THE ACTIVITY OF ENZYMES IN HUMAN DISEASE

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Much of our newer understanding of human disease has been acquired through the application to medical problems of established biochemical knowledge, procedures and techniques. The phenomenal growth of biochemistry was, in its turn, made possible through the contributions of organic and physical chemists towards the elucidation of molecular structure and reaction kinetics of an apparently endless number of cellular constituents. One notable recent achievement, in 1961, was the unravelling of the complete amino-acid structure of several haemoglobins, which only 2 years before had been confidently described as an impossible task. The future application of biochemical observations to the problems of human health and disease, irrespective of whether these are made on other animals, plants, or bacteria, will doubtless be even more rewarding than hitherto. We have learned that the affairs of mice and men are strangely similar at the molecular level.

Perhaps the most important recent developments from the practical point of view — on the assay of numerous enzymes, coenzymes and trace metabolites — are but the realization of Gowland Hopkins' vision 30 years ago of the cell as a dynamic, polyphasic multi-enzyme system. Because assays of enzymes provide important information about the cause and course of human disease often not available by any other means, patients' lives may depend on correct results. The following discussion is intended to emphasize some aspects of enzyme systems that are proving or promising to be important in human disease.

It seems advisable to stress, at once, that the activity of an enzyme as determined *in vitro* may be quite different from its activity *in vivo*. In general, enzymes within cells are aggregated into structures such as mitochondria and microsomes, in close proximity to one another, in considerable excess of substrate requirements. Substrates are passed smoothly from one enzyme system to the next and, normally, there is very little accumulation of intermediates of metabolism. The metabolic pools of most substances in the body are small. Under conditions of health *in vivo*, therefore, the rates of reaction will depend more on the amounts of substrate, coenzymes, activators and inhibitors than on the concentration of enzyme itself. Only in disease, either through lack of the appropriate gene, enzymic inhibition by noxious agents, or other adverse conditions that obtain, such as abnormal concentrations of hydrogen or

other ions, will enzyme activity be the rate-controlling step. In order to measure the actual quantity of an enzyme in a system *in vitro* by its specific action, it is necessary to provide an excess of its substrate so that all the active enzymic sites shall be made to work. Under these conditions, provided all the necessary cofactors are present in optimal quantity, the rate of change in the substrate brought about by the enzyme is proportional to the amount of enzyme present, and for a known quantity of enzyme the reaction rate is constant; that is to say, the reaction obeys zero order kinetics. Under such circumstances, at least until the accumulation of products, pH, temperature or other factors slow down the reaction, enzymes may bring about rates of change, so-called turnover rates, unlikely to obtain in the living cell. Thus, one molecule of crotonase activates the hydrogenation of 1,300,000 molecules of crotonic acid per minute.

Active transport mechanisms, negative feed-backs, and aggregation of enzyme systems, prevent the accumulation of such a preponderance of substrate, certainly within the normal living cell. We must be cautious, therefore, in extrapolating enzyme activity obtained by *in vitro* assay to obtain a measure of the concentration or activity of the enzyme in its natural locus in the cellular structure. The conditions are quite dissimilar. Even *in vitro,* missing cofactors, activators or inhibitors may vitiate activity in biological preparations as a measure of the actual weight of enzyme present. With these thoughts in mind, one can consider examples of enzyme systems, determination of one or more separate components of which has proved of especial interest or value in human disease.

