BRITISH MEDICAL JOURNAL

LONDON

SATURDAY MAY 10 1941

CRYSTALLINE ENZYMES

It is a commonplace nowadays that maintenance of the life of all living things depends upon physical and chemical processes which are made possible by a series of catalysts elaborated by the organisms themselves. From this point of view the living body as a whole may be called an autocatalytic machine. Whereas these catalytic agents deal primarily with the normal necessities of the organism, it is remarkable how readily they can be adapted to deal with substances administered for various purposes and normally foreign to it. Although it is inevitable that we should study the catalytic reactions in the body separately, no significant picture of them will be obtained until they can be fitted into the total scheme of the living unit. The construction of such an understandable scheme will be a laborious process. At present a number of aspects of the subject have been clarified. The complicated system of enzymes, about whose nature and action there has been so much controversy, are vital to the sequence of metabolic events in the organism. From the present standpoint all these arguments seem a waste of energy. Until recently all the experiments were performed with very crude preparations; thus, although there may have been much truth in what each disputant maintained from his experiments, no finality could be reached until preparation of the pure enzyme was made possible.

When, in 1926, Sumner isolated from soya bean a crystalline cell catalyst which appeared to be identical with urease, the enzyme which rapidly decomposes urea into ammonia and carbon dioxide, a new field of enzyme research opened. Pepsin was isolated and crystallized shortly afterwards, and in the succeeding years pepsinogen, trypsin, and other pancreatic enzymes; papain (kathepsin), amylase, lysozyme, and others were crystallized. Northrop,[1] who has been in the forefront of this work, states that, so far as is known now, these enzymes are simple proteins—that is, they do not embody groups other than amino acids. The enzymes are catalysts of hydrolytic processes; for example, trypsin acting on proteins with the characteristic linkages (–CO–NH–) splits them and adds the molecule of water thus: $(\text{–CO–NH–}) + H_2O \rightarrow (\text{–COOH}) + (\text{–}NH_2)$. Catalase, an enzyme which catalyses the reaction $2H_2O_2 \rightarrow 2H_2O + O_2$, and tyrosinase, which splits the amino acid tyrosine to form derivatives of catechol, have also been isolated and crystallized. They differ in structure from the hydrolytic enzymes in that they are conjugated proteins (haemoglobin is a conjugated protein—the protein globin + the prosthetic group haematin).

The significance of these isolations and crystallizations of enzymes depends upon whether it can be demonstrated unequivocally that the substances obtained are chemical individuals and possess the specific properties attributed to the enzyme. So long as the purity is in doubt it is open to critics to object that the active enzyme is adsorbed on the protein and responsible for the activity. The criteria for purity of an organic compound cannot be applied to these enzyme proteins, or indeed to any protein. Hence other criteria must be used (for example, solubility curves) which are not absolutely satisfied even by such well-studied proteins as haemoglobin and egg albumen. If the deviations from theoretical requirements are no greater than those observed for the purest specimens of the latter, then comparative purity can be inferred. In at least one case (chymo-trypsinogen) the correspondence to theoretical requirements is very close and indicates that this protein is one of the purest ever isolated. Other methods (sedimentation in the ultra-centrifuge; rate of migration in the electrophoresis cell) are also used to test for purity of proteins, but are perhaps not so reliable as the solubility test. These various methods do not always give results consistent one with the other: thus crystalline pepsin, which appears to be a chemical individual by the sedimentation and electrophoresis test, is shown to consist of two or more components by the solubility test. Clearly more work is necessary to establish once and for all that enzymes are definite chemical individuals, though it looks as if it is a mere matter of time before this is done.

The familiar fact that heat destroys the activity of enzymes and that they have an optimum range of reaction (*p*H) for their activity is demonstrable also with the crystalline preparations. The fact that the crystalline preparations can be inactivated by other proteolytic enzymes also points to a very close relation between the protein and the enzyme. These and other findings suggest strongly that the enzymatic activity is vested in the protein molecule itself, but it is, as Northrop states, impossible at present to say what chemical structure is first responsible. By introducing certain groups and elements—for example, acetyl groups and iodine—into the molecule, and by determining the effect on activity, it can be shown that the free amine groups are not necessary for the activity, but that the phenolic groups (for example, the –OH in tyrosine) are. A very interesting consequence of the crystallization of enzymes has been the clearer picture that can now be obtained of the puzzling phenomenon of the activation of pancreatic trypsinogen. The older experimenters showed that freshly secreted pancreatic juice is inactive and is activated by the so-called enterokinase present in extracts of the small intestine. This classical experiment, using other pancreatic juice or amorphous preparation of pancreas, is repeated by every class of chemical physiology. It has now been demonstrated that amorphous preparations contain traces of a substance (this has also been isolated in crystalline form) that inhibits trypsin. Crystalline trypsinogen, however, freed from such inhibitor, is activated in neutral solution by the most minute trace of trypsin, and autocatalysis proceeds uninterruptedly. In the case of *in vivo* activation of pancreatic juice by enterokinase, the old controversy whether this is an enzymatic reaction or whether active trypsin is a compound of trypsinogen and kinase (stoichiometric view)

