

Studies on the Processes Operative in Solutions (XXX) and on Enzyme Action (XX).—The Nature of Enzymes and of their Action as Hydrolytic Agents.

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Our object in the present communication is to utilise the experience gained in the course of two convergent series of inquiries carried on during the past 12 years in the hope of arriving at a satisfactory solution of the problems of hydrolysis whether effected either by ordinary agents—acids or alkalies—or by enzymes.

The nature of the hydrolytic process has been discussed broadly in Part XXIV of the one series and the phenomena attending the dissolution of salts in water and the behaviour of saturated solutions towards precipitants has since been considered in Part XXV of the same series: much that has been said in these two communications will be of consequence in the present discussion. The views put forward with regard to the composition of water (S. VI)* and with reference to the part played by the class of substances which we have termed collectively *hormones*,† in altering the state of water and of substances dissolved in it, will also be found to have a bearing on the problems presented by enzymes. The present communication, it should be stated, is in the main an amplification of the views expressed in Part II of the Studies on Enzyme Action.‡

The interpretation of the phenomena we shall offer will also involve taking into account the views on residual affinity of the negative elements in particular and on the nature of the process of chemical change advocated by one of us in a communication brought under the notice of the Society in 1886 and in a Presidential Address to the Chemical Society in 1895.

The present communication, in fact, is the outcome of an inquiry, lasting over a long period, carried out with the object of arriving at a rational solution of some of the most fundamental of chemical problems—especially

* It will be convenient to refer to communications of the one or the other series as papers of the S. and E. series respectively.

† 'Roy. Soc. Proc.,' 1910, B, vol. 82, p. 588. Cf. 'Annals of Botany,' 1911, vol. 25, p. 507. As used originally by Bayliss and Starling, the term *hormone* had a restricted significance; we have applied it more generally to compounds which penetrate the differential septa of animal and vegetable structures.

‡ *Ibid.*, 1904, vol. 73, p. 500.

those relating to the interactions of water, interactions which it is now generally admitted are often of great complexity.

Definition of an Enzyme.—At the outset we are met by the difficulty of defining an enzyme. The state of opinion is well brought out in the opening lines of the recently published English edition of Euler's 'General Chemistry of the Enzymes':—

"The name enzyme is given to animal or vegetable substances . . . which are able to accelerate chemical reactions. The term enzyme is thus included in the much more general term catalyst. By catalyst we understand a substance which, without being required by the accelerated reaction or appearing among the final products, alters the velocity with which a chemical system strives to attain its final condition."

As one of us is responsible for the introduction of the word *catalyst*,* we may be permitted to consider the significance of the term.

Catalysis.—It is noteworthy that the conception of catalysis, first enunciated by him in 1835, is discussed by Berzelius in his celebrated 'Jahresbericht' (vol. 15, p. 237), under the heading "Pflanzenchemie," in a section to which is attached the significant explanatory marginal note—"Some ideas on a hitherto unnoticed force active in living Nature in the formation of organic compounds."

At the outset, Berzelius refers to the difficulty of explaining the complex phenomena of organic life with the aid of the conceptions up to that time derived from the study of inorganic phenomena. He then draws attention to the discovery of a series of changes in which the agent appeared to take no permanent part in the change but was ultimately recovered unaltered in amount.

Thus he refers in succession to the formation of grape sugar from starch by means of dilute acids (Kirchhof—1814): to Thénard's discovery of hydrogen peroxide, a substance which is readily resolved into oxygen and water: to Humphry Davy's observations on the effect heated platinum has in inducing the oxidation of the vapour of alcohol or ether: to Edmund Davy's discovery of platinum black, a substance which induces oxidation at ordinary temperatures: to the use that Doebereiner made of this discovery in constructing his well-known lamp: to Dulong and Thénard's observations on induced oxidation, showing that not only the platinum metals but also gold, silver and even glass could produce similar effects if sufficiently

* 'Report of the British Association,' 1885, p. 953. We venture to deprecate the use of the expression "to catalyse"—both because it appears to us to lack euphony and to be unnecessary if not undesirable; for similar reasons, we regard catalyst as preferable to catalyser.

heated: finally, he refers to the discovery of diastase (Dunbrunfaut, 1830) and then discusses Mitscherlich's investigation of the formation of ether from sulphuric acid and alcohol and the manner in which this chemist had correlated with that of acids the action of diastase on starch.

On account of the evidence afforded by his observations that water passes over together with ether, leaving the acid unchanged, when a mixture of sulphuric acid and alcohol is heated, Mitscherlich had supposed that the acid exercises the same power over alcohol that alkali exercises over hydrogen peroxide, arguing that its influence could not be ascribed to its affinity for water as the water was vaporised with the ether: he was further led to conclude that sulphuric acid and diastase act similarly on starch.

Hence Berzelius came to the conclusion that many substances, simple as well as compound, have the property in the solid and also in the dissolved state, of exercising an influence on compound substances which is quite different from that of ordinary chemical affinity, as they influence the occurrence of changes without their own constituents being necessarily concerned in the change—though there are cases in which this may happen. But he took care to point out that whilst he preferred to speak of the force contemplated as new it was presumably only the manifestation in a special way of the ordinary electrochemical properties inherent in matter. His views are summarised in the following statement:—

“Die katalytische Kraft scheint eigentlich darin zu bestehen, dass Körper durch ihre blosse Gegenwart und nicht durch ihre Verwandtschaft die bei diesen Temperaturen schlummernden Verwandtschaften zu erwecken vermögen, so dass zufolge derselben in einem zusammengesetzten Körper die Elemente sich in solchen anderen Verhältnissen ordnen durch welche eine grössere electrochemische Neutralisierung hervorgebracht wird.”

Berzelius finally calls attention to the possibility of “thousands of catalytic processes” being operative under vital conditions.

The meaning attached to the word catalysis in the interval has not only been vague but as often as not the term has been used to cloak ignorance and simulate understanding.

The following definition is given in a well-known dictionary:—

“*Catalysis*—a decomposition and new combination supposed by Berzelius and other chemists to be produced among the proximate and elementary principles of one or more compounds by virtue of the mere presence of a substance or substances which do not of themselves enter into combination.”

There is no doubt that gradually the term has been interpreted as implying an *action of presence*, the *catalyst* being regarded as a material

which produces chemical change in another or other substances merely by contact, though it in no way has this significance etymologically. This idea has gradually supplanted that of a loosening down preparatory to and determining chemical change between substances in contact with the catalytic agent—the conception which appears to have been in the mind of Berzelius when he coined the term from the Greek *κατα* and *λυο*. The interaction of hydrogen and oxygen or of sulphur dioxide and oxygen in presence of platinum are cases in point: the “loosening down” of the molecules concerned is commonly overlooked and the combination effect alone thought of.

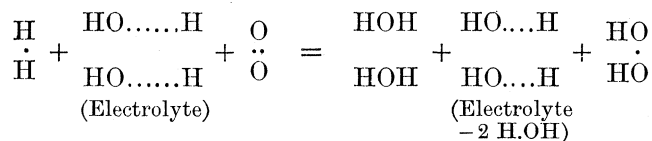
The definition which has been popular of late years is that quoted above from Euler but originally advanced by Ostwald. No proof whatever is forthcoming, however, that the catalyst merely alters and hastens the rate of a change already in progress. Whilst such an assumption is permissible, it is in no way necessary. A very large amount of evidence is on record showing that, in many cases in which it has been customary to speak of two substances as interacting, the change only takes place under special conditions in presence of a third substance of a particular type—in other words, that the catalyst determines the change: Brereton Baker's work, in particular, has afforded much proof of this kind but such evidence is entirely disregarded by the advocates of the view that the catalyst merely hastens change and it has nowhere received proper notice.

When chemical changes are regarded as electrolytic phenomena, as the several components of the system are all concerned in the change, it is obviously not an easy matter to decide which of the substances present is to be regarded as the catalyst. The interaction of hydrogen and oxygen, for example, is not determined by platinum alone but only takes place when an electrolyte is present to complete the circuit: the “loosening down” which Berzelius appears to have contemplated takes place immediately the circuit is formed and probably is consequent on the various attractions *reciprocally* exercised throughout the circuit. The platinum undoubtedly acts either by absorbing them at its surface or by combining with each of the two gases, thereby bringing them into circuit with the electrolyte.

On this account, the catalyst may well be defined as the agent which brings about the inclusion of the interacting substances in the circuit within which the change takes place so soon as the circuit is established, the electrolyte being the actual agent by which the change is effected.

Obviously, however, the electrolyte may, in some measure, be regarded as the catalyst and, as a matter of fact, it is generally so regarded in the case of the hydrolysis of ethereal compounds by acids.

The distinction between the view promulgated by Ostwald and that which we advocate lies in the fact that we do not admit that action is either possible or ever takes place between two non-electrolytes—such as hydrogen and oxygen, for example—but hold that action sets in only when a suitable electrolyte is present together with the two substances thought of as interacting directly, though in reality they interact only indirectly. Thus—



In such a case, the electrolyte is the catalyst, the occurrence of change being determined by and dependent on its presence: it does not merely accelerate the change but gives rise to it by making it possible. The change may be and is promoted, however, by the inclusion in the circuit of a substance such as platinum, which, by condensing or combining with the gases, promotes their association with the electrolyte. Therefore, if the term catalyst be restricted to materials which act merely by increasing the extent to which substances are brought into interaction and only as intermediaries, the definition given by the Ostwald school may be accepted as satisfactory: whereas, if the electrolyte be regarded as the effective catalyst, this is not the case, as the catalyst not only determines the occurrence of interaction but contributes, *ex hypothesi*, of its own substance to the change and is only recovered unchanged, *i.e.* undiminished in amount, because it is constantly being changed reversibly.

