# SUPPLEMENT TO "CURRENT SCIENCE".

## The Mechanism of Enzyme Action.

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### 1. INTRODUCTION.

THE first to assert the momentous law of the reign of a catalytic power in nature, whereby complicated chemical processes, for whose realisation the chemist requires powerful reagents and high temperatures, become easily possible under the mild conditions prevailing in plant and animal organisms, was Berzelius in his famous *Text-Book of Chemistry*. At an early date the action of this catalytic power was traced to the Ferments, or Enzymes as they were termed later. The action was specific,—given effects being obtained only with given fractions of secretions or organ extracts and only under definite conditions of medium. Biochemists were thus led to postulate the existence of a multitude of catalytic "substances," whose chemical action was well known, but whose chemical nature remained a mystery. The specificity of the enzymes was set forth most sharply by the investigations of Emil Fischer on stereoisomeric substrates, investigations which led this researcher to draw his famous analogy of "key and lock".

As might be expected, these actions have been an unfailing source of stimulation for the researcher, and have ever spurred him on in his efforts to determine the chemical structure of the mysterious substances causing them. As yet, however, the aim has not been attained with enzymes. R. Willstätter, H. v. Euler and their collaborators tried for many years to prepare pure enzymes, but only succeeded in enhancing the activity of their preparations by freeing the total mass of cell and tissue ingredients from those parts which, in their opinion, contained little or no enzymic substance. But the residue obtained by them was itself nothing else again than a further mass of cell ingredients united with active substance, the chemical nature of which still remained as unexplicable as ever.

It is very difficult under these circumstances to speak of a purification of the enzymes. It is an open question whether the above mentioned concentration process really constitutes a true purification at all. By the expression purification the chemist understands the liberation of a compound (A) from other compounds (B, C . .) which generally stand in no direct relation to it. No such purification can be reported for enzymes. Willstätter, by the ingenious method he has developed, abstracts from the total number of carriers, as the mass charged with active substance may be termed, only those carriers whose activity is most manifest. The other carriers, however heavily they may be charged with active substance, are neglected by this method. The essence of the Willstätter method is the autolytic breakdown of the original native carriers, which being mainly colloidal do not, as we shall see, reveal their true degree of charge with active substance, into low derivatives which freely manifest their association with the active principle and hence also their function as carriers.

Before drawing such a conclusion, it was necessary for us to prove that different carriers variously enhance or depress the activity of the active substance (the very nature of which is still a mystery to us), even as a definite quantity of electricity charges variously bodies of different dielectrical properties which thus act as a real determinant of the "electrical activity"

Carriers which manifest the activity of their active substance we shall term zymophoric. The degree in which the activity

of the active substance in respect of a given substrate is made manifest by the zymophore corresponds to the degree of zymolability (or inversely to the degree of zymostability) of the zymophore. Zymolability is merely a qualitative and relative concept, however. In reality it is revealed to us only by the relative ease with which the active substance (zy. s.) is transmitted from the carrier (C) to the substrate, as well as by the effect of this transmission process.

$$C \longleftrightarrow zy.s. + substrate \rightarrow C + substrate \longleftrightarrow zy.s.$$

Assuming that the substrate on association with active substance undergoes chemical alteration (hydrolysis, decomposition, etc.) at an infinitely great velocity, it must be concluded that in kinetic experiments it is this transmission effect which is really measured. If the measurements are carried out in the first phase of the enzymatic action upon the substrate, before the formation of the cleavage end products has so far advanced that these products themselves become significant as carriers of active substance, the quantity of the substrate transformed during this period may be taken as a measure of the enzymatic activity, *i.e.*, of the transmission effect. In respect of any given enzyme extract prepared under exact and constant conditions and allowed to act either upon several substrates present in equivalent amounts in the same medium, or upon the same substrate under varying conditions of medium, the presence of different substances, for example, the amount of substrate transformed during the first phase ($t$) of the reaction forms a valid measure for comparisons of enzymatic activity. In no case, however, are the *actual* "enzyme units" or "enzyme quantities" determined by kinetical measurements. What is really measured is the transmission effect of assumed enzyme quantities, but not as is the case in ordinary kinetic experiments, an effect which is governed solely by the actual concentration or quantity of the reacting compound. To be sure, the very nature of this transmission effect is not yet definitely known. Enzymic activity is only the outward manifestation. The transmission itself probably involves a distribution of active substance between the carrier and the substrate, a distribution dependent for its realisation upon the occurrence of a definite manner of contact betwen the two bodies. The frequency of this contact, moreover, depends upon the nature of the carrier involved. This view thus assumes that a chemical and physical, constitutional and perhaps configurative, relationship underlies the mutual association of substrate and carrier. This assumption also provides the explanation of that other cardinal feature of enzymic action: its specificity.

Though the assumption that the velocity of a given reaction is determined by the frequency of the collisions of the reacting compounds and that this frequency in turn is proportional to their concentration is quite permissible in ordinary chemodynamical considerations, this assumption is not justified in the case of enzyme action. In this case the collisions must be of quite a definite nature, if they are to result in a manifestation of the above mentioned "chemical and physical relationship" between carrier and substrate. It is necessary for the colliding bodies to form a definite space pattern which is the prerequisite of their combination. The frequency of occurrence of appropriate collisions, *i.e.*, collisions in which the participating bodies assume the right position in space, is governed by a certain degree of probability and is increased if the chemical structure of the colliding bodies causes them to tend to "catch" each other. Only when these suitable collisions occur, is enzymic activity (with the transformation of activated substrate proceeding at infinite velocity) manifested.

We regard as zymophoric only such carriers which can enter into the above mentioned relationship with the substrate. Their zymolability is the greater, according as their chemical structure permits of a greater frequency of "appropriate" collisions, according as, that is, the "attraction" between the two partners is the stronger.