THE ENZYME

The presence of amylase and lipase, alkaline phosphatase or prostatic acid phosphatase in the serum in abnormally high concentrations has been used for 25 - 30 years in the recognition of diseases of the pancreas, bone and liver, or prostate, respectively. They represent members of a group of enzymes readily extractable from their productive cells, in contrast to those many respiratory and other enzymes, e.g. succinic dehydrogenase, cytochrome oxidase, firmly bound to mitochondria or other cellular particles. These enzymes are normally being secreted through the normal intact endoplasmic reticulum — amylase and lipase obviously at an extremely rapid rate into the pancreatic duct. Thus, they appear as easily measurable concentrations in the serum of normal persons. Characteristically, amylase is liberated much more rapidly by acute lesions of the pancreas or parotid and passes *via* the lymph into the serum. All the evidence suggests that increased cellular permeability allows the escape of the enzyme from the microsomes, and that the rise in serum amylase is not accompanied by hypersecretion. Pancreatic amylase appears to be identical with salivary amylase, having a pH optimum of 6·5 - 7·0, needing chloride ions for maximum activity, and compared with some enzymes, being comparatively thermostable. It is an α amylase whose primary action is the degradation of starch to dextrins. Like many other enzymes containing sulphydryl groups as active centres, the enzyme is easily inactivated in an irreversible manner by heavy metals (mercury, lead, copper) and organic arsenicals and mercurials.

The enzyme is a small molecule rapidly cleared into the urine; hence, levels of serum amylase tend to fall rapidly to normal, by 48 - 72 hours. Lipase is secreted by the pancreas, not by the parotid; thus raised lipase is an indication that the pancreatic cells are damaged. Lipase has a longer life in the serum and may be elevated for days after an attack.

Apparently neither amylase nor lipase is accompanied in the serum by activators or inhibitors; this renders assay straightforward, in contrast to trypsin, which is inactive in serum. It should be remembered that a fall in serum amylase may be the consequence of failure of secretion in a necrotic gland. Special stimulatory hormones — pancreozymin and secretin — play an important role in the discovery of whether this is true, or whether chronic pancreatitis is present. So far as I am aware, no hypersecretory syndrome associated with hyperplasia of the pancreas or parotid glands has been described, nor are misleading results ascribable to reabsorption of the enzymes through lesions in the small intestine as, for example, duodenal ulcer.

Alkaline phosphatase is also a comparatively stable enzyme secreted by the osteoblast and possibly by biliary epithelial cells. Its value in bone disease and the differential diagnosis of jaundice is familiar to all. The enzymic activity in the blood is probably raised primarily owing to over-production in the osteoblasts or in the biliary epithelial cells, but one cannot discount the possibilities that (a) the increase is merely one of activity, not of concentration, and due to the appearance of some unknown activator, or (b) the concentration of enzyme may, indeed, be raised, but as a result of retarded catabolism of the enzyme. If the enzyme plays an important part in the body's economy, this latter suggestion cannot lightly be dismissed: often one finds that the concentration of an important metabolite is raised in a stressful situation by the failure of the catabolic pathway (e.g. raised cortisol in the dying), a device which economizes on energy-requiring biosynthetic mechanisms and, having obvious survival value, has been perfected during biochemical evolution.

Prostatic acid phosphatase is extremely thermolabile and, after removal from the patient, the serum must be kept cooled to 0°C. and the assay carried out as soon as possible. Presumably its concentration *in vivo* is maintained by a correspondingly high biosynthetic rate, or perhaps the enzyme is protected in some way and changes occur on shedding of blood which render it more susceptible to thermal denaturation. The enzyme is unusual, too, in its stability in dilute formaldehyde solution. The natural substrates of these phosphatases are unknown.

The only transaminases recognized in human tissues up to the present[1] are those concerned in the transfer of the α amino group from glutamic acid to pyruvic acid or to oxaloacetic acid (respectively glutamic-pyruvic transaminase — SGPT — and glutamic oxaloacetic transaminase — SGOT). Elegant spectrophotometric procedures have been devised by which pyruvate or oxaloacetate can be reduced to lactate or malate respectively in the presence of the appropriate dehydrogenase and reduced nicotinamide-adenine dinucleotide. The change in optical density of the coenzyme at 340 mμ, when oxidized, gives a measure of the amount of transamination that has occurred. The con-