The Nature of Enzymes.—The enzymes are peculiar as catalysts not only because they are agents derived from natural organic sources which determine the resolution of a variety of compounds by hydrolysis but, more particularly, on account of their specific and limited activity: it is in this respect that they differ from most other catalysts. Each particular enzyme corresponds, if not to a single hydrolyte, at most to a series of compounds of one particular type. But until their specific nature be deciphered, it will be difficult to arrive at any final definition of enzymes.

The view that we have gradually been led to form of an enzyme involves the assumption that it has a double function—that of attracting or holding the hydrolyte and that of determining its hydrolysis: in other words, that the enzyme retains the hydrolyte in circuit while hydrolysis is being effected through the agency of an electrolyte itself formed from an active radicle present in the enzyme.

This twofold action we attribute to the presence in the enzyme of an

acceptor together with an *agent*. According to this view, an enzyme is a composite agent in which the functions of a catalyst such as platinum black are combined with those of an acid catalyst.

It appears to us that the only interpretation that can be placed upon the facts as they are now known to us is that the acceptor is a radicle which is very closely allied to, if not identical with, a dominant group in the hydrolyte.

For example, we incline to the belief that the enzymes which cause the hydrolysis of the glucosides—the glucases—are themselves glucosides.

With regard to the agent, as the only hydrolytic agents known to us are either acids or alkalies and the latter act only on ethereal salts, not on etheric compounds such as the sugars, we are of opinion that, in all probability, the agent is an acid radicle so situated with reference to the acceptor that when the hydrolyte is attached to this latter it is in immediate or compatible proximity with it, a conducting path being formed between agent and acceptor by their association with the solvent and it may be also with a sufficient amount of some "salt" to render the intervening liquid an electrolyte.

In the case of enzymes which condition the hydrolysis of the carbohydrates and glucosides, the *agent* may well be the carboxyl radicle, CO.OH . It may be objected that the carboxylic acids are too weak—that the rate at which hydrolysis is effected by enzymes is far too great to be accounted for on the assumption that carboxyl is the effective catalyst. We shall discuss this point later on, merely remarking that we should not regard such an objection as a valid argument against the sufficiency of our postulate.

The efficiency of an enzyme depends, however, not only on the effective conjunction and simultaneous operation of the two elements we have termed acceptor and agent but also on its colloid character.

Manner in which Enzymes Act.—It is so generally held that the enzymes are colloids that we think it unnecessary to restate the arguments on which this conclusion rests but shall deal only with considerations derived from our own work.

In virtue of their colloid character, they are present in a liquid in suspension—their solubility being only apparent: results such as those obtained with urease, for example, cannot well be accounted for in any other way.

When hydrolysed by this enzyme, urea affords carbonic acid and ammonia. When the hydrolysis is effected in presence of an excess of either of these products, the rate is approximately a linear function of the time*: whereas,

* E., XIX, p. 334.

however, in presence of ammonia, the change is retarded, in presence of carbonic acid it is much accelerated.

A second point of importance to be noticed is the fact that the enzyme has maximum activity in solutions which are only moderately concentrated and that whilst dilution has but little effect, the rate of change becomes less and less as the concentration is increased.*

In no particular, therefore, is the change a "mass action effect," nor are the departures such that it can be supposed that the change is primarily "unimolecular" and subsequently varied owing to the occurrence of secondary changes: it is doubtful, also, if it be necessary to take the occurrence of reversible effects into account except perhaps in concentrated solutions.

[*Note added July 30.*—Bourquelot and Vardon's recent experiments† entirely justify this conclusion. These observers have digested aqueous solutions containing glucose and various proportions of methylic alcohol with emulsin and have determined the amount of glucose which remained unchanged when equilibrium was established. Their results are shown in fig. 1, in which the ordinates indicate the amount of glucose unconverted and the abscissæ the percentage by weight of methylic alcohol in the solutions.

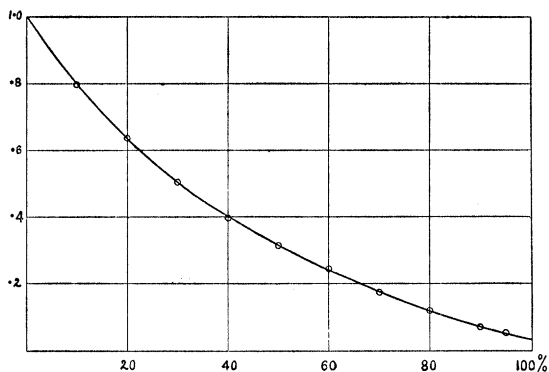


FIG. 1.

They have also shown that when solutions in 70-per-cent. methylic alcohol of varying amounts of glucose are digested with emulsin, the amount of glucoside formed increases proportionally to the glucose present up to about 12 per cent. of this latter but in more concentrated solutions at a diminishing rate: the proportion of combined glucose being 82.6 per cent.

* E., XIX, p. 336.

† 'Compt. Rend.,' 1913, vol. 156, pp. 957 and 1638. Cf. 'Ann. Chim. Phys.,' [8], 1913, vol. 28, p. 145; 'Bull. Soc. Chim.,' 1913, No. 14, pp. 1-xxviii.

in solutions containing up to 12 per cent. glucose and from 81.6 to 80.9 in solutions containing 16–30 per cent.

Moreover, the addition of β -methyl glucoside in advance serves to reduce the amount of glucose converted. Thus, when glucose alone was used (1 grm.), the amount converted was 0.826 grm. When an equal weight of glucoside was present, only 0.709 was converted and when 3 grm. of glucoside was used to 1 grm. of glucose, only 0.392 of the latter was etherified (*ibid.*, p. 1638).

It thus appears that the synthetic activity of the enzyme is affected in the same way as its analytic activity by changes in concentration. It is to be hoped that Bourquelot and Vardon will also determine whether the influence of the β -glucoside on the synthetic activity of the enzyme be in any way a preferential effect.]

But these are all conclusions at variance with the doctrine of the textbooks. So far as we are able to judge, the analytic activity of enzymes is exercised more nearly in the manner first pointed out by Duclaux in 1898 and subsequently in 1902 by Adrian Brown and also by Horace Brown and Glendinning: *in each successive interval of time, the enzyme determines the hydrolysis of the same amount of the hydrolyte*; the observed departures from this rule may be attributed to the influence of the products of change.

The mental picture of the process we have been led to frame involves the following suppositions.

Firstly, that a colloid surface in water is necessarily a hydrolated surface, *i.e.* a surface to which molecules of hydrone—the fundamental molecule of water—are attached in such manner that their activity at the surface is greater than the average activity of the water in the neighbourhood. Secondly, that as a consequence of this property of the surface the hydrolyte is absorbed* from the solution, so that the colloid surface remains highly charged with the hydrolyte probably almost up to the point at which the supply in the solution is exhausted. Our assumption being that the enzyme

* We venture to think that this term is sufficient for all purposes and that it is undesirable and unnecessary to introduce a special term (*adsorption*) both because the uninstructed reader cannot attach any special meaning to this latter different from that conveyed by the familiar term and because the meaning which it is sought to give to it is not in reality different from that conveyed by the familiar term: no distinction is drawn by implying that a substance is sucked in *towards* a body *in* a solution rather than *from* a solution *by* a body, the process being one in which solution and surface are reciprocally concerned. The growing tendency to introduce special terms which the reader cannot understand unless specially instructed, whose meaning cannot be discovered easily, is to be deprecated on all grounds.

is effective within a particular region, not over its whole surface, it is only necessary that the hydrolyte should be determined to this active region.

The partial or complete saturation of the surface of the colloid particles would serve, therefore, to promote the maintenance of a sufficiently constant supply of hydrolyte to this special region.

The hydrolyte attached to the acceptor, however, would not be permanently held but would oscillate between it and the liquid, so that only a certain proportion of effective contacts would be made—contacts during which the circuit would be completed wherein hydrolysis could and did take place. The rate of change would be determined by the rate at which these effective contacts occurred but would be relatively slow, in all probability.

After discussing the matter with Dr. Horace Brown, who has given special attention to such problems, we are inclined to think that the rate at which liquid diffusion takes place is probably so great that it is not necessary to take this into account as a limiting factor.

Influence of the Products of Change.—As the products of change accumulate in the solution, they affect the enzyme in various ways. It is to be supposed that the product immediately allied to the acceptor enters directly into competition with the hydrolyte and more and more takes its place as the amount present becomes greater. The carbohydrates and many glucosides are cases in point.

Products having no special configurational relationship to the acceptor section of the enzyme may act upon it in various other ways such as the following :—

(a) By neutralising it, as in the case of urease and doubtless also of pepsin and trypsin.