This is the interpretation which should be placed upon the fact that in peptidase action glycocoll behaves as a non-zymophoric carrier whose presence hinders the hydrolysis of the dipeptids. For higher polypeptid substrates, on the other hand, glycocoll appears to be an appropriate carrier, and its addition increases the rate

of the hydrolytic cleavage of such a substrate. Conversely certain proteins and their derivatives have been found to be fit carriers for the cleavage of dipeptids but less fit for higher peptids.

These observations may be brought to a head under a simple formula that proteins and their high derivatives enter more easily into the above described relationship with low protein derivatives such as amino acids or dipeptids than with higher protein derivatives. The low protein derivatives moreover evidently possess no affinity for each other which could bring about the association of carrier and substrate. The higher products are thus seen to constitute the best carriers for dipeptids, the amino acids for higher polypeptid substrates. The fact that the higher derivatives seem to exercise almost no attraction for each other, is probably due more to the large, hence cumbersome size of their molecules, which lowers the frequency of "appropriate collisions", than to any lack of affinity.

Still a few words more on the chemical nature of the carriers. It is a striking fact that in peptidase action only substances whose chemical nature is much akin to that of the substrate, *i.e.*, substances of polypeptid structure, act as carriers. This is not a mere coincidence in our opinion but rather a significant peculiarity which is inherent in the nature of living matter. As an ingredient of the living plasma, a protein is charged from the very beginning with a definite quantity of active substance (zy. s.) which, on the decomposition of the protein to lower derivatives, is transmitted (in the sense defined) to the latter. It may be laid down as one of the main criterions of the substance of the living plasma that they are equipped with the catalytic agents of their own decomposition, the products of which beginning with the higher and lower intermediate derivatives and ending with the lowest final products, serve as carriers of the active substance which is successively transmitted to carriers of various zymolability and is thus enabled to fulfil the various biological functions. This is true not only of proteins but also of other cell ingredients of high molecular structure. The seed which contains protein, starch, lipins, etc., as reserve substances is at the same time thus provided with all the enzyme systems necessary for the decomposition of the substances under the condition of germination. Moisture and the proper temperature act upon these systems as activators. In this way the ingredients of the living substance provide above for the implements of their dissimilatory decomposition, acting themselves as the carriers of these implements and transmitting them also to decomposition products which in turn act as new carriers. In the light of these considerations, the chemical kinship which is generally displayed by the carriers of a given enzyme system, such as polypeptidase, and its substrate no longer appears astonishing. All are equally decomposition products of the same mother substance.

The existence of a multiplicity of carriers in the enzyme action, and the possibility of a change of carriers, whereby the appearance of new enzyme specificities may be explained, are the fundamental ideas of the conception advanced above. In view of the fact that the isolation of pure enzymes has not met with success despite manyfold attempts and that at every trial the researchers only succeeded in isolating new carriers, it seems plausible to suppose that enzyme systems are in essence but multitudes of carriers charged with as yet unknown active principles, and that specific action is the result of chemical adaptation in varying degrees of completeness between carrier and substrate. The adaptation is the greater, the greater the specificity, the latter being the measure of the possibility of combination, either between numerous kindred substrates and some highly specialised, configuratively adapted and irreplaceable carrier, as is the case with $\alpha$-glucosidase. We have found no legitimate reason however for assuming as several researchers (Willstätter, v. Euler, etc.) have done that there are as many active substances as there are specific actions in a given group of kindred substrates, that in other words several "dipeptidases" and a multitude of polypeptidases exist. The experiments mentioned in Part 2 of this paper seem to show the relative truth of our view-point.

## 2. Some Proofs of the Multiple Character and Interchangeable Nature of the Carriers in Peptidase Systems.

In this section some experimental results are described which are only comprehensible

on the assumption that there exists a multiple of carriers for one and the same enzyme action, *e.g.*, the hydrolytic breakdown of polypeptids, that the appropriate individual carriers are mutually interchangeable, and that by the interchange the system's activity becomes altered.

The hydrolytic cleavage of polypeptids, both dipeptids and higher peptids (beginning with tripeptids and proceeding to higher forms) can be effected both by peptidase—wealthy yeast extracts and by pancreas extracts, or pancreatin preparates, *i.e.*, by the dried substance of the pancreatic gland. It hardly needs to be mentioned that these extracts also act on peptons which substances are known to be a mixture of higher polypeptids.

It was found (Abderhalden and Fodor, 1916) that fresh-water extracts of yeast on standing one or two days at room temperature, show a strikingly increased activity in respect of many dipeptids, but that this increased activity sharply drops again after five or six days more of preservation. The cause of this variation in activity which was invariably found to occur in extracts of dried Munchen yeast, could only be traced later to an autolytic breakdown by virtue of which the "active substance" of the polypeptidase system is transmitted from its native carrier—the yeast phospho-protein—to the products of this carrier's autolytic breakdown. Since the higher autolytic breakdown products (carriers K) which are appropriate, *i.e.*, zymolabile carriers for the splitting of dipeptids are formed first, the ability of the extracts to breakdown dipeptids at first increases. When in more advanced stage of autolysis however products of low molecular size (carriers X) and zymostable, as far as the hydrolysis of dipeptids is concerned, begin to predominate and the same activity falls. As has already been pointed out, however, we only arrived at this explanation later.