centrations of the transaminases are high in heart and skeletal muscle, liver, kidney, and pancreas, and there is some evidence for different forms of transaminase in various tissues. In human transmural myocardial infarction the SGOT usually begins to rise about 6 - 12 hours after the onset of cardiac pain and reaches a maximum about 12 - 36 hours later. Return to normal levels usually occurs within 4 or 5 days. The serum-enzyme level is not significantly raised in patients with angina pectoris or heart failure due to other causes. This enzyme assay provides valuable information on which an early diagnosis of myocardial infarction can be based. It is possible to recognize many posterior infarctions not detected by electrocardiography. Measurement of transaminases in the serum has also provided important aid to diagnosis and assessment of cellular damage in early viral hepatitis, toxic hepatitis, and the Duchenne type of muscular dystrophy.

In a recent review, Bodansky has listed a further 18 important serum enzymes reported during the last decade. It is quite impossible to mention even the major advances, but perhaps a list of titles may serve to indicate the biochemical revolution that is taking place: 6-phospho-gluconic dehydrogenase raised in vaginal fluid of patients suffering from uterine cancer; aldolase activity in liver homogenates increased in primary hepatoma; lack of galactose 1-phosphate-uridyl transferase, the cause of galactosaemia and diagnosable at birth in the erythrocytes of cord blood; poisoning by organic phosphorus insecticides recognizable by inhibition of serum pseudocholine-esterase; neonatal jaundice due to deficiency of UDP glucuronic-acid bilirubin transferase; congenital adrenal hyperplasia the consequence of genetic lack of 11, 17 dihydroxy-progesterone 21 hydroxylase; acetazolimide ('diamox') as a carbonic-anhydrase inhibitor in peptic ulceration; the increased fragility of erythrocytes due to missing glucose-6-phosphate dehydrogenase; and hyperammonaemia, citrullinuria and arginosuccinuria due to metabolic lesions discovered in the ornithine-urea pathway. This *pot-pourri* can give only the slightest impression of the endeavour that now makes it possible to describe in biochemical terms well over 300 inborn errors of metabolism in man.

COFACTORS

A few examples will be discussed simply to illustrate the importance of cofactors in enzyme systems both *in vivo* and *in vitro*. We preserve glucose in our blood specimens by addition of sodium fluoride; enolase, which requires $Mg.^{2+}$ as cofactor, is inactivated by fluoride ions, which form the unionized magnesium fluoride. The coenzyme phosphopyridoxal (vitamin B_6) is of particular interest since it is a cofactor not only for transamination but for decarboxylases and desulphydrases. Inadequate amounts in the diet have been shown to lead to epilepsy in very young children, cured by giving the vitamin. The underlying biochemical lesion is associated with the decarboxylation of glutamic acid in the brain to give γ amino-butyrate, a compound whose presence is needed in the brain to inhibit haphazard neuronal discharge in the cortex. In phosphopyridoxal depletion, the concentration of γ amino-butyrate in the cerebrospinal fluid is lower than normal. This obtains in the 40% of patients treated for tuberculosis with large doses of isonicotinamide hydrazide (isoniazid) who also become epileptic. Isoniazid is very similar in chemical structure to pyridoxal and acts as an antimetabolite to it, by competitive inhibition of glutamic decarboxylase. Further, the primary focus of disturbance in Jacksonian epilepsy has been shown to be an area of cortical tissue where this enzyme is either not active or not being produced.

Another important substance with an influence on cerebral activity generally is 5-hydroxy-tryptamin (serotonin), and its biosynthesis, too, is much dependent on vitamin B_6. Serotonin is formed by the decarboxylation of 5-hydroxy-tryptophan, a key step in the excessive production of the metabolite in the carcinoid tumour. Alpha methyldopa is an effective competitive inhibitor of 5-hydroxy-tryptophan decarboxylase; hence its role as a cerebral depressant. On the other hand, lysergic-acid butanolamide (chlorpromazine) produces similar effects by stimulating the monoamine oxidase, which causes serotonin breakdown. Monoamine-oxidase inhibitors such as marsalid and parnate raise the concentration of serotonin in the brain tissues, and therefore function as psychic stimulants.