(b) By converting it into a derivative which is different in structure and no longer compatible with the enzyme—the action of some aldehydes and of quinone are cases in point.

(c) By changing the osmotic conditions in the solution and thereby altering the state of “hydrolation” at the enzymic surface of the acceptor and also of the agent. Probably any substance dissolved in the solution will act to some extent in this manner but such effects are specially noticeable in the case of “inert” materials such as the alcohols (hormones). The diminution in the rate of change which is noticeable when the concentration of the hydrolyte exceeds a certain maximum is to be accounted for, apparently, in this way. In explanation of this contention, we may point out that it is based on the assumption that in aqueous solutions all interactions take place at hydrolated surfaces—in other words, we regard both acceptor and hydrolyte as hydrolated and assume that they are brought into conjunction at their hydrolated surfaces.

Having thus called attention to the various factors concerned in the hydrolysis, we may now point out that our hypothesis involves the assumption that the relationship of the acceptor section of the enzyme to hydrolyte is not that of lock and key but that of a superposable and therefore practically identical radicle. As a matter of fact, the lock and key relationship, taken strictly, is unknown and even inconceivable: the only known relationship among similar substances is that of object and image: this is clearly not the relationship which holds between enzymes and their correlated hydrolytes.

We use the expression practically identical advisedly, in view of the fact that the tetramethylated methyl- β -glucosides* and certain glucosamine† derivatives are all hydrolysed by "emulsin" (prunase). Apparently therefore the spatial arrangement of the several radicles in the hydrolyte and the correlated acceptor must be the same in so far as the relative distribution of negative and positive is concerned but the negative groups admit of variation within certain limits—*i.e.* methoxyl may take the place of hydroxyl and apparently even the radicle NH_2 may to some extent be substituted for hydroxyl.

The enzymes which hydrolyse the glucosides generally may well be compounds of the glucoprotein class containing either α - or β -glucosidic radicles and capable therefore of hydrolysing either α - or β -glucosides, as the case may be, because their configuration harmonises either with that of the one or with that of the other type of compound.

To take another example, it appears not improbable that urease is an enzyme in which the urea residue in arginine is in suitable relationship with the carboxyl group.

Special Efficiency of Enzymes.—With regard to the efficiency of saccharo-elastic‡ enzymes, two points have to be considered—the efficiency of the carboxylic radicle which we assume to be the agent and the special efficiency of a colloid mechanism such as we have pictured. It is well known that the efficiency of the carboxylic radicle varies greatly, being very low in the majority of acids but relatively high in formic acid and considerably higher in the substituted acetic acids, especially in trichloroacetic acid; it is therefore conceivable that it may have a relatively high efficiency in the enzymes, especially if the region within which it is immediately active be one of high concentration.

As to the special efficiency of a colloid mechanism, as we have elsewhere

* Irvine and Cameron, 'Chem. Soc. Trans.,' 1905, vol. 87, p. 900.

† Irvine and Hynd, *ibid.*, 1913, vol. 103, p. 41.

‡ We regret that in earlier communications we have been guilty of using the indefensible terms "sucrose" and "sucroclastic" in place of "saccharose" and "saccharo-elastic."

pointed out, there is reason to suppose that when the sugars are hydrolysed by means of acids the proportion of acid effectively associated with the hydrolyte at any one moment is probably very small—as both are attracted by the solvent and therefore subject to constant separation at its call. Presumably, therefore, the efficiency of acids, though relatively very low, is actually very high (S. VII, XXIV).

The colloid is little subject to such attraction and only the hydrolyte is specially attracted by the water; but owing to the fact that the colloid is present in an excessively finely divided state, the hydrolyte tends to accumulate at its surface and probably the attractive influence of the solution as a whole is largely overcome. A relatively large proportion of the hydrolyte is therefore brought into effective conjunction with the acid radicle; consequently this is placed under specially favourable conditions. The argument is applicable to enzymes generally whatever the nature of acceptor and agent.

Our hypothesis is one which renders it unnecessary to assume that enzymes obtained from a variety of sources which all function in a particular manner are one and the same substance; it is probable that the same acceptor and agent may be differently attached so long as they are appropriately placed to act in conjunction. It is conceivable, in fact, that a variety of enzymes may exist which are all capable of hydrolysing only one particular compound or type of compound but differ in activity. If, as appears to be the case, a given enzyme will act on compounds so different as say β -methyl glucoside and the corresponding β -methyl glucosamine derivative, it is clear also that a series of equivalent acceptors may give rise to corresponding enzymes which would all function similarly though probably with different degrees of readiness.

Our point of view is also one which admits of the existence of several classes of enzymes: for example, of enzymes which are compatible with the whole of the molecule they attack—it is not improbable that invertase belongs to this class—as well as of enzymes in which the acceptor is a group compatible with the one or the other section of the glucoside or other compound which it can hydrolyse.

Specific Character of the Enzymes.—The rigidly selective activity of the enzymes is in itself sufficient proof of their essentially specific character.

The fact that enzymes are known, such as α - and β -glucase (derived from yeast and the almond fruit respectively), each capable of hydrolysing a series of glucosides, is in no way subversive of this argument; it is easily accounted for by the assumption that each such enzyme carries an acceptor compatible with a group common to all the members of the series of glucosides and is, indeed, a corollary of the hypothesis.

Our conception of an enzyme is embodied in the two diagrams, figs. 2

and 3, representing the two amino-glucosides formed by the interaction of α - and β -glucose with the amino-radicle in a molecule of a complex albuminoid material.*

The models used serve to show the spatial arrangement of the atoms in the

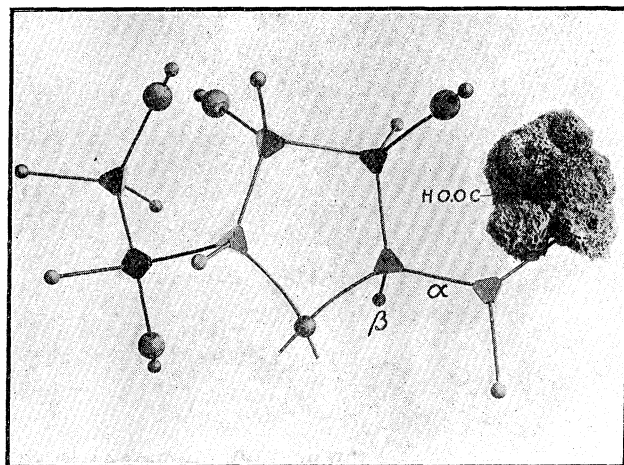


FIG. 2.— α -glucose.

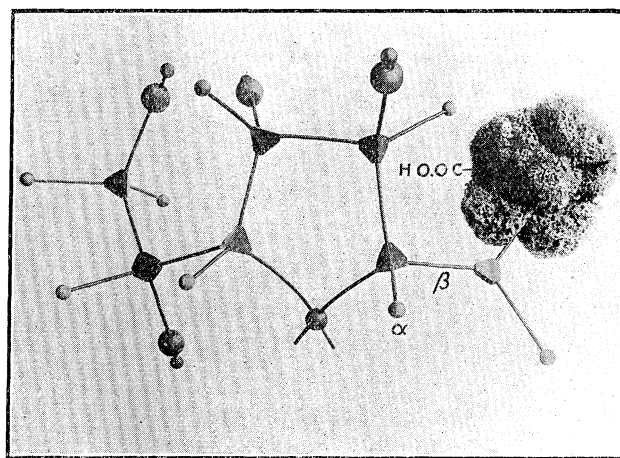
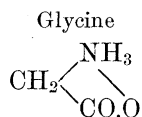
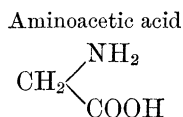


FIG. 3.— β -glucose.

acceptor: actually, the atoms in the glucoside would be close packed and in immediate proximity to the main mass of the colloid represented in the diagram by a sponge and probably would constitute but a slight excrescence on its surface.

* *Cp.* van Laer, "Sur la Nature de l'Amylase," 'Bull. Acad. Roy. Belg.,' 1913, p. 395.

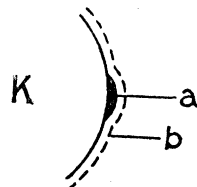
If both the carboxylic radicle and the amino-radicle to which the carbohydrate radicle is coupled formed part of some one amino-acid residue in the colloid complex, they would be in close conjunction and therefore self-protective; the "resting" enzyme may be thought of, in fact, not as an acid proper but as an internal salt of the glycine type:—



To unlock and render the "zymogen" active, it would be necessary to add a substance of superior acidic power—just as in the case of an indicator, which "indicates" only when either an acid or an alkali is added which is of superior strength.

It will be obvious that if brought into the proper apposition the molecule of an α -glucoside would fit the α -enzyme and the molecule of a β -glucoside the β -enzyme shown above and would fit it in such manner, moreover, that not only would contact be secured over the carbohydrate surface but the junction at which hydrolysis takes place in the glucoside would be in close proximity to the carboxylic radicle in the enzyme: it cannot be doubted that if an electrolyte intervened, hydrolysis would at once take place in such a system. The manner in which the electrolyte operates in such cases has been discussed in S. XXIV, p. 617, § 26.