It was afterwards shown (Fodor, 1920-1922) that the original carrier of peptidase action in yeast extracts is phospho-protein a substance which these extracts coutain in abundance. If the fresh maceration juices of yeast are precipitated with dilute hydrochloric acid, the protein coagulates in so delicate a form that, after filtration and cleansing with water, it may be redispersed by stirring with the latter forming a persistent suspension which exhibits all the characteristic features of a grossly dispersed colloidal system. The ultramicroscopical picture of the suspension shows extensive aggregate formation with but few single particles in a state of Brownian movement. Such an enzyme sol has been found to show under optimum conditions of concentration, temperature and pH a low but definite activity in respect of dipeptids. On carefully adding dilute sodium hydroxide to the sol, its activity increases, simultaneously, the ultramicroscopic picture obtained discloses a great increase in the degree of dispersion with single particles in strong Brownian motion, beginning to predominate over the aggregated forms. If however the addition of sodium hydroxide is carried too far, the field becomes empty, due to the formation of invisible alkali protein, and simultaneously the zymatic activity of the sol falls.

In this way was the existence of a definite carrier of enzymic substance proven, and the dependence of this enzyme's activity upon the physical state, *i.e.*, the degree of dispersion of the carrier demonstrated. In the case of a colloidal carrier, such as for example phospho-protein, an optimal degree of hydration is necessary for the manifestation of maximal degree of activity.

We were unable to decide at the outset whether the phospho-protein merely acts as a carrier of the peptidase action in the yeast and pancreas extracts (Fodor, 1922) or is itself the active substance or the enzyme. This question might have remained unanswered moreover had we not by a stroke of good luck found that glycocoll was a suitable elution agent for the kaolin adsorbate of yeast extract. All other elution means tried, aminoacids, other than glycocoll included, were fruitless. Stimulated by the adsorption and elution method developed by Willstätter. we (Fodor, Bernfeld and Schoenfeld, 1925) treated yeast macerates with a suitable form of kaolin which adsorbed their protein content. The adsorbate, after having been thoroughly washed, could be eluted with glycocoll. The eluate (A) proved to be active in respect of both dipeptids and peptons, particularly the latter. It was found to be nearly free of protein (with Esbach reagent, a very slight clouding resulted) but gave several of the colour

reactions, that with glyoxylic acid for example, and must therefore have contained some protein derivatives. Under aseptic conditions we were able to preserve this eluate without any change in its activity several months. An attempt to carry out a new kaolin adsorption and subsequent glycocoll elution did not succeed. The kaolin adsorbate of eluate A was itself active but admitted of no elution with glycocoll. At this juncture we felt justified in setting up the following scheme as representation of these adsorption and elution processes:

First adsorption: Protein ⟷ zy. s. + Kaolin →
→ Kaolin ⟷ Protein ⟷ zy. s.;

First elution: Kaolin ⟷ Protein ⟷ zy. s. +
Glycocoll → Glycocoll ⟷ zy. s.

Second adsorbtion: Glycocoll ⟷ zy. s. + Kaolin →
→ Kaolin ⟷ zy. s.

Second elution: Kaolin ⟷ zy. s. + Glycocoll →
→ no elution

The elution process is only successful if the active material (zy.s.) is directly linked to the protein and not to the adsorbent.

At the later date (Fodor and Frankenthal, 1930, 1931), when we carried out the adsorption on kaolin of phospho-protein coagulated from yeast extracts by dilute acids, and eluted the adsorbate with glycocoll, we obtained an eluate B which was active in respect of the tripeptid leucyl-glycyl-glycine, but inactive in respect of the dipeptid leucyl-glycine.

Evidently, a carrier of category K (see above) which promotes the splitting of the dipeptid, must be assumed as present in eluate A. Since such a carrier is lacking in the much purer eluate B the latter is unable to hydrolyse the dipeptid.

Starting from this assumption we were led to the idea of attempting to bring about the activation of the eluate B in respect of the dipeptid by the addition of a carrier of the category K. With this aim in view we permitted yeast to undergo brief autolysis in the presence of acetic ether so as to obtain an increased yield of the high protein derivatives (K). When this operation was completed, an ultrafiltrate of the autolysate was prepared in order to separate the original unaltered protein from their newly formed K-derivatives. When the clear ultrafiltrate, which exhibited no enzymic activity whatsoever was added to eluate B, the latter was activated, splitting fully 32% of all the leucyl-glycine present in the course of 21 hours at the temperature of 25° C. In this way therefore eluate B aquired a capacity it had formerly lacked. The experiments thus furnished proof that for the cleavage of the dipeptid, glycocoll is an inappropriate carrier of the active substance of peptidase system. The dipeptid can only be split if carriers K, such as are contained in fresh yeast autolysates, are present.

It was shown by analogous experiments that pancreas extracts totally inactive in respect of glycyl-leucine but active in respect of glycyl-leucyl-leucine acquire an activity in respect of the dipeptid after an induction period of about 3–4 hours (37°) which is necessary for autolysis. It would be demonstrated, moreover, that the curve of the dipeptid hydrolysis followed the course of an autocatalytic process. This result admitted of no further doubt concerning the truth of the conclusion that the first products of the autolysis are the activators of the dipeptid hydrolysis. Moreover this conclusion furnished us with a convincing explanation of the results of our experiments of 1916 (s. above) which showed that the ability of yeast extracts to cleave dipeptids (and also peptons) increases with preservation.

This increase is evidently connected with an autolytic process wherein apparently not only carriers K but also products X of a further degree of breakdown are formed. For this reason apparently the ability to split peptons is also increased (even by 55%) on preservation under these conditions, *viz.*, for a period of three days at room temperature (Fodor and R. Cohn, 1928).

Some proof must still be furnished that the deep breakdown products (X) of the protein, among them amino acids, increase the activity in respect of higher peptids (and peptons) but not in respect of dipeptids. Experiments with this aim in view were carried out with maceration juices of pancreatic glands (Schoenfeld-Reiner, 1930).

The results showed that on the addition of pepton hydrolysates, rich in X carriers, to a mixture of this pepton and the macerate, the pepton cleavage was augmented, but that of the dipeptid decreased. Apparently the cleavage of dipeptids is obtained by carrier X.

