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ON THE PHENOMENA OF VITAL CHANGE.

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“Enzyme Action as bearing on the Validity of the Ionic-Dissociation Hypothesis and on the Phenomena of Vital Change.” By HENRY E. ARMSTRONG, Ph.D., F.R.S. Received April 5,—Read April 28, 1904.

*D 8030 Formation of carbohydrates in protoplasm.

D 1810 }
Q D 1810 } glucosamine, significance of, in enzymes.
Q 1440 }

On several occasions of late years, I have protested against the dogmatic attitude assumed by the advocates of the ionic-dissociation hypothesis of chemical change and have remarked on the danger of allowing a purely mathematical treatment to supersede a careful, unbiassed consideration of the facts as these present themselves to the chemist. I have insisted on the limited application of the hypothesis—especially in explanation of the behaviour of the large majority of organic compounds; and have contended that an association hypothesis is preferable and of far wider application: yet, in so doing, I have always recognised that the dissociation hypothesis is often susceptible of numerical treatment in a way which places it at a great advantage.

Twenty years ago, I contended that the solvent played as important a part as the dissolved substance in electrolytic changes; then and for some time afterwards the dissociationists regarded the solvent as a mere screen. Gradually they have been led to recognise that the solvent plays an active part and “ionising” solvents are now freely spoken of: the admission has been made, however, tacitly and without recognition of the fact that the difference of opinion is now reduced to the one question—whether ions enjoy separate existence in solution: whether, for example, in a solution of hydrogen chloride, free hydrogen

* [Index supplied by author, classified according to the schedule of the International Catalogue of Scientific Literature.]

and chlorine ions are present to the extent of 70 odd per cent. I have ventured to characterise such an assumption as not merely unnecessary but eminently improbable: the contradictions involved in the conception being extraordinary—for while we are asked to believe this of hydrogen chloride, we are assured that a substance of such very inferior stability as mercuric chloride exists in solution practically “unionised.” There is danger that under the guidance of mathematicians anxious to negotiate numerical agreements, we may lose our sense of proportion as chemists; I believe the danger to be a very serious one, against which it is necessary to make some protest. We shall fail in securing the object we aim at if we allow the element of authority to intrude in any way into our work: it will cease to be scientific; it will be impossible to claim for it any special educational value; an attitude of doubt rather than one of conscious certainty is essential to progress.

Although the solvent is now regarded as of importance, its peculiar importance and functions are far from being sufficiently recognised. It is necessary, in fact, to urge that the nature of the correlated processes of electrolysis and chemical change need to be considered most carefully, from every point of view, especially with reference to the functions of the solvent and the part played by residual affinity. Results such as have been obtained by Brereton Baker—proving that interactions occur only when a somewhat complex conducting circuit is established—must be fully taken into account: the observations of this chemist on the formation of ammonium chloride, for example, are of the utmost consequence; yet the advocates of the ionic hypotheses simply disregard all such evidence.

In my paper presented to the Society in 1885, I contended that the special influence exercised by water is to be attributed to the residual affinity possessed by the oxygen atom of the water molecule; this view has gradually grown in popularity of late years in consequence of the discovery by Collie and Tickle, von Baeyer and others of compounds in which oxygen apparently functions as a tetrad. There has naturally been a tendency to extend the view to other elements and to attribute the “ionising” power of liquid ammonia, hydrogen cyanide, etc., to residual affinity. But it is necessary to be cautious in accepting the conclusion that these solvents are strictly comparable with water. Water is perhaps peculiar in the extent to which it conditions the electrolysis of substances which are not electrolytes *per se*. In at least a large proportion of the cases in which conducting solutions in solvents other than water have been obtained, substances have been used which are conductors *per se* in the liquid (fused) state: it may be, therefore, that the conductivity of such solutions is but a consequence of the presence of the liquid substances and is largely if not wholly independent of the solvent, the relatively high conductivity of some of

the solutions being perhaps conditioned by the slight viscosity of the solvents. On the other hand, it is highly probable that in many cases in which slight conductivity has been observed insufficient care has been taken in the purification of the materials used.