In amplification of the argument advanced on p. 571, it may be pointed out that if each enzyme particle, in virtue of its colloid character, tend to absorb the hydrolyte so that the solution at its surface is relatively concentrated, the concentrated layer (*b*) would necessarily extend across the active enzymic area (*a*), as may be illustrated thus:—



The mechanism postulated is therefore such that the concentration of the hydrolyte would be raised and preserved in the neighbourhood of the active enzymic centre.

Action of Acids and Alkalies.—If we seek to interpret the effects produced by enzymes in the light of the hypothesis now advocated, it is obvious that one of the main conditions to be fulfilled is the maintenance of the freedom of the acidic (or alkylie) radicle which is the active hydrolytic agent.

It is probable that the amount of actual enzyme present in the preparations which are ordinarily used is so minute that the quantity of acid (or alkali) which would render it active initially, assuming that it is present either as an internal or as an ordinary salt, must be very small: therefore, if

any further amount be required and the presence of acid (or alkali) in excess serve to accelerate the action of the enzyme, it is to be supposed that the acid (or alkali) neutralises some product or products of the change. This has been shown to be true in the case of urease, as ammonia retards the hydrolysis of urea by the enzyme whilst weak acids accelerate the change.

Assuming that it is a derivative of arginine, the enzyme urease may be represented by the following diagram, the section which the urea molecule would fit being that within the figure:—

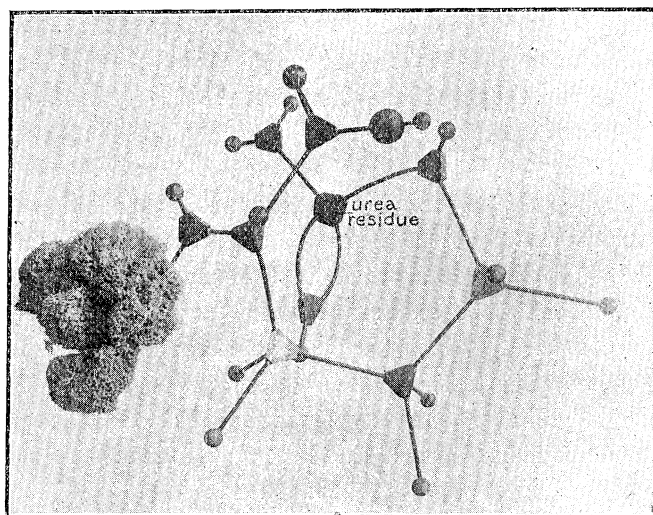


FIG. 4.—Urease.

The carboxyl group in such a compound would tend to unite with one of the contiguous basic NH groups, thus forming an internal salt. It would remain free if sufficient acid were present to hinder the formation of the salt but neutralisation would set in as ammonia was liberated from the urea: the enzyme would not be entirely thrown out of action, however, as the neutralisation would be more or less incomplete, as the ammonium salt would be dissociated by the water: its activity would be in great measure if not entirely preserved if an acid were present which neutralised the ammonia, so long as the effective acidity of the solution did not exceed a certain maximum—beyond which the acid would itself interfere by combining with the enzyme and perhaps also with the hydrolyte.

Apparently a not inconsiderable degree of acidity is essential in the case of peptic digestion. It is therefore not improbable that the active radicle in the enzyme is stronger than carboxyl and that it may even be a phosphoric

residue—either $\text{PO}(\text{OH})$ or $\text{PO}(\text{OH})_2$. As the products of change are more basic than the primary hydrolyte, in such a case the presence of acid would be necessary to maintain the freedom of the enzyme.

A similar argument may be applied to tryptic digestion in presence of alkali. The fact that the action takes place in presence of alkali cannot well be explained, in terms of our hypothesis, except by the assumption that the active agent of change is a basic radicle—perhaps an “ammonium” hydroxide. As the products of change—the glycines—are capable of neutralising bases as well as acids, the presence of alkali would serve to maintain the freedom of the basic radicle in the enzyme.

The saccharoclastic enzymes, as a class, appear to be active only in almost neutral solutions, a very slight degree of acidity being the most favourable condition. Faint acidity rather than alkalinity appears to be the natural condition of most living structures in which enzymes are present. But, as a rule, under natural conditions, enzymes are not subject to control by the products of their own action, as these are removed by diffusion more or less rapidly and cannot accumulate as they do in laboratory experiments.

Assuming that the enzymes are formed from internal salts (zymogens) by neutralisation, it is to be supposed that the degree of acidity or alkalinity which would be most favourable initially will vary according to the “strength” of the salt to be neutralised; at subsequent stages, variations may arise owing to the formation of products of varying “strength.”

It has long been recognised that quantity alone is not the only factor to be taken into account in using acids but that these vary greatly in “strength.” Thus, if cane sugar be subjected under strictly similar conditions to the action of quantities of chlorhydric and acetic acids which neutralise the same amount of alkali, the amount hydrolysed in a given time by the latter will not be 1 per cent. of that hydrolysed by the former. It is therefore not sufficient to determine the mere amount of “acid” present in a liquid by determining the amount of standardised alkali required to neutralise it—the *apparent acidity* of the solution: but the *effective acidity* must be ascertained.

Two methods of determining the effective acidity or strength of dilute solutions of acids are in use—the one is electrical and involves the determination of electromotive force, the other colorimetric; but the two have been applied in such a way that they are interdependent. A custom is growing up, which is much to be deprecated, of stating the results in terms of hydrogen-ion-concentration. Undoubtedly this method is not only one which can be understood by those alone who are instructed in terms of a special hypothesis—that of ionic dissociation—and have the necessary mathematical knowledge to grasp its significance but it is misleading in more than one

respect. It has been contended, indeed, throughout this series of studies that the doctrine itself is purely hypothetical and not in accordance with facts generally.

The upholders of the dissociation hypothesis assume that the characteristic activity of acids is due to the constituent all acids have in common, the hydrogen ion (H) and that of alkalies to the hydroxyl ion (OH). Everything goes to show, however, that acids act as acids—that is to say that the negative or acid ion is as much concerned as is the positive or hydrogen ion; in fact, that the characteristic properties of an acid are in the main due to the negative radicle, the hydrogen radicle being no more characteristic of an acid than it is of an alkali.

Ex hypothesi, the passage of an electrical current through a solution takes place only through the agency of the dissociated ions. The slight conductivity of highly purified water is therefore attributed to the presence of a small proportion of free hydrogen and hydroxyl ions. The results are so interpreted that about 1 molecule in every 10,000,000 (1.05×10^{-7} at 25°) is supposed to be in this condition; further, that if produced in a larger proportion than this under any conditions, hydrogen and hydroxyl ions at once unite to form neutral water. If a solution be acid, it is therefore supposed that hydrogen ions are present in excess of the proportion in which they are contained in water: if it be alkaline, the assumption is made that they are present in a smaller proportion.

It is difficult enough for non-mathematical readers to appreciate values stated in terms of the expression $x \times 10^{-7}$ or 10^{-y} but it is still more difficult for them to follow the method adopted by Sørensen,* the first to introduce order and one of the chief workers in this field, who uses the indices alone (the y values) as the *exponents* of the hydrogen-ion-concentration, so that values below 7 indicate alkalinity and those above 7 acidity. Such a system, moreover, has the disadvantage that when curves are plotted to indicate the relation between the effective acidity (or alkalinity) of the solution and enzymic activity, as logarithmic values are used instead of actual values, an altogether false and misleading shape is given to the graph.

Recognising the unsuitability of the method followed by Sørensen and others, James Walker† has recently advocated that acidity and alkalinity be referred to water as a standard. He puts the acidity and likewise the alkalinity of pure water as equal to 1: hence the product of the acidity and

* S. P. L. Sørensen, "Études enzymatiques. II.—Sur la mesure et l'importance de la concentration des ions hydrogène dans les réactions enzymatiques," 'Comptes rendus des Travaux du Laboratoire de Carlsberg.' 8me volume. 1re Livraison, 1909.

† 'Journ. Soc. Chem. Ind.,' 1912, p. 1013.

alkalinity of any solution is always unity. He proposes the use of a series of standardised solutions of the two phosphates KH_2PO_4 (acid) and Na_2HPO_4 (alkaline) in certain proportions. The relative acidity and alkalinity of such mixtures being known, it is easy to determine that of any given solution by matching the tint which it produces when mixed with azolitmin with that produced by one of the mixtures. The values given by N/15 solutions vary between a relative acidity of 300 and a relatively alkalinity of 20. Inasmuch as most enzymes show maximum activity within this range, such solutions afford very convenient standards. As illustrating the delicacy of the control, it may be added that whereas the relative acidity of a solution of N/15 acid phosphate is 300, that of N/10 acetic acid is 13,000.

In the past we have emphasised the need of carefully excluding all alkaline impurity in studying the action of saccharoclastic enzymes and have shown that the addition either of faintly acid or of amphoteric substances such as glycine was of material advantage in the case of invertase. Other workers have since used an acid phosphate for the same reason.