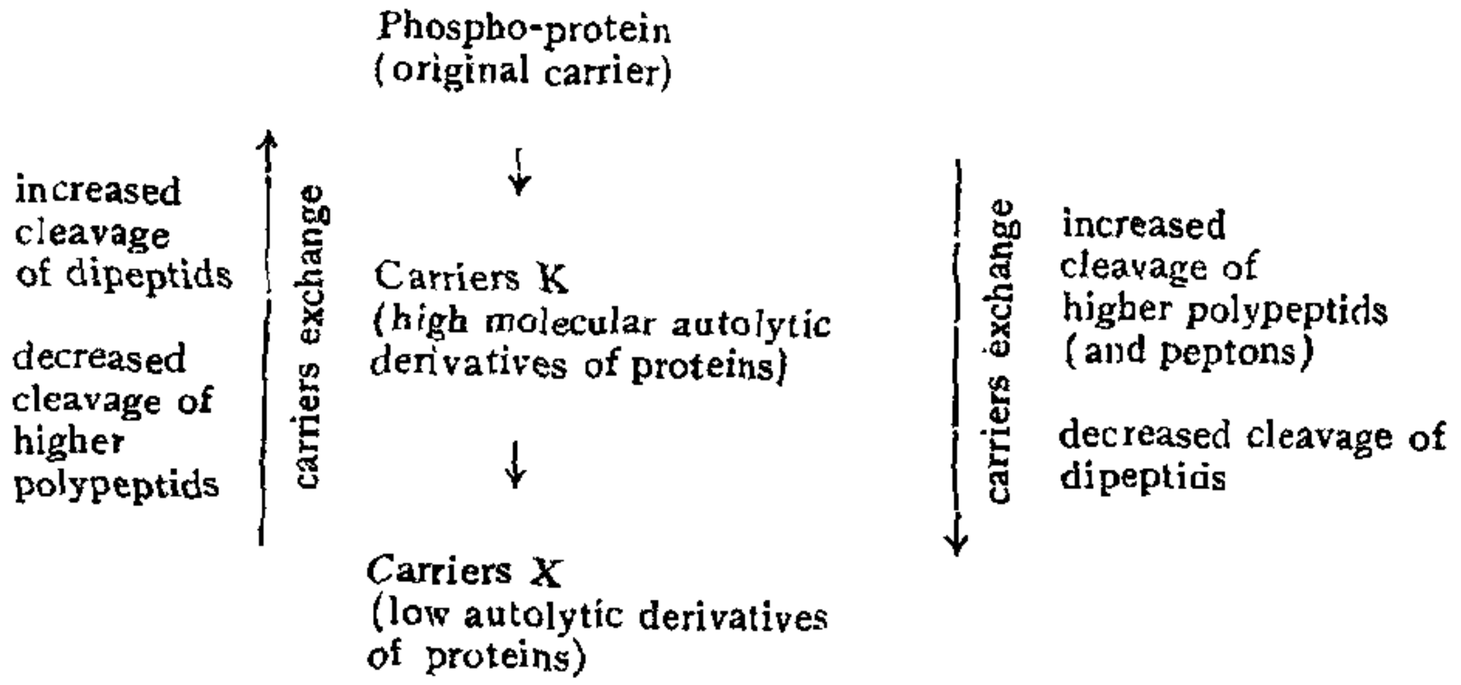
On the other hand, it could be demonstrated that pancreatin macerates which were deprived of the greater part of their carriers X content by a short dialysis, suffered a loss of activity in respect of peptones, but gained in activity in respect of dipeptids.

We are clearly led to expect from these results that the addition of amino acids which represent the extreme of the carrier X type, to a yeast extract would cause an activation of the latter in respect of peptones. We actually succeeded moreover repeatedly to demonstrate this activation (Fodor, 1928, Fodor and R. Cohn, 1928). Especially striking results were obtained with fresh maceration juices relatively poor in carriers X. Extracts which were not so fresh and contained relatively greater amounts of carriers X and K owing to the advanced state of the autolysis, exhibited a lesser degree of activation in respect of peptones on the addition of the amino acids.

On the basis of the experimental results described above, we felt justified in proposing the following carrier scheme (Scheme I).

The explanation advanced by the Willstätter school ascribes these activation and depression effects to the removal and appearance respectively of "detaining bodies" (Hemmungskorper), but this is merely a tautological circumscription of facts, and no true explanation. We only possess this latter when we have comprehended that the "detaining bodies" are nothing else than zymostable carriers. Their exchange for zymolabile ones brings an activation in its wake; exchange *vice versa*—a depression. The active substance is distributed, according to this view-point, between its original zymostable carriers and new zymolabile ones, such as those that are contained in various autolysates for example, or such substances as we added directly. A depression in activity signifies an exchange of one carrier for another with a contrary effect. The new carriers formed during the autolysis of protein might also be regarded as intermediating carriers (Scheme II).

## Scheme I.




## Scheme II.

by a slow transmission

(*a*) Carrier X ⟷ *zy. s.* + dipeptid ⟶ dipeptid ⟷ *zy. s.*

by a rapid transmission

(*b*) Carrier X ⟷ *zy. s.* + carrier K ⟶ *carrier K* ⟷ *zy. s.*

by an accelerated transmission

Carrier K ⟷ zy. s. + dipeptid ⟶ dipeptid ⟷ zy. s.

It is evident that the relative quantities of zymostable and zymolabile carriers present will have a significant influence upon the effect of the transmission of the "active substance" to a given substrate and consequently also upon the zymotic activity in respect of the latter.

