered to a new stable level. This re-adjustment occurs whenever the supply of oxygen to the body becomes inadequate; under these circumstances more of the haemoglobin is converted to its deoxygenated form which has a greater affinity for DPG. The concentration of free (i.e., unbound) DPG in the erythrocyte is increased, hexokinase is no longer inhibited and more DPG is produced. Even a brief period of oxygen deficiency can increase the amount of DPG in the erythrocyte and the quantity attached to haemoglobin; more oxygen can be released in the tissue capillaries and counteract the oxygen deficiency. As Dr. Peter Jacob pointed out in a recent faculty lecture, these changes occur during circulatory or respiratory failure, in anaemia and at high altitude.

A similar physiological compensation, which also depends on DPG, occurs when erythropoiesis is stimulated by the renal hormone, erythropoietin, during these same conditions of oxygen shortage. New, rather immature erythrocytes enter the general circulation; they have an abnormally high DPG content and are therefore more efficient in carrying oxygen to the tissues. As mentioned above, the erythrocytes have a limited life because they are unable to replace their enzymes. One consequence of this enzyme deficiency is that the older cells are unable to stabilise their DPG content and fail to release their oxygen as they pass through the tissue capillaries. This same defect develops in stored blood; although a patient's haemoglobin concentration may be raised by transfusion, this is no guarantee that the oxygen supply to the tissues has been restored.

One should enquire whether an evolutionary advantage accrues from DPG's control of oxygen transport. Why is DPG not always present in high concentration to help unload oxygen at the tissues? May I suggest that there are two reasons: first, under normal resting conditions, only the fourth molecule of oxygen is detached from oxyhaemoglobin in the capillaries; an increased level of DPG could not influence resting oxygen transport, its presence would confer no advan-

tage and it would be wasteful to convert glucose to this otherwise useless metabolite. The second consideration is that the ability of the erythrocyte to increase its DPG content forms a reserve for use in an emergency. Under normal resting conditions 70 per cent. of the oxygen in the arterial blood is never used; it forms a reserve to be used when oxygen is in short supply. In the same way, the increased production of DPG during conditions of oxygen deprivation contributes to this emergency system and assists the final molecules of oxygen to reach the tissue cells.

CONCLUSION

If there is one lesson to be learnt from the modern study of enzymes, it is that their molecular *shape* is all-important. We are only just beginning to understand how the shape of these protein molecules is determined by their chemical structure[19, 53] and how, in its turn, the shape of the molecule controls the enzyme's chemical and catalytic activity.[54, 55]

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