The method we have adopted in the experiments now to be referred to has been to extract dried yeast powder with the phosphate solution and after filtration to add a certain portion—about 20 c.c.—of the extract to a solution of α -methyl glucoside in 80 c.c. of the same phosphate mixture. The acidity of such a mixture is approximately, though not strictly, that of the original phosphate. On account of the yellow colour of the solution, it is impossible to determine the acidity exactly by the colorimetric method practised by Sørensen nor were we concerned to achieve such a degree of accuracy: for our purpose, it was sufficient to know that the solutions used varied in acidity.

As might be expected, an extract of dried yeast contains sufficient soluble material to provide a highly favourable medium. Thus, a solution made with ordinary distilled water containing carbon dioxide had a relative activity of 83 towards α -methyl glucoside; when distilled water free from dissolved carbon dioxide was used, the slightly lower value 75 was obtained; when a mixture of acid and alkaline phosphate in the proportions necessary to give neutrality was used the relative activity of the extract was 84.

The great variation in the results obtained with solutions of various degrees of acidity and alkalinity is well shown in the table on p. 578.

In the experiments lasting four hours, the activity was clearly at a maximum in faintly acid solutions.

The maximum is less marked in the case of the experiments lasting 21 hours. The limits within which action takes place are obviously very narrow.

Activity towards α -Methyl Glucoside of Solutions prepared by extracting Dried Yeast with Solutions of various Mixtures of Monopotassium and Disodium Phosphate.

Solution	Sørensen values	Walker values	Glucose formed 4 hours' action	Glucose formed 21 hours' action
β	9.3	200.0	grm. nil	grm. nil
α	8.93	67.6	0.003	0.06
A	8.3	20.0	0.16	0.49
B	7.35	2.3	0.34	0.87
C	6.81	1.55	0.37	0.90
D	6.24	5.8	0.54	0.91
E	5.3	50.0	0.47	0.88
F	4.53	300.0	0.31	0.80
G	4.0	1000.0	0.23	0.72
H	3.3	5000.0	nil	nil

Similar results obtained with other enzymes are shown in the following table:—

Enzyme	Hydrolyte	Time in hours	Relative amounts of action in solutions of								
			Alkalinity			Acidity					
			6S	20	2.3	1.5	5.8	50	300	1000	5000
Maltase* ...	Maltose	4	1.6	2.2	2.9	3.5	3.4	3.35	3.0	2.9	0.15
Emulsin ...	Salicin	4		0.54	2.6	6.2	9.4	11.0	11.2	10.3	2.0
Aucuba leaf powder	"	5		0.15	9.5	17.3	24.9	24.3	17.0	10.6	nil
" "	"	24		1.1	30.8	71.1	89.0	87.6	57.2	36.7	"

* The yeast extract used in this experiment was a portion of that used in the experiment with α -methyl glucoside.

[*Note added July 30.*—A particularly instructive series of observations, represented in fig. 5, were made in the course of our experiments with urease. They illustrate the remarkable sensitiveness of the enzyme to acid and alkali.

The action of the enzyme on urea alone is represented by Graph 1. Graph 2 represents the action in presence of M/25 monopotassium phosphate, Graphs 3 and 4 representing the effect produced by M/5 and 2M/5 solutions of this salt. It will be noticed that the smallest proportion produced an acceleration of the change from the outset; the intermediate proportion at first caused a retardation, but the action soon set in at a greater rate than was observed in the case of the smaller

proportion. In the case of the highest proportion, the retardation was at first very great, but it is obvious that recovery then set in. The effect of the alkaline salt, $M/5$, Na_2HPO_4 , was prejudicial from the beginning.

It was noticed that the solution became cloudy on mixing the enzyme with

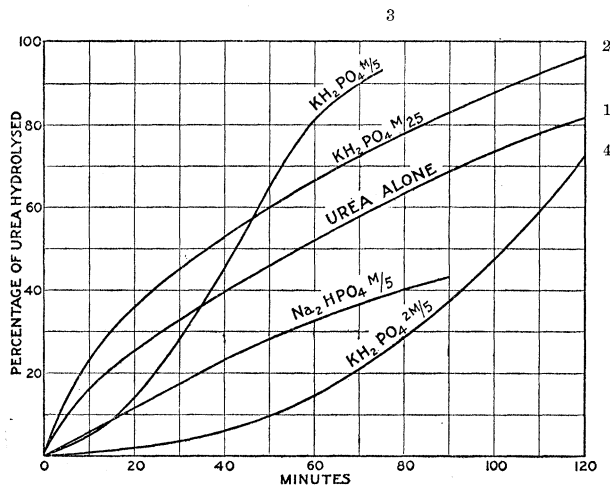


FIG. 5.

the strong solution of the acid phosphate but that it afterwards became clear. Evidently the enzyme was at first in large part neutralised and "coagulated" but as alkali was formed it became redistributed and as the acid phosphate served to neutralise the alkali produced in the change the enzyme preserved its activity to a greater extent than it did when no phosphate was present.]

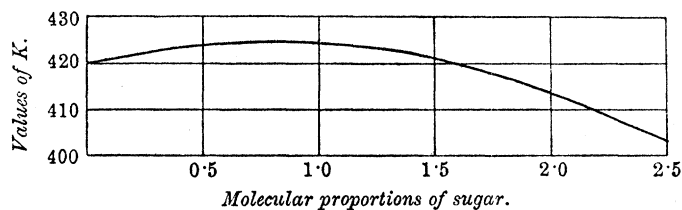
Influence of the Products of Change and of other Substances.—In these studies, stress has always been laid on the influence which certain of the products of change exercise in retarding the action of an enzyme and the special influence of one or the other of the products of change has often been advanced as an argument of consequence in favour of the conclusion that the enzymes are strictly specific agents.

This, in fact, is to be regarded as a principal argument in favour of the hypothesis now advocated and we have therefore been led to reconsider very carefully the evidence brought forward in this connexion at an early stage of this inquiry. We now desire to amend it in important particulars, having in the interval arrived at the conclusion that it is necessary to be very careful in interpreting the effects produced by various substances and not to regard them always as specific influences.

In the first place, it is necessary to take into account the manner in which the proportion of the hydrolyte present influences the result and to realise

and producible also by substances which must be inert in this respect. In fact, a similar effect is produced whenever a substance is added which increases the "osmotic tension" in the solution. Moreover, not only enzymes but all hydrolytic agents are affected.

Thus Mr. Worley's experiments (S. XII) prove that the rate of hydrolysis of cane sugar by acids is not proportional to the concentration of the former at all strengths of the solution but soon reaches a maximum, as shown in the following graph representing the results obtained with nitric acid in experiments in which the sugar was the only variable :—



These results, which correspond strictly with those obtained when the concentration of the hydrolyte is varied relatively to an enzyme, are of special importance as showing that the diminished activity must be due to an alteration in the osmotic state—not to mechanical causes.

That salts should retard hydrolysis by enzymes is only to be expected on account of the "concentrating" effect they exercise.

Neutral substances generally, however, also exercise an inhibiting effect which not only varies according to the proportions in which they are present but is also the greater the less soluble the substance. As slightly or moderately soluble substances are often formed in cases of enzymic hydrolysis this behaviour is of consequence. Results obtained with cane sugar, illustrating the effect referred to, are given in No. XIII of the Solution Studies and also in Nos. XI and XXV. It is shown that, in the case of a considerable number of neutral substances used as precipitants of salts, the less soluble precipitant is always the more efficient. It is difficult to explain the effect they produce otherwise than by the assumption that in presence of the neutral substance the water becomes more active, in the sense that by their interposition the proportion of hydrone molecules in the liquid is increased: consequently the degree of hydrolation of any substances that may be present is lowered both because the solution is more attractive and the surface therefore less attractive of hydrone and because the neutral molecules also interfere directly at the hydrolated surfaces and promote dehydrolation mechanically. The less they attract hydrone and the more easily they can move about in the liquid, the more

active they are apparently : it is on this account that the higher alcohols, chloroform and similar substances are so specially active.

The same explanation may be given of the effect produced by neutral substances in reducing the electrolytic conductivity of solutions. Moreover, it has been shown in S. XX and XXVI that chemical changes are similarly influenced. Thus, in the former, it is proved that the rate at which urea is formed from ammonic cyanate is influenced by alcohols of the ethylic series and more promoted by the higher slightly soluble alcohols than by the lower easily soluble. In the latter, it is shown that the equilibrium between the two isodynamic forms of fructose is affected by a variety of neutral substances and that the effect produced is greater the less soluble the substance.

Preferential Retardation of the Activity of Enzymes by Compatible Materials.—The effect of various substances on the hydrolysis of α -methyl glucoside by α -glucase is shown in the following table:—

	Weight of glucose liberated		Percentage of glucose hydrolysed
	Exp. I	Exp. II	
M/5 α -methyl glucoside	gm. 0·97	gm.	
" + M/5 α -methyl glucoside	0·945	0·99	
" + M/5 glucose	0·2	0·2	
" + M/5 galactose	0·82	0·8	
M/5 α -methyl glucoside (3·6 gm. per 100 gm. water)	2·03		57·2
(7·2 " ")	2·69		37·3
" + M/20 glucose	1·67		46·5
" + M/10 "	1·40		39·0
" + M/5 "	0·94		26·0
" + M/5 galactose	1·47		41·0
" + M/20 saligenin.....	1·47		40·8

It will be noticed that glucose has an effect which is out of all proportion large in comparison with that produced by other materials. Galactose has not nearly so great an effect though its inhibiting power is considerable in comparison with that of α -methyl glucoside, which is practically without influence.

