Studies on Enzyme Action. X.*—*The Nature of Enzymes.*

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The study of the action of enzymes has now reached a stage at which it may be well, in view of their importance as vital agents, to consider what is established and to call attention to some of the principal issues which remain to be elucidated.

Specific Activity of Maltase and Emulsin.—As the investigation is extended, the evidence becomes more and more convincing that the action which an enzyme exercises is specific; in other words, that it is limited to compounds of a particular type—to a greater extent, indeed, than is recognised in earlier communications of this series, which in some few particulars need modification.

In No. III a table is given (p. 520) in which it is indicated (on the authority of Emil Fischer) that maltase hydrolyses α-galactosides as well as α-glucosides and that galactose has a slight retarding effect. At the time when the experiments to which the table relates were carried out, no opportunity had been found of verifying Fischer's statement. Subsequently, when experiments were made with methyl-α-galactoside, extracts from both top and bottom yeast proved to be entirely without action. It was then ascertained that the summarised account of Fischer's work which had been consulted† contained an incorrect statement and that the results we had obtained were in harmony with those recorded by Fischer in his original papers.‡

The slight retardation of the action of maltase by galactose did not appear surprising so long as it was supposed that galactosides were hydrolysed by the enzymes; when, however, this was found not to be the case, it became

* *Former Communications.*—I, 'Chem. Soc. Trans.,' 1903, vol. 88, p. 1305; II, 'Roy. Soc. Proc.,' 1904, vol. 73, p. 500; III, *ibid.*, p. 516; IV, *ibid.*, p. 526; V, *ibid.*, 1904, vol. 74, p. 188; VI., *ibid.*, p. 195; VII, *ibid.*, 1905, B, vol. 76, p. 592; VIII, *ibid.*, p. 600; also *ibid.*, 1904, vol. 73, p. 537; 1906, B, vol. 78, p. 376.

† 'Zeit. Phys. Chem.,' 1898, vol. 26, pp. 66 and 69.

‡ 'Ber.,' 1894, vol. 27, p. 2987, and 1895, vol. 28, p. 1430.

necessary to re-examine the action: ultimately, the retarding effect of galactose was traced to the presence of an alkaline impurity.*

In No. V, in which the action of emulsin on milk sugar is discussed, attention is called to the evidence in favour of the view that emulsin is capable of acting on β-galactosides (*i.e.*, milk sugar) as well as on β-glucosides; also to Bourquelot and Hérissey's contention that probably lactase is present in ordinary emulsin. Experiments to be described in a later communication, made specially with the object of clearing up this question, leave no doubt that the activity of emulsin *as ordinarily prepared* is due, as Bourquelot and Hérissey and also Pottevin have contended, to the presence of a distinct enzyme capable of affecting the β-galactoside, on which emulsin proper has no action.

To state our opinion in brief, maltase is capable of hydrolysing α-glucosides alone, whilst emulsin hydrolyses β-glucosides.

Correlation of Enzyme with Hydrolyte.—In view of these considerations, the suggestions should be cancelled which were put forward in the third communication† with reference to the extent to which configuration may be modified without nullifying the action of an enzyme.

Attention was directed in this communication to the evidence of a close correlation in configuration between enzyme and hydrolyte afforded by the controlling influence on the rate of change of a glucoside which the hexose derived from it exerts; welcome confirmation of this conclusion is afforded by Ter Meulen's observations.‡

A further series of experiments has been made with specially purified materials which enable us to confirm and extend the earlier observations; it is unnecessary to describe these in detail, as the method followed has already been described. The results are summarised in the following table:—

Enzyme.	Hydrolyte.	Inhibitants.	Destitute of inhibiting power.
Maltase	Maltose	Glucose, methyl-β-glucoside	Galactose, fructose, mannose, sorbose, arabinose, mannitol, dulcitol
Invertase	Cane sugar	Glucose, fructose...	Galactose, mannose, mannitol, dulcitol
Lactase	Milk sugar	Galactose, methyl-α-galactoside	Fructose, mannose, arabinose, xylose, mannitol

The evidence that enzyme and hydrolyte must be in complete correlation appears to be little short of absolute in the case of the sucroclasts.

* *Cf.* Caldwell, 'Roy. Soc. Proc.,' 1906, vol. 78, p. 283.

† Vol. 73, p. 525.

‡ 'Rec. Trav. Chim.,' 1905, vol. 24, p. 444.