From the biochemical point of view, however, probably the most sensitive index of phosphopyridoxal deficiency is at another point of action of this protean compound, viz. the conversion by kynureninase of hydroxy-kynurenine to 3-hydroxyanthranilic acid. By default, xanthurenic acid is formed in vitamin-B_6 deficiency and this is found in abnormally large quantities in the urine.

In the practical assay of transaminases, the importance of phosphopyridoxal must be remembered, since it is remotely possible that the serum may not contain sufficient vitamin to activate the enzyme. On the other hand, a serious source of error in the spectrophotometric technique was discovered when a commercial batch of malate dehydrogenase was found to contain glutamic oxaloacetic apotransaminase—GOT from which the prosthetic group, phosphopyridoxal, had been removed. When this batch of malate dehydrogenase was used, erroneously high transaminase activities were recorded because the phosphopyridoxal concentration of the sera was sufficient to activate the contaminating apo-transaminase.

The dehydrogenases comprise a large group of enzymes that catalyze oxidation-reduction reactions in the presence of either of the coenzymes, nicotinamide-adenine dinucleotide (NAD) or nicotinamide-adenine dinucleotide phosphate (NADP), which serve as hydrogen-donors or acceptors. Those systems of diagnostic importance include lactate, isocitrate, malate, glutamate and glucose-6-phosphate dehydrogenase, and glutathione reductase, and these must all be involved to varying extent in pellagra, which arises owing to nicotinamide deficiency. An important enzyme, β-hydroxybutyric dehydrogenase, which can be prepared from liver, kidney, heart and skeletal muscle, is NAD-dependent and brings about the progressive conversion of acetoacetic acid to β-hydroxybutyrate in diabetic ketosis. This change is of great practical importance, since β-hydroxybutyrate may be excreted in large amounts in the urine, and these are not detected by the colour tests for acetoacetic acid. Hence, the value of procedures such as titratable acidity, which include β-hydroxybutyrate, as an index of the severity of ketosis. Although the specificity of the enzymes resides in the enzyme protein, it should be

remembered that the coenzymes themselves may exist in either free, active or bound inactive forms intracellularly, and, although little is at present known concerning the factors (e.g. hydrogen ions) and processes that liberate the coenzymes, such processes must obviously play an important part in the activity of the enzyme system generally.

One other example must suffice. The hormone insulin increases the rate of uptake and metabolism of glucose in liver, muscle, and adipose and other tissues, probably as a necessary cofactor for glucokinase and also fatty-acid synthesis. Young and his colleagues have demonstrated a wide distribution of insulin and its inhibitors throughout the various fractions of serum proteins. Of especial interest was their finding that marked differences of insulin activity, in the rat diaphragm test, were apparent between serum prepared from capillary and from venous blood. Oxygenation in the lungs changes the inhibitors in some way so that they no longer bind to the hormone, which is thus set free for its action in the peripheral tissues. Here, the insulin inhibitors revert to binding forms which may be partially protective against hepatic insulinase. Thus, changes in oxygenation, by means of this insulin shuttle, can influence general metabolism.

THE SUBSTRATE

Seventy years ago Emil Fischer first demonstrated that the relationship between an enzyme and its substrate may be highly specific. The substrate molecule requires to have a spatial configuration such that it may become intimately bound to the enzyme surface to form an enzyme-substrate complex. The closeness of this fit between the substrate molecule and the active centre of the enzyme determines the specificity of the enzyme towards chemically related compounds. The spatial relationships are three-dimensional, as was brilliantly deduced by Ogston, who explained how aconitase can distinguish between the terminal carbon atoms of a molecule, citric acid, which is perfectly symmetrical. Aconitase acts asymmetrically upon the symmetrical citric-acid molecule through a 3-point (group) attachment of the molecule to the enzyme surface, by which the symmetry of the substrate is abolished. Similarly, the condensing enzyme that facilitates the formation of citric acid from oxaloacetate and acetyl coenzyme A makes a definite distinction between the two valencies of the oxaloacetate-carbonyl double bond. The asymmetric action of enzymes upon various symmetrical substrates is well established.