[1] *Johns Hopk. Hosp. Bull.*, 1940, **66**, 207.

seems at least in part to have been cleared up. By using purified materials Kunitz has found that confusion arose because an inert protein and the active trypsin are formed simultaneously. The relative amounts of the two depend upon the acidity of the reaction mixture. The active trypsin preponderates in slightly acid condition, while the inactive protein appears in greatly increased quantities in neutral or slightly alkaline conditions. The isolation and crystallization of pepsinogen were followed by the proof, confirmatory of the earlier work on crude preparations, that it has no proteolytic activity, and that it is converted to active pepsin in slightly acid solution. The progress of the activation is made possible by the initial formation of a trace of pepsin, which then starts the activation of the remaining pepsinogen—that is, the reaction is autocatalytic. This activation is accompanied by a splitting-off of nitrogenous compounds, so it is probable that the formation of the active enzyme from the inactive precursor involves rupture of some of the peptide linkages in the latter.

Thus, with the progress made in the isolation and crystallization of what is likely to be established as the enzyme proper, and with the elucidation of the processes of activation of inactive precursors, enzyme action will be reduced to the physics and chemistry of a special kind of catalysis. These enzyme proteins must possess peculiarly active groups or configurations, perhaps made available for action by the characteristic temperatures and *p*H requirements, which permit of the formation of combinations with the substrates so that rupture at certain points of the molecule of the latter occurs rapidly in an aqueous medium. Much of the old ground will, we imagine, have to be gone over, using crystalline enzyme proteins, before a reasonable hypothesis is forthcoming. The great advance so far has been that enzymes need be regarded no longer as mysterious agents working in a way which puts the chemist to shame, but as clear-cut chemical entities which are amenable to the methods of investigation already familiar to us but requiring a much more profound understanding of protein chemistry and physics than we yet possess.

SOCIAL FACTORS IN MEDICINE

The correspondence which has appeared in our columns under the title "Medicine in a Changing World" reveals some difference of opinion as to the functions of the medical profession in relation to the community it serves. There can, at least, be no doubt about our duty to find out if individual patients are so affected by their physical environment or by mental stress that their recovery from somatic disease is hindered; and to compile and broadcast such scientifically ascertained facts as indicate the extent to which the well-being of the people is adversely influenced by social circumstances. In the field of nutrition, for instance, it is our task both to keep our patients right and to make known to the whole community the physiological facts and their relation to economic and social circumstances without knowledge of which they cannot properly order their lives. It was the work of James Lind that enabled Captain Cook to make his famous voyages with an unprecedented freedom of his crews from scurvy, and Lind and John Pringle showed by their rules of hygiene for the navy and army how typhus could be controlled in any community. About the same time Percival, Ferriar, Currie, Haygarth, and Thackrah were piling up evidence of the deleterious effect on health of the evil state of the towns and factories. The medical observations of these men led to their open advocacy of reforms which were later effected through the political efforts of men like Shaftesbury and the administrative persistence of Chadwick and Simon, himself the first medical man to hold high public office. But perhaps no single individual had more influence on the character of the great public health movement of the nineteenth century than John Snow, who appears to have been less concerned with propaganda than with proving by scientific inquiry that cholera was a water-borne disease. Whatever may be the best attitude for the profession in these matters there can be no question that the effect of environment on health and recovery from disease is a subject of intimate concern to them and that it ought to be in the forefront of their minds from the very beginning of their student days. In some of the provincial schools, and especially in the Scottish universities, where there was intimate contact with civic life, the older clinicians who had lived and worked through the squalor and epidemics of the industrial revolution carried their experience to the classroom and the bedside. But when sanitation had solved its grosser problems and public health became a specialty, students, if they were taught preventive medicine at all, were often bored with disquisitions on the technique of a subject they foresaw no prospect of practising. In spite of lip-service to the unity of preventive and curative medicine the practice of hygiene has increasingly parted ways with the practice of clinical medicine.

That there is a close connexion between successful therapy and the domestic and occupational surroundings of patients has been recognized in hospitals by the establishment of almoners, whose business it is to gather the facts for the use of the clinician and to try to establish so far as possible the milieu which will favour the maximum restoration to full health and least exposure to relapse. The Americans have accepted more whole-heartedly than we have the idea of the social service department in a hospital, and, above all, its place in the teaching of medical students.[1] And yet Dr. Edward L. Bortz, physician in the Lankenau Hospital, Philadelphia, deplores the fact that still only eleven medical schools in the U.S.A. arrange for their social workers to participate in the training of medical students.[2] From a study of 200 patients suffering from cardiovascular, diabetic, gastro-intestinal, respiratory, nervous, and other conditions, he concludes that 75% were affected by social circumstances aggravating their medical state and impeding their recovery. These ranged from absence of economic security, bad physical surroundings, physical strain, and lack of assistance, which are of the same order as those defects which engrossed the minds of our forerunners, to marital or family conflicts, fear of dependancy from sickness, uncongenial employment, solitude, and personal inadequacy (which presumably

[1] *British Medical Journal*, 1936, **2**, 869.
[2] *Ann. intern. Med.*, 1940, **14**, 1065.