It may also be deduced from this statement that the constancy of an enzyme system is dependent upon the nature of the carriers of the active substance. As long as the carrier is a colloid, in which state as a rule, it is subject to incessant change, no constancy of activity can be expected. If active substance is transmitted to a protein derivative carrier which itself suffers cleavage as a result of this transmission, the breakdown will produce new carriers that will compete with the substrate, by acting themselves as substrates. In this competition however the substrate proper, owing to its greater concentration, gains the lion's share of active substance. Nevertheless it is a significant fact that such a system undergoes perpetual changes in activity, changes which are already revealed in the self-alteration of enzyme extracts and which researchers take into account by setting up parallel controls.

True constancy can only be expected where the carrier is not subject to changes of the nature mentioned above. This condition was fulfilled for example in our glycocoll eluates. The experimental verification of this fact provides additional proof that the eluting substance, *i.e.*, the glycocoll, is itself the actual carrier of active substance in the eluates.

### 3. THEORETICAL CONSIDERATIONS.

The following paragraphs deal with the nature of the zymoactive substance. This is still as has been pointed out above, a mystery to us. In discussing its chemistry therefore we enter a domain of pure hypothesis.

Our claim that zymoactive substance (zy. s.) distributes itself between the substrate and one or several carriers contains the implication that zy. s. possesses an affinity both for all the different but chemically kindred carriers and for the substrate which, as we have shown, also ranks among these chemical relatives.

This conception involves a further assumption. The chemical constitution of the zymoactive substance must admit a combination both with the multitude of carrier and with the substrate which itself acts in this case as a carrier. Consequently it was concluded that zymoactive substance possesses a common affinity for all these carriers, substrate included. But granting this, the existence of at least as many zymoactive substances as there are protoplasmic carriers of fundamentally different chemical constitution must also be granted. For it is inconceivable that the same active substance enters into combination both with the totality of proteins and protein derivatives and with such a substance as starch for example or other cell and tissue constituents.

We are thus led to conclude that the ground substances of the plasma are equipped above with zymoactive substances which are closely attuned to them chemically. When the ground substances are broken down into breakdown products of a kindred chemical constitution, the zymoactive substances become transmitted to them (see above).

These conclusions follow from our main premises. It follows further from the investigations of many researchers on enzymes that the active principle is impermeable to dialytic membranes and must therefore be in a colloidal state. Where the dialysis brings about the inactivation of the enzyme system, its activity can, as a rule, be restored by the re-addition of the dialysate to the dialysed solution even though both these fractions were themselves inactive. It may be concluded therefore that the active principle of the system does not diffuse through the membrane and that inactivation by dialysis is merely due to the removal of permeable activators.

On the other hand, we are compelled to conclude from the experimental results of several researchers that the separation or isolation of the active substance is an impossibility, and that its connection with a carrier is necessary for the manifestation of activity. When we attempt to attain the active material in pure form, all we ultimately get is inactive chemical substance. The zy. s., whose quantity is extremely minute, is lost during the purification procedure in the mass of purifying reagents.

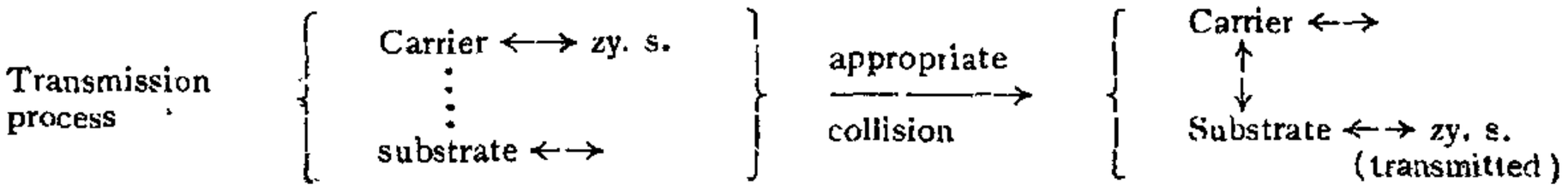
It seems right therefore to conclude that the colloidal active material depends for its existence on its connection with a carrier or substrate, *i.e.*, that the activity is only preserved in connection with the latter, and that separation brings inactivation in its wake, probably as a result of some change in colloidal state, such as coagulation. It is a commonplace experience of the colloidal chemist that substances which in a high degree of dispersion possesses enormous surfaces and fill correspondingly great volumes, are almost invisible to the naked eye and hardly measurable by weight after coagulation.

Consistent with our view that the active material is a colloid of high disperse degree which is linked to carriers and preserves its dispersion only as long as this linkage endures, we may go a step further and concede that the linkage to the carrier is to be ascribed to a compensation of the great surface energy, and that liberation from the carriers invariably involves the loss of this compensation and hence a coagulation. The carrier thus functions as a protective colloid : it preserves the colloidal state of the substance which is linked to it.

It is permissible for us to draw still another conclusion of a colloidal kind, *viz.*, that a partial annulment of the surface energy of the active material can be attained by a change of carrier. If we assume, as indeed we do below, that a parallelism exists between the surface energy and the activity, we may regard as zymophoric only such carriers as allow to maintain the high energy state ; carriers which bring about its decrease may not be regarded as zymophores. On the other hand, the measure of zymolability is largely dependent upon the efficiency with which diminution of the surface energy maintained by a given carrier, is effected by the combination of this carrier with the substrate. According to energetical principles, the surface energy liberated by this combination must manifest itself as thermal, electrical or chemical activity (depending upon the conditions prevailing in the system under consideration), and hence we may also attribute the chemical change of the substrate to the partial liberation of surface energy which is brought by the combination of the carrier with the substrate.