In 1939, Woods provided a rational explanation of the antibiotic action of the sulphonamides, as being due to their similarity in chemical structure to the bacterial growth factor, p-aminobenzoic acid, the drug being sufficiently closely related chemically to the normal substrate to compete with it in forming complexes with the enzyme, which is thereby inactivated. This hypothesis has been amply substantiated by a host of examples of antimetabolite action at the substrate level by Woolley, McIlwain and others, and it has provided a new and fruitful approach to the preparation of therapeutic agents. Good examples of this are aminopterin and amethopterin, which are antimetabolites to tetrahydrofolic acid and inhibit the synthesis of nucleic acids. Other well-known competitors are malonate and succinate for succinic dehydrogenase, ethionine and methionine in protein synthesis in the pancreas, and methyl tryptophan and tryptophan in the renal tubular cells; all produce dire effects due to competitive inhibition at the substrate level.

In contrast to the irreversible binding of heavy-metal ions (Ag, Hg, Pb, Cu), organic mercurials, arsenicals, phosphorus and iodine compounds, most of the inhibitions at the substrate level are competitive and reversible. An unusual type of competitive inhibition, of especial interest in South Africa, is that arising from fluoroacetate. This compound occurs in the leaves of the plant *Dichapetalum toxicarium*, which grows in the Transvaal and is responsible for the death of sheep in that part of the country. The toxic agent was identified by Marais, working in the laboratories at Onderstepoort. Peters and his colleagues in Oxford showed that fluoroacetate itself is not toxic until it has been further metabolized in the body to fluorocitrate, which is a competitive antimetabolite to citrate for the enzyme aconitase. The competition leads to the accumulation of citrate and blocking of the aerobic oxidative pathway. This poisoning has been aptly named by Peters a lethal synthesis. Fluorosuccinate, the toxic principle of the ordeal bean widely used by witchdoctors in Central Africa, is a powerful poison through its direct competition with succinate.

These examples typify what, in fact, is a general phenomenon in living cells, the control of biochemical systems at the substrate level, which can, perhaps, be illustrated by reference to the following simple scheme.

Each of the biochemical steps from A to B, from B to C and so on (Fig. 1) represents a relatively small change in chemical structure. This provides many opportunities for competitive inhibitions of enzymes, and the exploitation of these has provided the basis for biochemical and biological evolution. The equilibrium of many biosynthetic reactions so greatly favours synthesis as to be irreversible, and the effects of end-products on reaction rates can only infrequently be ascribed to a mass-action effect. As a rule, biosynthetic pathways are regulated by subtle enzyme-substrate interrelationships. Let us suppose that early in

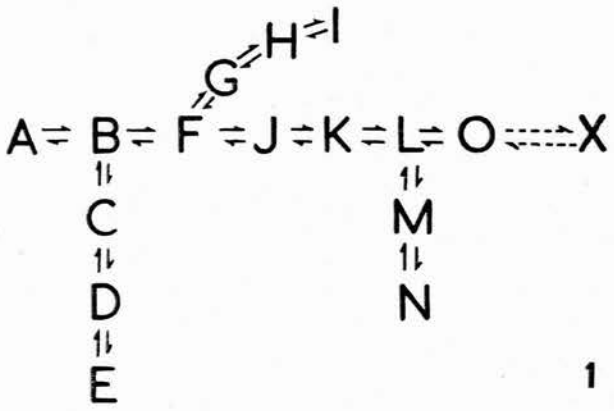

Fig. 1. Scheme to illustrate the control of biochemical systems at substrate level.