The Hydrolysis of Cane Sugar.—The evidence as to the manner in which the hydrolysis of cane sugar is affected by the products of change is conflicting. O'Sullivan and Tompson stated, in 1890,* that the products of inversion are without influence on the rate of change. In 1902, however, Henri asserted† that invert sugar retards the hydrolysis and ascribed the inhibiting effect almost entirely to the fructose.‡

In the same year, Adrian Brown§ also showed that invert sugar had a marked effect, whilst milk sugar had but little influence in retarding inversion.

Barendrecht has since published two communications‖ dealing with the influence of a variety of substances. His results are of a most contradictory character and scarcely admit of rational interpretation; it is difficult to regard his radiation hypothesis seriously.

Apparently, the difficulties which attend experiments on the subject are not sufficiently appreciated. Invertase being extraordinarily active, it is necessary to work with highly dilute solutions of the enzyme: minute quantities of impurity therefore exercise an important influence. Unless hard glass vessels are used, it is impossible to obtain consistent results. A very large number of experiments have been made at various times (chiefly in 1904—05) to test the influence of glucose, fructose, galactose, mannose, dulcitol and mannitol: in many cases the results were conflicting, so that the problem cannot be regarded as solved: the general impression has been gained, however, that glucose and fructose alone retard the action, probably to an equal extent.

Conclusions similar to ours have been arrived at by Ford with reference to the action of diastase on starch,¶ and he has used amino-acids in order to neutralise impurities. From this point of view, we had used glycine and

* 'Chem. Soc. Trans.,' vol. 57, p. 927.

† 'Compt. Rend.,' 1902, p. 917.

‡ This statement was incorporated in the table given in No. III.

§ 'Chem. Soc. Trans.,' vol. 81, p. 382.

‖ 'Zeit. Phys. Chem.,' 1904, vol. 49, p. 456; 1906, vol. 54, p. 367.

¶ 'Soc. Chem. Ind.,' 1904, vol. 23, No. 8; 'Chem. Soc. Trans.,' 1904, vol. 85, p. 980. Ford expresses the opinion that the extraordinary results obtained with starch by various workers are due to a lack of recognition of the important influence of traces of impurity on the course of the change. The results of recent French workers justify this view. According to Ford, the action of diastase attains its maximum in neutral solution and is not augmented by asparagine unless there has been a previous restriction; this is true also of the various salts which are said to accelerate the action.

The accelerating influence of traces of acid on the activity of invertase (*cf.* O'Sullivan and Tompson) is probably a consequence of the neutralisation of alkalinity. We are inclined to think that the effects produced by salts are also of a secondary character and that they do not influence the action of invertase in any specific manner.

asparagine in experiments with maltase prior to the appearance of Ford's communication: finding them to be practically without influence, we felt safe in using them with invertase. As a rule, the activity of invertase is much increased by amino-acids; the experiments on which we rely were all made in presence of glycine.

Nature of Enzymes.—Granting that both glucose and fructose inhibit the action of invertase on cane sugar, it would seem to follow that this enzyme is so constituted that it can adapt itself to both sections of the biose. Unfortunately, no satisfactory expression of the structure of cane sugar has been arrived at hitherto, so that we are unable to formulate the changes which the biose undergoes on hydrolysis in a definite manner. It is an altogether peculiar substance, its extraordinary instability in presence of acids being most remarkable in comparison with that of other bioses* from which it does not differ markedly in most respects.

Invertase, the enzyme correlative with cane sugar, is equally remarkable among enzymes on account of its extreme activity.

Apparently, cane sugar is a derivative of α-glucose, to judge from O'Sullivan and Tompson's observations;† nevertheless it is not attacked by maltase and as this acts on all the *simple* α-glucosides, it cannot well belong to their class. Moreover, taking into account Pottevin's statement‡ that methyl-fructoside is not hydrolysed by extracts of *S. octosporus*, *Mucor mucedo* and *Mucor alternans*, which hydrolyse cane sugar and maltose, it must be supposed, in like manner, that cane sugar is not a *simple* fructoside.

These conclusions are in entire harmony with that derived from the study of inhibiting agents, so that there is little room for doubt that during hydrolysis the enzyme extends its influence over the whole of the cane sugar molecule—being intimately correlated in all its parts with the carbohydrate in the manner postulated in No. III§ of these communications.

The question arises whether this is true of other biases.‖ The experimental material is not yet sufficient for the discussion of the problem, but there are already indications which make it probable—at all events, in the case of milk sugar—that what is true of cane sugar and its correlative enzyme applies generally.