We can now understand why that which above was termed "transmission effect" should not be represented as a kinetical problem only, for it is in the same time one in energetics. The quantity of the liberated energy depends both upon the nature of the combination which is effected between the carrier and the substrate and upon its intensity. It is easily comprehensible that a decisive rôle is played in this connection by the nature of the carrier which confronts a given substrate. Different carriers free different quantities of surface energy of zy. s. on combination with the same substrate. Not only relations which arise from chemical reactions between atoms come up for consideration in this connection, but mainly rather relations which are conditioned by the colloidal state of the zymoactive material, and arise from the space pattern and atomic constellation of the carriers and the substrate at their mutual association. Zymolabile carriers produce a considerable decrease in the surface energy of the zymoactive substance by this association, thereby calling forth the correspondingly great "transmission effect". Zymostable carriers, on the other hand, have but a small effect.

The protective action of the carriers upon the zymoactive substance is partially removed by the association of the carriers to the substrate. Consequently a corresponding dislocation which is conditioned by the intensity of the combination between the carrier and the substrate, and which liberates zy. s. occurs. The zy. s. thus liberated can fix itself upon the substrate and by this fixation some of the surface energy of the zy. s. is undoubtedly transformed into manifest chemical energy :



The transmission process can only take place of course, if the fixation of zy. s. by the carrier is sufficiently loose to allow the dislocation. If the zy. s. is closely fixed however, a zymophoric behaviour by the carrier is quite out of the question. Thus:

| | |
|---|---|
| Carrier K ⟷ zy. s<br>(high derivatives)<br>↕ easy linkage<br>Dipeptids<br>(low derivatives) | Easy dislocation of zy. s. and consequently an easy transmission. |
| Carrier X ⟷ zy. s.<br>(low derivatives)<br>↕ difficult linkage<br>Dipeptids<br>(low derivatives) | Difficult dislocation and difficult transmission. |
| Carrier X ⟷ zy. s<br>(low derivatives)<br>↕ easy linkage<br>Higher polypeptids<br>(peptones) (high derivatives) | Easy dislocation and easy transmission. |

When high protein derivatives meet low derivatives, the dislocation and transmission are facilitated, since the association between carrier and substrate in this case is particularly easy.

On the basis of all that had been pointed out above, the answer to the question whether a given substance can act as a suitable carrier, is seen to depend upon the possibility of an association, in the sense already demonstrated in Part 1, between the carrier and the substrate in question, as well as upon the ability of the carrier to have entered into combination with the zymoactive substance without causing entire loss by this combination of the surface energy which the zymoactive substance possesses as a colloid and which it must liberate subsequently when the carrier and the substrate combine, if it is to effect chemical action such as hydrolysis or other cleavages.

The kinetic equation of an enzyme system must therefore take into account two factors: (1) the frequency of the "appropriate collisions" (see above) and (2) the quantity of surface energy ($\Delta\pi$), set free in a unit of zy. s. after the above mentioned collisions. With one and the same substrate both factors, the frequency as well as the $\Delta\pi$, vary according to the nature of the main and the subsidiary carriers.

Yet it may occur that a carrier T which is itself now zymophoric in respect of a given substrate is made zymophoric by the presence of a second, for its part zymophoric, carrier $T_1$ (Fodor and Frankenthal, 1930):

$$T \longleftrightarrow \text{zy. s.} + \text{substrate} \longrightarrow \text{no transmission}$$

$$T \longleftrightarrow \text{zy. s.} + T_1 \rightarrow T_1 \longleftrightarrow \text{zy. s.}$$

$$T_1 \longleftrightarrow \text{zy. s.} + \text{substrate} \longrightarrow \text{substrate} \longleftrightarrow \text{zy. s.}$$

The part played by $T_1$ is thus that of a mediator or even "activator".

The conception of the existence of mediators of this kind was advanced by the author (Fodor, Kuk and Frankenthal) as early as 1928. The fact of their existence has recently been confirmed by O. Warburg (1935), who ascribes a similar part to the "yellow flavin enzyme," as mediator of the hydrogen transmission in the alcoholic fermentation system of yeast.

The ideal form of the kinetic equation of, for instance, the hydrolysis of a dipeptid to amino acid molecules

$$\begin{array}{l} \quad\quad R \qquad\quad R_1 \\ NH_2\text{-}CH\text{-}CO\text{-}NH\text{-}CH\text{-}COOH + H_2O \\ \quad\quad R \qquad\quad R_1 \\ = NH_2\text{-}CH\text{-}COOH + NH_2\text{-}CH\text{-}COOH \end{array}$$

must be postulated under the following premises:

Let us conceive a single highly zymolabile carrier T. Let us further assume that none of the hydrolytic products act as carriers. Let the unit of zymoactive substance, whose quantity Z may be assumed to remain constant during the initial time interval $t$, call forth an energy change $\Delta\pi$ equivalent to the surface energy loss $\Delta\omega$.

Then the quantity of energy transmitted to the substrate will be $E = \Delta\pi . Z$ (= effect) and

$$\frac{dx}{dt} = E.k\,(a-x)^n = \Delta\pi.Z.k\,(a-x)^n.$$

In this equation $a$ represents as usual the concentration of the substrate at the beginning of the action, $(a-x)$ its concentration

after a time $t$ ($x$ being the quantity transformed), $k$ and $n$ are constants of which the latter depends upon the frequency of the "appropriate collisions" and generally has a value of about $\frac{1}{2}$ but varies with the pH of the whole enzyme system. Under certain experimental conditions $n$ may even be equal to 1. In this case E really being constant the equation would assume the external form of a reaction of the first order. We may easily see also from this equation how one and the same quantity Z can produce radically different degrees of zymotic activity (as measured by the quantity $\frac{dx}{dt}$) according to the quantity of the effect E, a quantity which is determined by the nature of the carrier.

To be sure, this kinetic equation is in practice rarely realised in its ideal form. Numerous disturbing factors intervene. Such for instance are the products of enzyme action which may either accelerate or slow the hydrolytic process, thus changing the value of both E and the constants.