biological time the substance A played a role in the metabolism of a primitive organism. By genetic mutation and enzyme synthesis, A was changed to B, a compound which

endowed the cell with certain advantages with regard to energy metabolism, cell division, and so on. At later stages, C and D might have arisen, E representing the limit of advantageous specialization of the molecule along that particular pathway. Subsequently, or perhaps, to some extent simultaneously, a new type of change took place leading to F, G, H and I, which in turn was superseded by a branching at F to L, each in its turn being invoked by the need for survival. We arrive at the present. Let us suppose that compound X is an important metabolite in the pathway. Not only is the formation of X dependent on the inheritance of the genetic-enzymic systems leading from A to X, but the compound X itself must be the most suitable heir to that inheritance; witness how often compound X has a profound inhibitory action upon the enzyme converting L to O on the first step of the enzyme pathway leading to X, thereby avoiding unnecessary synthesis of compounds O to X. In such a manner does the concentration of cholesterol control its own biosynthesis in the rat liver by inhibiting the formation of mevalonic acid from β-hydroxy-β-methyl-glutaryl coenzyme A, 17 precursor steps away; and cholesterol added in the diet impedes *de novo* synthesis. This explains the widespread inhibitory effect of end-products on the accumulation of their precursors.

On the contrary, exhaustion of the important metabolite due to an enzymic block at a precursor stage may facilitate little-used pathways as, for example, the activation of amylo 1-4 glucosidase (γ amylase), which splits free glucose from glycogen in injured muscle and in McArdle's syndrome, giving rise to the 'second-wind phenomenon' encountered in that disease.

A final example, the control of glycolysis by nicotinamide-adenine dinucleotide phosphate (NADP), may serve to illustrate the interrelationships between substrates, enzymes and cofactors that obtain in living cells and influence the activity of any individual enzyme in a multi-enzyme system.

Addition of NADP to cell-free preparations of rat brain leads to decreased glycolysis and the inhibition is further enhanced by addition of fresh or aged mitochondria, which will oxidize $NADPH_2$.

In the metabolism of glucose, NADP is the required coenzyme of glucose-6-phosphate dehydrogenase, and of the next enzyme 6-phosphogluconic dehydrogenase. In cell-free preparations, the latter enzyme is much less active than the former, and 6-phosphogluconate accumulates — in the presence of NADP but not in its absence. The

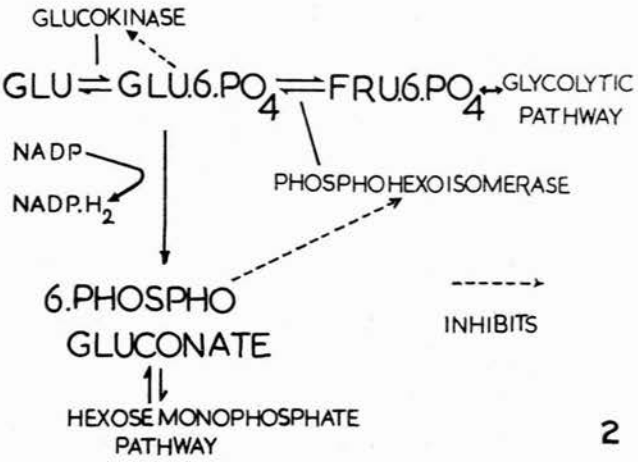

Fig. 2. The control of glycolysis by nicotinamide-adenine dinucleotide phosphate (NADP). GLU=glucose. GLU.6. PO_4=glucose 6-phosphate. FRU.6.PO_4=fructose 6-phosphate.

accumulated 6-phosphogluconate competitively inhibits phospho-hexo-isomerase, by which there is a hold-up at glucose-6-phosphate, which in turn inhibits glucokinase. Thus the coenzyme NADP by its effect on the first stage of the hexose-monophosphate (aerobic) pathway exerts a powerful controlling influence on the rate of entry of glucose into the cell and the total rate of glycolysis in the body.

REFERENCE

1. Wilkinson, J. H. (1962): *An Introduction to Diagnostic Enzymology.* London: Edward Arnold.