In discussing the nature of chemical change in my Presidential Address to the Chemical Society in 1895,* I specially drew attention to the difficulty of explaining the behaviour of enzymes—particularly their selective action—as hydrolytic agents by the ionic-dissociation hypothesis: the view was then advocated that their function consists in bringing water into conjunction with the carbohydrate *by combining with both*. The evidence brought forward in the two previous papers† on enzyme action appears to me to be of consequence in this connection: and I venture to think that the case is very materially strengthened by the considerations advanced by E. F. Armstrong and R. J. Caldwell in discussing the action of acids on the sugars.‡ The selective character of the effect produced by enzymes, which has now been demonstrated in several ways, may almost be regarded as final proof that action is determined by association—not by dissociation. And if in such a case we are led to admit that change is the immediate consequence of effective association, there can be no difficulty in regarding chemical changes generally as of this character.

In their behaviour towards acids, the sugars exhibit peculiarities which are so little in accordance with the ionic-dissociation hypothesis that we are almost compelled to admit that in this case also hydrolysis is dependent on association. The explanation which this latter view affords of the great influence which temperature changes have on the rate of change is in itself striking evidence in its favour—especially as the explanation set forth in their paper by E. F. Armstrong and R. J. Caldwell is applicable to chemical changes generally.

In advancing proof that the enzymes are built of dimensions which enable the molecule to become associated *at several points* with the carbohydrate molecule, E. F. Armstrong has carried a step further the argument which I advanced in explanation of fermentation proper in 1895.‡ The statement then made was as follows:—"Supposing that the protoplasmic hydrolyst were to condition the formation of a conducting circuit in which any two of the carbon systems (CH_2OH , CHOH or COH) of the glucose molecule and water molecules were included, if the total hydrolytic change which could take place in such a circuit were exothermic, even if the change affecting the one group involved an expenditure of energy, water could be electrolysed and its hydrogen would effect the withdrawal of OH from the one group and its displacement by hydrogen, while oxygen would be added to the other

* 'Chem. Soc. Trans.,' vol. 67, p. 1122.

† *Supra*, pp. 526—537.

‡ *Loc. cit.*, p. 1137.

group, it might be either directly or in consequence of the displacement of H by OH. The different effects produced by different organisms, on this hypothesis, would be the consequence of the hydrolysis affecting different systems. As most, if not all, fermentable compounds are asymmetric and the enzymes are undoubtedly also asymmetric bodies, the direction of attack would depend on the character of the asymmetry of both hydrolyte and hydrolyst; moreover only compatible hydrolysts, *i.e.*, those compatible with the hydrolyte, would condition hydrolysis and fermentation."

I then considered that fermentative changes were "presumably functions of the protoplasm." Buchner's researches, however, would seem to necessitate a non-vitalistic interpretation of the phenomena. He has unquestionably proved that fermentation can take place to some extent outside the living cell, but the effect observed has always been of a very transient character, unlike enzyme action in general; this is the more remarkable, as the amount of energy liberated during fermentation is by no means inconsiderable; and also because Buchner's observations would lead us to correlate alcoholic fermentation more closely than we have hitherto done with the ordinary sucroclastic action of enzymes. A system such is pictured of enzyme and hexose might well break down into the ordinary products of fermentation, if energy from outside were in some way initially impressed upon the system, as might well happen if it were a part of the protoplasmic complex. It is conceivable that Buchner may have dealt with systems intermediate in complexity between the enzymes proper and the protoplasmic complex in which exothermic change is still in progress; but ephemeral systems only. On the other hand, the presence of proteoclastic enzymes in the expressed fluid may be a cause of the decay of the fermentative activity. But I venture to think the case is not yet fully proved in Buchner's favour; and however willing we may be to regard alcoholic and lactic fermentations as enzymic changes, such an explanation appears to be altogether inapplicable, for example, to butyric fermentation or to fat formation: we have no reason at present to suppose that the various kinds of fermentation are brought about in essentially different ways.

In discussing the changes which attend fermentation, I pointed out, in 1895, that "Such changes are known to occur entirely within the cell and are presumably functions of the protoplasm; in other words, they probably occur within very complex molecular systems of extreme instability, perhaps under the influence of, in contact with, the very same hydrolyst (enzyme) which is so active, when separated from the cell, in promoting the hydrolysis of cane sugar; or if not, of substances of a similar nature." This explanation has gained greatly in probability now that it is established that the sucroclastic enzymes are very closely related to the alcoholic ferments.