From the point of view of kinetics, the enzyme action is thus seen to be a highly complex, and a but little elucidated process. It was only great optimism which moved former researchers to hope that they could make the kinetics of enzyme action conform with those of so simple a process as the cane sugar inversion found by Wilhelmy.

Natural phenomena are found to be more complex and intricate the deeper we penetrate into their profundities. To this fact the mechanism of enzyme action is an abundant testimony.

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A peculiar property of such an "Eloxal" (Electrolytically Oxidised Aluminium) is its porosity, the film being covered with a network of pores the diameters of which are of colloidal dimensions. It is this porosity of the Aluminium film in Eloxal that is responsible for its many valuable applications.

This porosity enables Eloxal to absorb liquids. The Eloxal plate can be charged with light-sensitive chemical either by tanking it in an aqueous solution of the reagent or (with water-insoluble chemicals as silver halides) by causing the reagent to be precipitated within the porous film. The Eloxal plate is now ready for the SEO photograph employing the usual photographic processes. Only, the pores in the oxide film have to be closed finally by rubbing the plate with either a suitable fat or wax. The cost of the Seo Eloxal photo is stated to be no higher than the ordinary photograph.

The Seo photo possesses many useful properties. It is not affected by temperature, by the common organic solvents, by seawater, and by the ordinary corroding factors. The Seo photo can be heated up even to the m.p. of Aluminium (658°) when the photograph is still clearly distinguishable on the oxide film. These properties give the Seo photo a large field of utility. It is excellently adapted to the manufacture of Placards, Scales and other Measuring Instruments, Notice Boards, Passports and other Legal Documents, Maps and Plans, etc.

The porous Eloxal film allows the deposition of various colouring matters enabling the manufacture of "Coloured Aluminium". When Eloxal, deposited with colloidal gold, is heated to various temperatures, the surface of the material takes on various shades of rich brown-red tints. Beautiful imitations of the grain and colour of timber and marble can be imparted to Eloxal which can be used as fire-proof panelling in the interior decoration of ships, æroplanes, railway carriages, etc. It can also be used in the wide field of applied art and in the manufacture of fancy-goods.

The great variety and extent of use indicated above, to which Eloxal can be put, it is claimed, is of national importance to Germany which does not possess copper deposits and is endeavouring to minimise the use of the metal and its alloys. The development of Eloxal is, therefore, another step in the German effort towards national self-sufficiency.

Eloxal was invented and developed by Jenny and Budilhoff, two Engineers on the staff of Siemens & Halske, A. G., Berlin, who have protected the invention by a series of patents.

EMMENNAR.

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## Silica Fluff.

A NEW form of hydrated silica, having unusual properties, prepared by slow drying at low temperature of the transparent gel resulting from the action of silicon tetrafluoride on water has been described by Jacobson (*J. Phys. Chem.*, 1936, **40**, 413) and is called Silica Fluff on account of its fluffy nature. This extremely light, opaque, white powder has low specific gravity, contains inclusions of air bubbles (1 to 1·5 microns) which give colour phenomena with polarised light and which cannot be dislocated by a vacuum pump. Its percentage composition leads to the empirical formula $(SiO_2)_{12}3H_2O$ and the graphic formula has also been speculated.

K. S. RAO.

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## The Movement of Protoplasma in Plant Cells.

THE movement of protoplasma within the plant cell as a result of irritation is a well-known phenomenon, the wounding of Vallisneria and allied plants furnishing striking examples. Prof. Hans Fittig of the University of Bonn has investigated (*Forschungen und Fortschritte*, 1936, **12**. 160), whether in such cases, the cause of the movement is due to purely mechanical influences or whether it is traceable to any chemical stimulant formed as a result of the injury. Fittig, by careful experimentation, shows that the extract from crushed Vallisneria leaves contains a substance which is stable towards heat but susceptible to bacterial decomposition and which in dilutions as low as 1 in 2 millions causes the movement of protoplasma in plant cells. As a first step in the elucidation of the nature of this active principle, Fittig sought to find if any known substances are capable, in dilutions of a comparable order, of causing such protoplasma movement. He finds that Amino acids are capable of such irritation. $\alpha$-Amino acids are the most active, the potency rapidly diminishing with the $\beta$, $\gamma$, etc., isomers. Further, the Amino acids in their naturally occurring

optical isomeric forms are very much more active than their artificially prepared optical antipodes.

The effects of different Amino acids on a given plant cell vary enormously. By far the most active are Histidine (effective in concentrations as low as 1 in 600 millions) and Methyl Histidine (1 in 3,000 millions). Fittig considers it probable that the Vallisneria extract used in his experiments contains either about 1% Histidine or about 0·2% Methyl Histidine. The extract did not respond to the diazo colour reaction with Diazobenzenesulphonic acid which is sensitive to Histidine in concentrations of about 1 in 100,000. Fittig does not, however, consider this as conclusive or even significant because the reaction is easily masked by the presence of Methyl Histidine and other foreign substances which presumably are present in the Vallisneria extract. Work is in progress to find other methods of detecting Histidine. The importance of Fittig's work lies in the fact that α-Amino acids which till now were considered to be physiologically indifferent in plant life are shown to be in reality very active substances comparable to the physiological potency in animals of Thyroxin, another (though highly complex and Iodine-containing) Amino acid.

EMMENNAR.