The following results obtained with the β -glucoside salicin and the enzyme emulsin are in harmony with the above conclusions:—

	Percentage hydrolysed		
	Little enzyme	Much enzyme	
M/10 salicin	28·3	88·8	85
„ + M/10 glucose	20·8	78·7	77
„ + M/20 saligenin	23·5	84·0	80
Glucose obtained			
	gram.		
M/10 salicin alone	0·388		
„ + M/10 α -methyl glucoside	0·385		
„ + M/5 „	0·368		
„ + 2M/5 „	0·364		
„ + 4M/5 „	0·331		
Complete hydrolysis	0·450		
M/10 salicin	1·52		
„ + M/10 α -methyl glucoside	1·50		
„ + M/5 „	1·44		

It will be noticed that both glucose and saligenin have the greater effect when the amount of enzyme present is small. It is very noteworthy that saligenin has so marked an effect: but both salicin and saligenin are slightly soluble substances and it is to be expected therefore that the former would be specially sensitive. We do not regard the influence on the activity of emulsin exercised by saligenin as in any way a case of preferential retardation by a compatible material—both on general grounds and because it has so marked an influence both on α -glucose and on urease.

The influence of the small quantities of alcohol produced during hydrolysis is subordinate, although at a higher concentration the alcohol soon exercises a marked influence. The following tables show the magnitude of this effect:—

M/10 α -Methyl Glucoside + Yeast Extract.

Medium	Relative change	Medium	Relative change
Water	90	Water	91
10 p. c. ethylic alcohol ...	44	10 p. c. methylic alcohol...	51
20 „ „ ...	25	20 „ „ ...	21
40 „ „ ...	10	40 „ „ ...	0
60 „ „ ...	2	60 „ „ ...	0

Methylic alcohol is apparently more toxic than the higher homologue.

2 per cent. Salicin + Emulsin.

Medium	Relative change
Water	86·0
20 p. c. ethylic alcohol	38·0
40 " " "	14·0
60 " " "	10·0
80 " " "	4·8
90 " " "	2·0
95 " " "	0·2

The enzyme is largely precipitated from yeast extract when 40 per cent. of alcohol is present. Emulsin is also largely precipitated from a solution of this strength but the enzyme remains active as a hydrolytic agent, even in very strong alcoholic solution; attention has recently been drawn to this fact—of which we have long been aware—by Bourquelot.

The manner in which the rate at which α -methyl glucoside is hydrolysed is affected over a considerable period by glucose is shown in the following table and in the corresponding graph.

Action of α -Glucase on α -Methyl Glucoside Alone and in Presence of Glucose.

Time	Weight of glucose formed	
	Glucoside alone	Glucoside + glucose
	gram.	gram.
2 hours	0·43	0·15
3½	0·66	0·24
5	0·85	0·29
7	1·03	0·37
8½	1·21	0·41
10	1·31	0·45
24	1·82	0·82

The slight influence exercised by α -methyl glucoside on the hydrolysis of salicin may be attributed entirely, we think, to its concentrating or de-hydrolating effect, in view of the inappreciable effect produced by an equivalent amount and the gradual increase of the effect as the concentration is raised.

In our former experiments, E. III,* the solutions used were highly concentrated. Thus, 10 gram. of β -methyl glucoside, together with 10 gram. of maltose, were dissolved in water to 100 c.c. (including the enzyme). Under these conditions, the action of the α -enzyme was retarded by the β -glucoside :

* 'Roy. Soc. Proc.,' 1904, vol. 73, p. 516.

similar results were obtained with emulsin and milk sugar, showing that α -methyl glucoside controlled the action. Our recent observations recorded above show no such effect in the case of dilute solutions. We therefore think that our former conclusions were erroneous, as they were based on results obtained with solutions so concentrated that the point at which the enzyme has maximum activity was exceeded and, consequently, the addition of any substance would cause a retardation of hydrolysis.

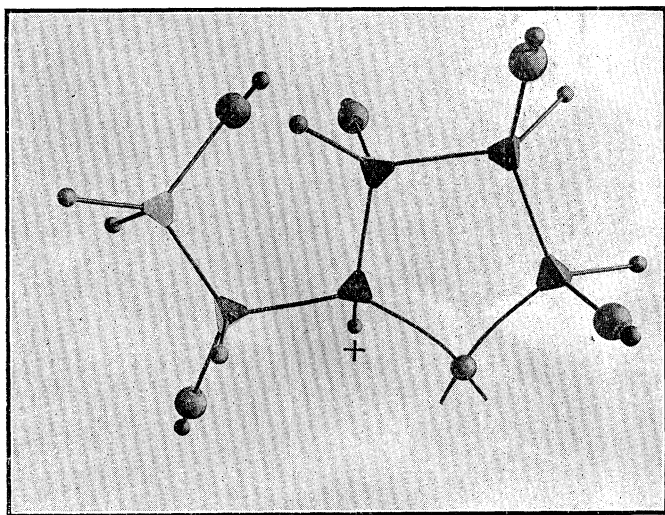


FIG. 6.—Glucose.

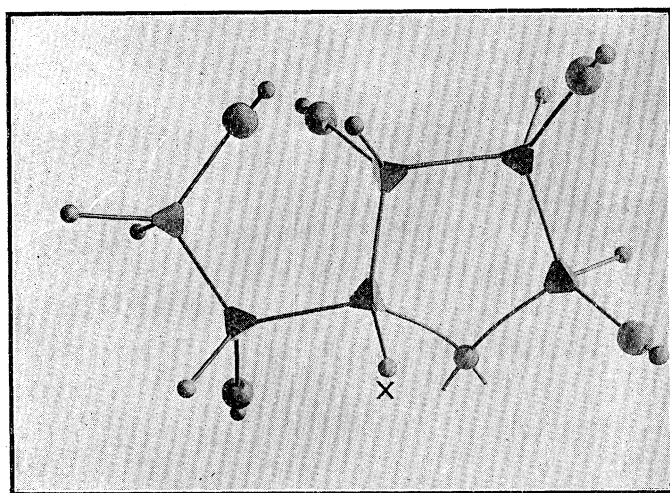


FIG. 7.—Galactose.

It remains to consider the distinct effect produced by galactose.

On reference to the diagrams figs. 6 and 7 representing the spatial arrangement of the atoms in the two sugars, it will be noticed that the only difference between them is that the hydrogen atom X shown to the left of the oxygen atom in the ring is in a plane behind the ring in the one case, and in a plane in front of the ring in the other; there is also a corresponding difference in the relationship of the linking oxygen atom to the ring plane. The difference between the close packed assemblages, therefore, would probably be small: though sufficient perhaps to reduce the compatibility of the two molecules, some degree of compatibility might still persist.

This is one of those cases of minute difference which it will be important to study further, especially in view of the observation made by more than one worker that some yeasts "acquire" the power of fermenting galactose if habituated to its presence. The question of the presence of a distinct enzyme in emulsin capable of hydrolysing milk sugar and presumably of inducing the synthesis of β -galactosides must also be reconsidered from this point of view: we are at present engaged in this inquiry.

Studies on Enzyme Action. XXI.—*Lipase* (III).

By H. E. ARMSTRONG, F.R.S., and H. W. GOSNEY, B.Sc.

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On account of the part which Lipase plays in promoting the resolution of fats generally into fatty acid and glycerol, one of the most important processes in animal nutrition, it is desirable that a clear picture should be obtained of the manner in which the activity of the enzyme is exercised.

The material hydrolysed—the fat—being practically insoluble and the enzyme presumably a colloid, the interaction to be considered is that of substances insoluble in water and therefore presents unusual features.

Two brief communications on the subject were made to the Society in 1905 and 1906.* In the first of these, which had reference to the enzyme in castor oil seeds, it was stated that Connstein's contention had been confirmed that the presence of acid is necessary to condition the hydrolysis of a fatty oil by the enzyme and that practically any acid was effective provided a sufficient amount were used. As acids did not act equally in

* 'Roy. Soc. Proc.,' B, vol. 76, p. 606; vol. 78, p. 376.