It is commonly assumed that maltose is an α-glucoside, because maltase, which hydrolyses it, also acts on simple glucosides such as methyl-

* *Cf.* IV, p. 530.

† *Cf.* E. F. Armstrong, 'Chem. Soc. Trans.,' 1903, vol. 88, p. 1308.

‡ 'An. Inst. Past.,' 3903, vol. 17, p. 43.

§ Pp. 522—524.

‖ To avoid periphrasis, it appears desirable to term those enzymes which act on simple glucosides *monases* and those which attack the biose sugars *biases*.

α-glucoside. But independent proof of its character is afforded by the fact that the primary product of the hydrolysis of maltose is α-glucose (I.1307). Maltose and apparently lactose also appear to differ from cane sugar in that they are hydrolysed by enzymes which also act on the corresponding simple glucosides (monosides).

The existence of this difference affords further proof that the configuration of cane sugar and of its correlative enzyme are peculiar and altogether different from that of other bioses and biases. To account for the wider activity of a biase such as maltase, it is only necessary to suppose that not only are the simple glucosides of the same general type as the correlative bioses, but that this resemblance also extends to the biases and that it is sufficient in some cases if the attachment of the biase be secured over one section of the hydrolyte. Thus, representing the glucose radicle by G and other radicles such as are present in simple glucosides by R, E^g being the hypothetical radicle of the enzyme correlative with the radicle G, the following diagram may be used, the horizontal line representing the section of the molecule over which attachment of enzyme to hydrolyte takes place—

$$\frac{G\,.\,O\,.\,G}{E^g\,.\,E^g}$$

$$\frac{G\,.\,O\,.\,R}{E^g\,.\,E^g}$$

Formation of Enzymes.—It is conceivable that the enzymes themselves are subject to hydrolysis and simplification—in other words that a biase may give rise to a monase. The existence of monases in admixture with biases is, therefore, to be expected. Attention has already been called in the previous communication to evidence indicative of the presence in extracts of dried yeast of an enzyme (α-glucase) capable of hydrolysing methyl-α-glucoside, but without action on maltose; this discovery may well prove to be significant from the point of view now advanced.

There can be little doubt that the sucroclastic enzymes are products of hydrolytic changes conditioned by enzymes (mainly by proteoclasts, in all probability); the very different results obtained with extracts prepared under different conditions (at different temperatures, etc.) are scarcely to be accounted for in any other manner. And it is to be supposed that several enzymes may arise in this manner, all of one type, yet all capable of hydrolysing a particular hydrolyte and differing only in activity, much in the same way that the various simple glucosides represented by the general formula R′.O.G. (such as methyl-, ethyl-, phenyl-glucoside, salicin, etc.) differ in stability towards hydrolytic agents.

In the case of enzymes other than those which affect carbohydrates, the range of activity would appear, however, often to be greater than is ever manifest in the case of enzymes of the sucroclastic class. Thus lipase hydrolyses not only fats but also numerous ethereal salts; and many of the polypeptides prepared by Emil Fischer are hydrolysed by trypsin, which clearly exercises a wide range of activity.

As the only radicles common to the ethereal salts and to the polypeptides respectively are in the one case the group—CO.O—and in the other the group >N.Ċ.CO—present in the amino-acids represented generally by the formula $NH_2.CHR.CO.OH$, it is to be supposed that these simple groups are the active centres and that the specific influence of the enzyme is exercised at these centres. The variations observed in the activity of an enzyme towards a variety of hydrolytes are, from this point of view, to be ascribed mainly to differences in the stability of the hydrolytes; a single key, as it were, is provided which fits a variety of locks almost equally well; but as the locks are constructed with springs varying in stiffness, the effort which must be exerted to open the locks varies from lock to lock. But the interesting problem to be solved in this connection is whether, in the case of compounds such as the proteins, several junctions may not be resolved practically simultaneously by "compound" keys, as it were.

Bertrand's remarkable observations* on the limiting oxidising power of *Bacterium xylinum* have shown that configuration is of consequence even in the case of oxydases. It is conceivable that the oxidation of compounds such as the higher fatty acids is a regulated process consequent on the attachment of an oxidase to the terminal carboxyl group, the which oxidase serves to bridge over the interval between this group and some more or less distant CH_2 group and to locate oxygen against it.

* 'Ann. Chim. Phys.,' 1904, ser. 8, vol. 3, pp. 181—288.