## A New Mode of Fixation of Nitrogen in Soils.

DHAR AND CO-WORKERS, in their extensive investigations, have shown the importance of light in many oxidation processes taking place in soils. In a recent paper (*J. Indian Chem. Soc.*, 1936, **13**, 155–179) N. R. Dhar and S. K. Mukherji have pointed out a new possible way in which light is helpful in the fixation of nitrogen in soils. When some sterilised soil suspended in a medium containing dissolved carbohydrates (like glucose, cane sugar, molasses, etc.) is exposed to sunlight for a long time, it is found that there is an appreciable increase in the available and the total nitrogen contents of the soil. The energy set free during the photo-oxidation of the carbohydrates seems to fix the atmospheric nitrogen. The efficiency of nitrogen fixation obtained in the induced oxidation of sugars is of the same order as that with cultures of Azotobacter thriving in flasks containing solutions of energy-rich compounds. These observations have been correlated with the results obtained in some field experiments undertaken for studying the effect of molasses on soils.

K. S. G. D.

## Thermal Decomposition of Talc.

EWELL, BUNTING AND GELLER (*J. Research National Bureau of Standards*, 1935, **15**, 551–556) describe the changes which accompany the careful heating of a specimen of nearly pure talc from Manchuria. The furnace employed for the purpose could be heated up to 1435° C., and the temperature rise adjusted to about 6° per minute.

Observations with a differential thermocouple revealed two endothermic effects (both irreversible) at about 530–572° and 860–953° respectively. Determinations were simultaneously made of the weight losses suffered by the sample at different temperatures ranging up to 1435°, the period of heating at each temperature being 2-22 hours.

X-Ray and microscopical examinations were made of the unheated material as well as of the material heated to different temperatures. The X-Ray camera was divided so that comparison patterns of the test and reference specimens could be obtained on the same film.

It was found that between 380–500° all the water present in excess of 1 molecule was driven off from talc but without any alteration in crystal structure. The molecule of combined water was removed at 800-840° and this was accompanied by breakdown of the talc into enstatite and amorphous silica and also by an increase in specific gravity. On further heating up to 1300°, the enstatite changed into clinœnstatite and amorphous silica into cristobalite. The final products of the thermal decomposition of talc were found to be clinœnstatite and cristobalite, and the specific gravity of the fully decomposed material was found to be 3·01 as compared with the value 2·83 for the unheated specimen.

K. R. K.

## Histophysiological Study of Testis.

PROF. JACQUES BENOIT has a very interesting paper in the Actualités Scientifiques et Industrilles (*Exposés de Biologie*, 1935, **3**, 3–64) on "Le Tèsticule" as an organ elaborating the male sexual hormone. He has studied the testis from the morphological and histophysiological view-points. This

study has enabled the author to divide the testicular cells under three heads, *viz.*, the *lignée seminale* forming the reproductive cells, the cells of Leydig and the cells of Sertoli. The latter two sets of cells are probably derived from the same cellular layer in the Gallinacea and the same is also likely to be the case in mammals and the other groups of vertebrates. The transformation of the Sertoli cells into glandular interstitial cells is accompanied by the loss of Sertoli characters and the acquisition of a secretory activity. The cells of Sertoli play *vis-a-vis* to the seminal elements the rôle "d'un terrain somatique trophique". The interstitial cells secrete endocrines, which hormone is responsible in the development and functioning of the secondary sexual characters and the accomplishment of reproduction.

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### The Arteries of the Chimpanzee.

As a result of the examination of ten specimens of the Chimpanzee (Pan spec ?), E. M. Glidden and C. F. De Garis (*Amer. Jour. Anat.*, March 1936, **58**, No. 2, 501) have distinguished five types of aortic arches. In one, which exemplifies the normal human sequence, three trunks, the *a. anonyma*, the *a. caroticus communis sinistra* and the *a. subclavia sinistra* arise from aortic arch. In the second type, *a. truncus communis* gives rise to *a. anonyma* and *a. carotis communis sinistra*. This condition is found in a single specimen out of the ten examined. The third pattern occuring in four specimens, simulates the first except that a small *a. thyroidea ima* arises directly from the arch between *a. anonyma* and *a. carotis communis*. The fourth type is characterised by the *a. thyroidea ima* arising from the *a. carotis communis* instead of directly from the arch. This occurs in two specimens. The last condition is characterised by the fusion of the bases of *a. carotis communis* and *a. subclavius sinistra* to form *a. anonyma sinistra*. The *a. thyroidea ima* always arises from this *anonyma sinistra*. This occurs in two specimens.

In the single specimen which the authors have dissected for the arteries of the descending aorta and of the pelvis and the extremities, it is seen that *a. subclavia* lacks the *a. thyroidea* inferior and the relation between the *a. axillaris* and *plexus brachialis* is seen to be different from that obtaining in man. The visceral branches of the descending aorta are in the main similar to the human pattern. In the pelvic region the obturatoria is rudimentary and the *circumflexa femoris medialis* arises from the external iliac.

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### The Idea of Contrasted Differentiation.

PETROLOGISTS are aware that during the last few years S. R. Nœkolds has been developing his hypothesis of contrasted differentiation to account for the variation in igneous rocks. His main contention is that in intercrustal magma reservoirs, differentiation yields two contrasted magma types—acid and basic, which manifest as intrusions, with the basic member invariably coming out first, and that most of the intermediate rock types are commonly the products of contamination. Recently Aurther Holmes (*Geological Magazine*, May 1936, No. 863) has shown that the idea of contrasted differentiation as advocated by S. R. Nœkolds is beset with many inconsistencies and objections so fundamental as to be in direct variance with the physico-chemical principles of crystallisation differentiation established by Bowen. In discussing Nœkolds's evidences in support of contrasted differentiation, Holmes observes that the occurrence of contrasted rock types in close proximity does not constitute any direct proof for contrasted differentiation especially in view of Fenner's observation where he has shown that the alternate outpourings of typical basalt and typical rhyolite with little or no lava of intermediate composition. Hence according to Holmes preference must be given to the hypothesis of progressive crystallisation differentiation for which there is at least a convincing experimental basis rather than contrasted differentiation for which there is no real evidence at present.

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