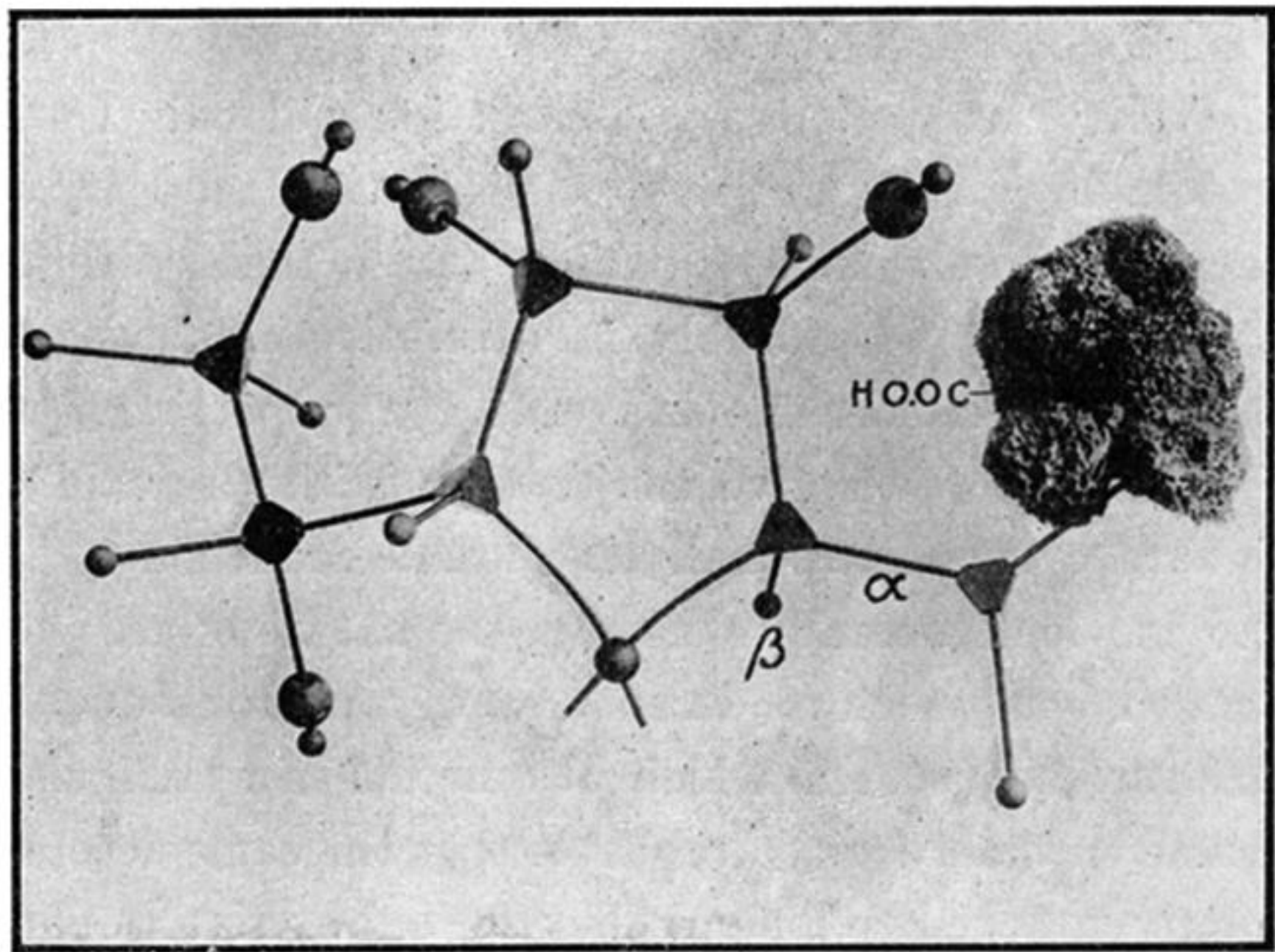


FIG. 2.— α -glucose.

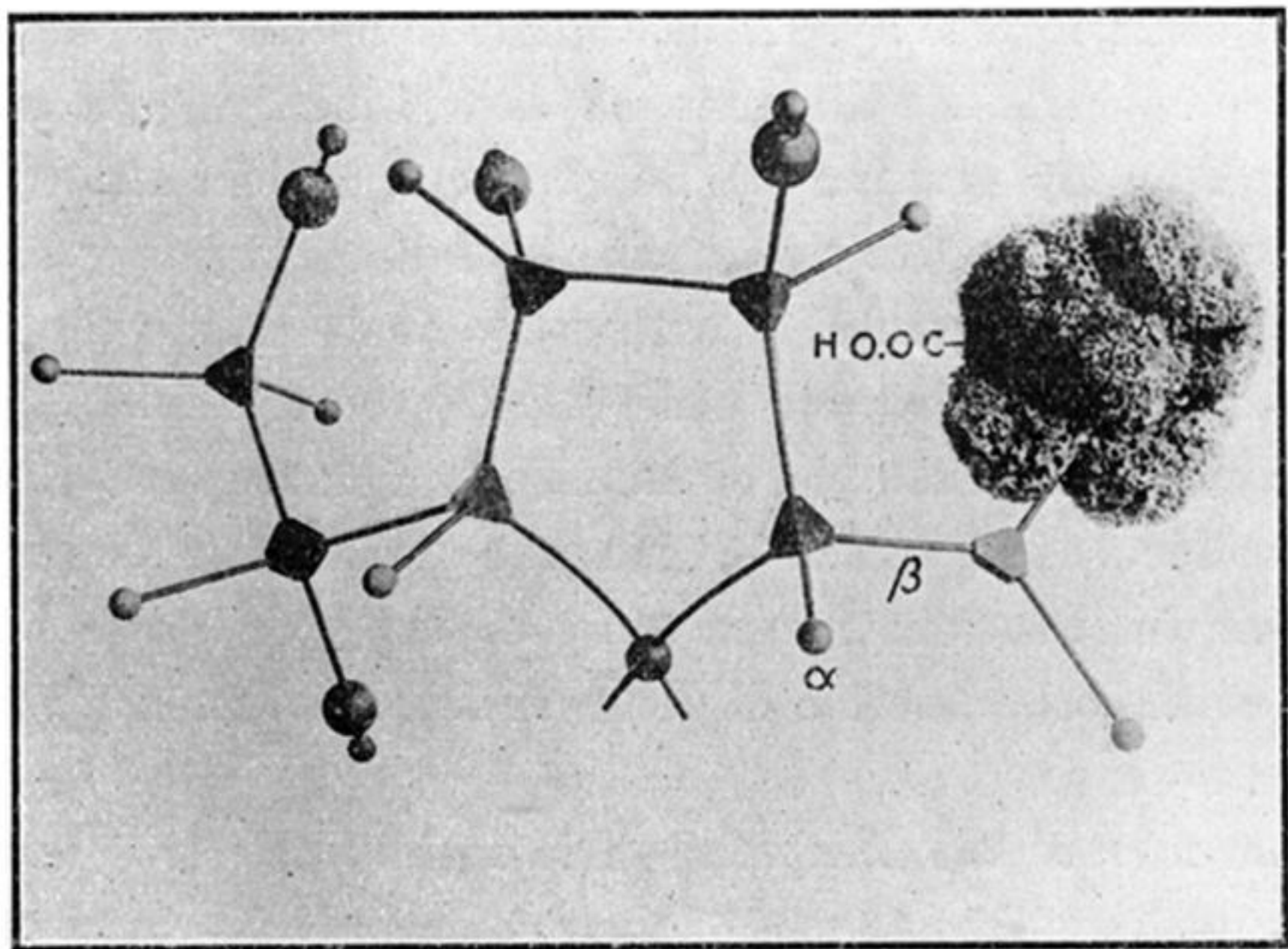


FIG. 3.— β -glucose.