If invertase were present as a branch of the protoplasmic complex, any hexose compatible with it would be attracted by it; and when once in association with the complex, the sugar molecule might undergo changes such as are contemplated in Baeyer's explanation of fermentation, the energy required to initiate these changes being derived from exothermic changes proceeding simultaneously in some other part of the complex.

But it is possible to extend the conceptions which we owe to van't Hoff and Emil Fischer still further. My son and I agree in thinking that the assumption that enzyme and hydrolyte become associated will make it possible to understand a variety of phenomena which have not yet received any satisfactory explanation. For example, when condensed under laboratory conditions, formaldehyde gives rise to an inactive mixture of *d*- and *l*-hexose; but under natural conditions only *d*-fructose and *d*-glucose are produced. *If condensation took place in immediate contact with a compatible enzyme, it is conceivable that a bias would be given to the synthesis sufficient to determine change wholly in the one of the two possible directions.*

It is a striking fact that yeast, which so readily ferments the hydroschists of cane sugar, contains the two enzymes—invertase and maltase—which are respectively compatible with the two hydroschists. Assuming that these enzymes are present as branches of the protoplasmic complex, it is easy to understand, from the point of view now set forth, why yeast should be able to ferment both fructose and glucose easily.

On the other hand, the existence of contiguous maltase and invertase branches in the protoplasmic complex might determine the formation of glucose and fructose in contiguity; and these might then unite—thus giving rise to cane sugar. This conclusion is of special interest, bearing in mind the observations of Horace Brown and Morris* that cane sugar rather than maltose is a primary product of plant metabolism. Both invertase and maltase are known to be present in plants.†

The formation of starch may be looked at from a somewhat similar point of view. It may be supposed that glucose is produced initially as an open chain compound and that the formation of the γ -oxide ring confers stability on the molecule; it may well be that starch is formed by condensation of a number of such merely potential glucose

* 'Chem. Soc. Trans.' 1893, vol. 63. p. 604.

† I am still inclined, however, to favour the view which I expressed in 1890 ('Chem. Soc. Proc.', p. 56), that maltose is the precursor of cane sugar in the plant: it is difficult to avoid this conclusion in view of the fact that cane sugar is formed when barley embryos are fed on maltose but not when they are fed on glucose, although in the latter case the plantlet is found to contain invert sugar.—[Note added May 28, 1904.]

molecules prior to the closure of the oxide ring. The formation in this manner of starch rather than of the far simpler glucose or maltose might be conditioned by the production at protoplasmic surfaces in close contiguity and in suitable orientation of the necessary number of glucose or it may be maltose elements to form the starch molecule.

The origin of albuminoids may be regarded from a similar point of view; and if carbohydrate elements were associated with the protoplasmic complex, they might serve to determine the formation of compatible enzymes in the same way that the enzyme elements may be supposed to determine the formation of compatible carbohydrates.* *In short, the protoplasmic complex may be regarded as built up of a series of associated templates which serve as patterns to determine change in the various directions necessary for the maintenance of vital processes and of growth.*

There are many directions in which such a principle can be applied so as to form the basis of experimental inquiry. Its bearing on the formation of anti-toxins, for example, is obvious; and it may be worth while to ascertain, if possible, whether in cases of acute diabetes the failure of the organism to utilise glucose may not be the consequence of the disappearance of the maltase element from the protoplasm of the organs which ordinarily condition its utilisation.

* This assumption almost necessarily involves the conclusion that a carbohydrate in some way enters into the composition of the sacroclastic enzyme. From this point of view, it is perhaps significant that glucosamine is obtainable from many albuminoids, especially as mucin from human saliva is said to afford 30 per cent. of its weight of this substance. The production of levulinic acid from yeast nuclein is equally noteworthy. But if the activity, for example, of the amyloclastic enzyme should prove to be referable to the presence of a carbohydrate component and this be glucosamine, it will follow that the influence is exerted by a compound in some measure similar to the carbohydrate in configuration—not by one opposed to it in symmetry.—[*Note added May 28, 1904.*]