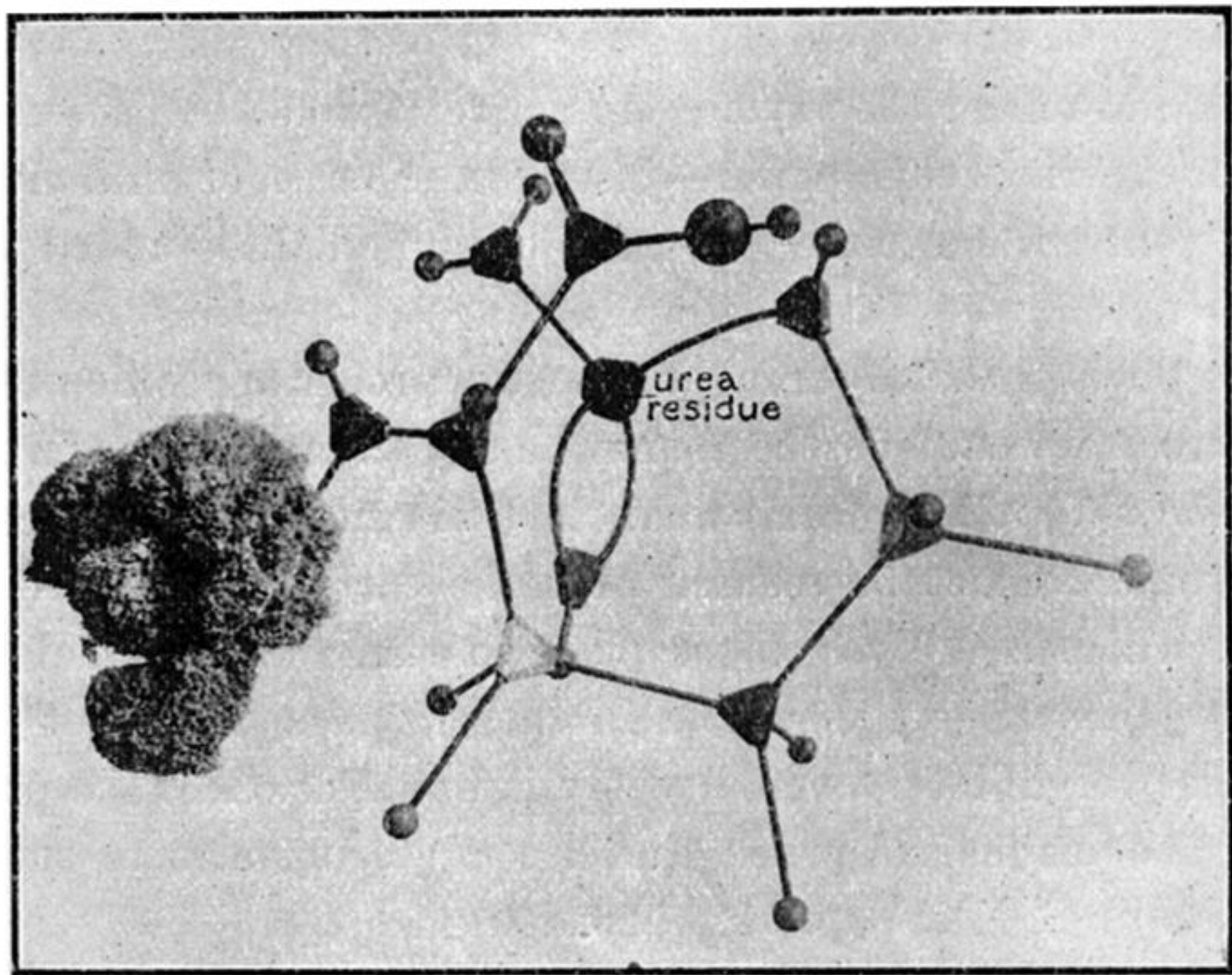


FIG. 4.—Urease.

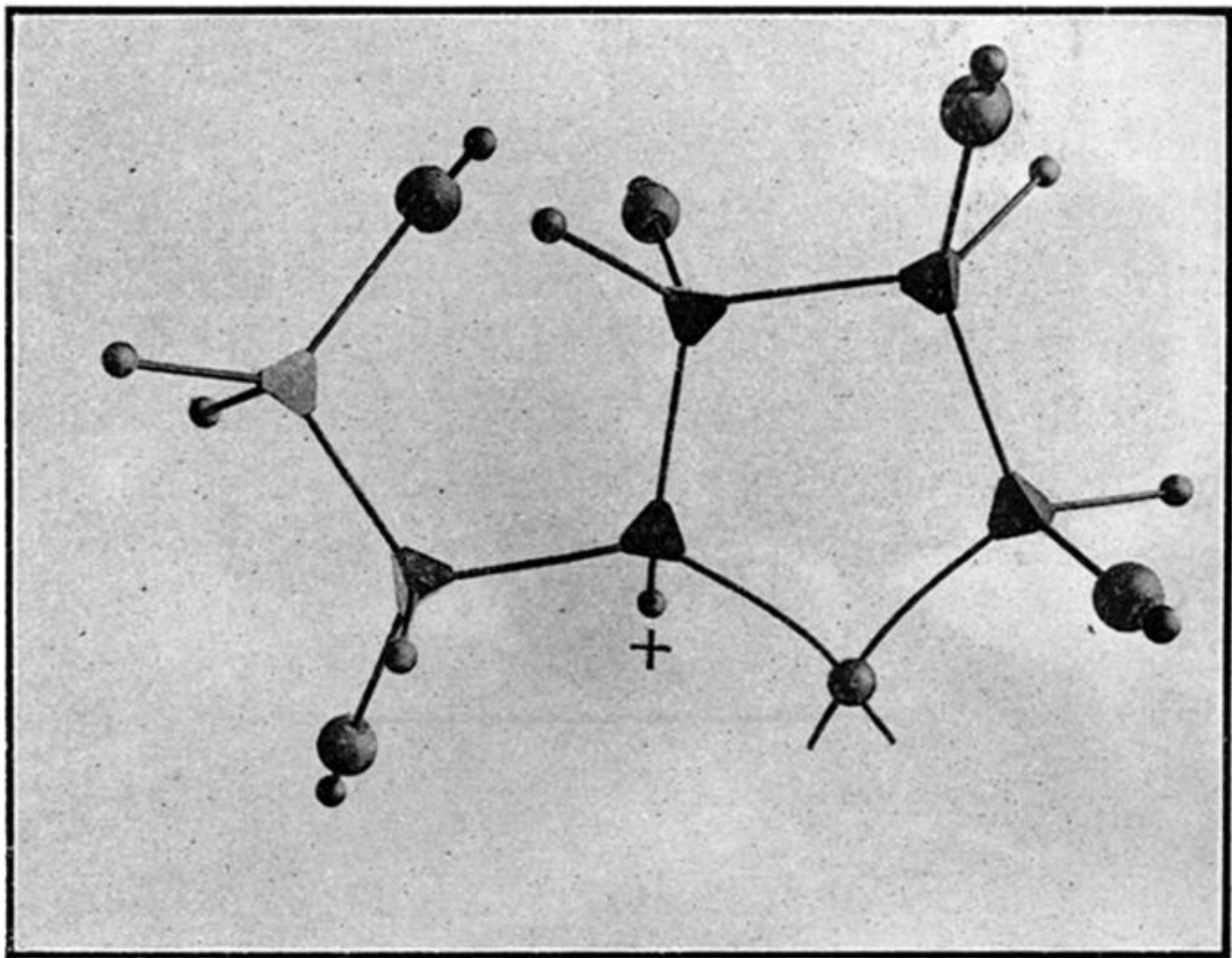


FIG. 6.—Glucose.

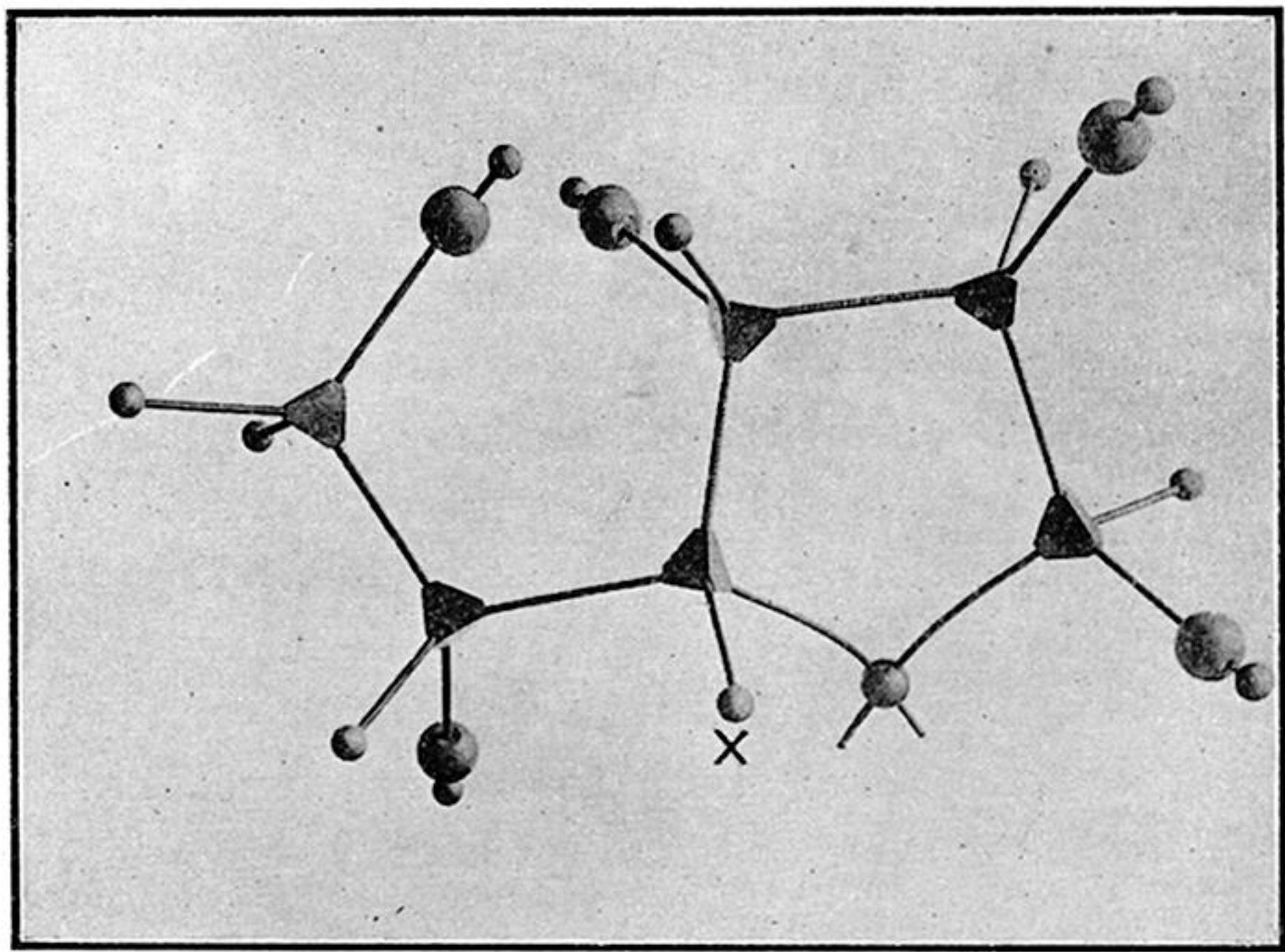


FIG. 7.—Galactose.