CHEMICAL PROCESSES IN FERMENTATIONS[1]

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[1] Revision of a paper prepared to be read at the Los Angeles meeting of the American Chemical Society, August, 1925.—Certain circumstances prevented my arrival in the United States in time to attend the meeting.—The greater part of the German manuscript was translated by Floyd D. Hager.

"It seems to me that perhaps the only advantage of advancing age is that one is able to recall what the old masters thought."—*Graham Lusk.*

1. INTRODUCTION

One of the most important ways in which the products of agriculture are used is the utilization of carbohydrates through their decomposition by the various fermentations, alcoholic, lactic acid, butyric acid, and acetic acid, brought about by microorganisms. We knew what the original materials and end products are in these biochemical reactions, but we are just in the development of a period whose task it is to penetrate the mechanism of these reactions by use of the tools of biochemistry.

The very great importance and significance of investigations concerning the transformations of carbohydrates and the relation of those transformations to biological science can be indicated by reference to a few facts. The carbohydrates are formed in the assimilation processes of plants and serve as the starting product for the chemical and physical performances of work by the plants. Further, nearly all bacteria take up some form of sugar from their nutrient media. Not only that, but in the animal body carbohydrates can be responsible for the synthesis of protein and fat.

All are familiar with the experiment of two naturalists performed some sixty years ago. They climbed the Faulhorn in Switzerland, a peak 1956 meters high. Seventeen hours before starting they ate the last nitrogenous food taken during the experiment. During eight hours, with one interruption, they climbed, and were in motion a total of thirteen hours. They carefully collected the urine excreted during that time and showed, by its analysis, that the work done corresponded to three times the energy which could be derived from the protein used. This experiment was the starting point of all observations which have shown that, in the case of animals as well as of other more highly developed organisms, the source of energy is not in protein but primarily in fat and carbohydrate.

Workers in this field have shown that the carbohydrates are hereby, in general, burned to carbon dioxide and water. But

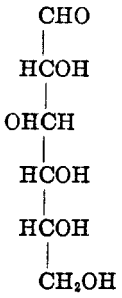
the yeasts use for alcohol production, even in case of increased aeration with oxygen, less sugar material than they have at their disposal for this purpose. This shows that in this process there is not only a partial resynthesis of sugar from the intermediate products but from numerous observations made on various organisms, we know that in this disintegration, analogous to purely chemical oxidation, there are intermediate stages.

Excluding, obviously, the case of a biological oxidation, if we are inclined to consider fermentation as a process through which, by a biochemical action carbon chains are broken down or united (see particularly pages 56, 70), it is intelligible that for many decades the efforts of naturalists have been directed toward an interpretation of the decomposition of sugar, using as an example the long known phenomenon of alcoholic fermentation, a process typical of one that stops, to a certain degree, at an intermediate stage.

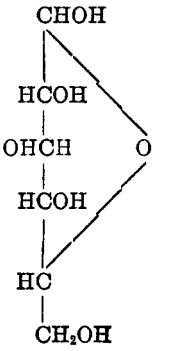
The decomposition of carbohydrate by yeast is expressed, in its quantitative proportions, by the equation of Gay-Lussac:

$$C_6H_{12}O_6 = 2\ C_2H_5OH + 2\ CO_2 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

At first, we are unable to interpret these proportions. The practical quantitative course of the fermentation process produces from each mol of sugar two mols each of alcohol and carbon dioxide. The old familiar formula of glucose,



and the newer one proposed by Haworth for discussion,



give nothing at all of an indication of ethylidene or carbon dioxide groups which occur in the fermentation products. Here, then, is a typical example in which the final condition comes about only through intermediate steps, a process which involves a gradual dissolution of the hexose carbon chain.

The decomposition of fermentable initial materials in the course of enzymatic alcohol production seems to rest, in the ultimate analysis, on the fact that the substances formed in the course of the reaction are very convertible and difficult to intercept. These substances must, however, in every case, be such that the yeast can further act upon them and render possible in the end the production of compounds which are in equilibrium with each other and which give the end products of the fermentation.

2. THE RÔLE OF ZYMOPHOSPHATES

In regard to the first phase in the decomposition of the sugar complex, i.e., the processes which probably initiate the disintegration of the hexose molecule by the action of the yeast enzyme, to substances containing three carbon atoms, it is difficult to reach any clear cut conclusions from the existing experimental data. It is, however, supposed that in this case the alkali phosphates have an important rôle.

The first proposal, to add to a 15 per cent fermentable sugar solution 0.2 gram per liter of monocalcium phosphate, came from

Pasteur. After Buchner and Hahn had ascribed initial acceleration of fermentation by alkali phosphate to the alkaline reaction of these salts, starting in 1905 L. Iwanow, as well as Harden and Young, began fundamental investigations in connection with this question. The British investigators noted that the speed[2] of the fermentation increased if one added, in the presence of phosphates, boiled or ultrafiltered yeast juice. This action apparently should be ascribed to the phosphates, as similar observations were made when solutions of the alkali salts of orthophosphoric acid were used. At the end of the fermentation, no phosphate could be detected in the usual way. It must be supposed, therefore, on the basis of later investigations, that in the alcoholic fermentation always two molecules of sugar are concerned, of which one combines with two molecules of phosphate to form hexosediphosphoric acid while the other molecule of sugar breaks up to form carbon dioxide and alcohol. In the further course of the reaction a special enzyme, the phosphatase continually breaks down the hexosediphosphoric acid to inorganic phosphate and fermentable hexose, whereby the repetition of the process becomes possible:

$$2\,C_6H_{12}O_6 + 2\,PO_4HM_2^* = 2\,CO_2 + 2\,C_2H_5OH + 2\,H_2O + C_6H_{10}O_4\,(PO_4M_2)_2 \quad (2)$$

$$C_6H_{10}O_4\,(PO_4M_2)_2 + 2\,H_2O = C_6H_{12}O_6 + 2\,PO_4HM_2 \quad (3)$$

Previously, however, Harden and Young had made, by means of kinetic measurement and analysis, the important observation that the increase in the amount of carbon dioxide or alcohol formed was, within definite limits, in direct proportion to the amount of phosphate added: (see fig. 1). In the graph, curve A shows the normal course of fermentation in the case of yeast juice (l. c., p. 416), curve B the effect of adding phosphate. In the latter case the velocity of fermentation amounted to 9.5 cc. per 5 minutes, i.e., some six times the normal value, and then again reached almost exactly the original value of 1.4. Curve C

[2] According to Slator (Chem. Soc., **89**, 133, (1906)), the speed of fermentation is directly proportional to the quantity of yeast and only very slightly dependent on the sugar concentration between 0.5 and 20 per cent.

*M = metal.

shows the repetition of the whole phenomenon after a period of 70 minutes when the phosphate was renewed.

This organic phosphoric acid compound, which is not precipitated by magnesia mixture, was isolated, as the copper salt, by L. Iwanow in 1905; it can also be precipitated by lead acetate. This Russian investigator considered it a triose derivative hav-

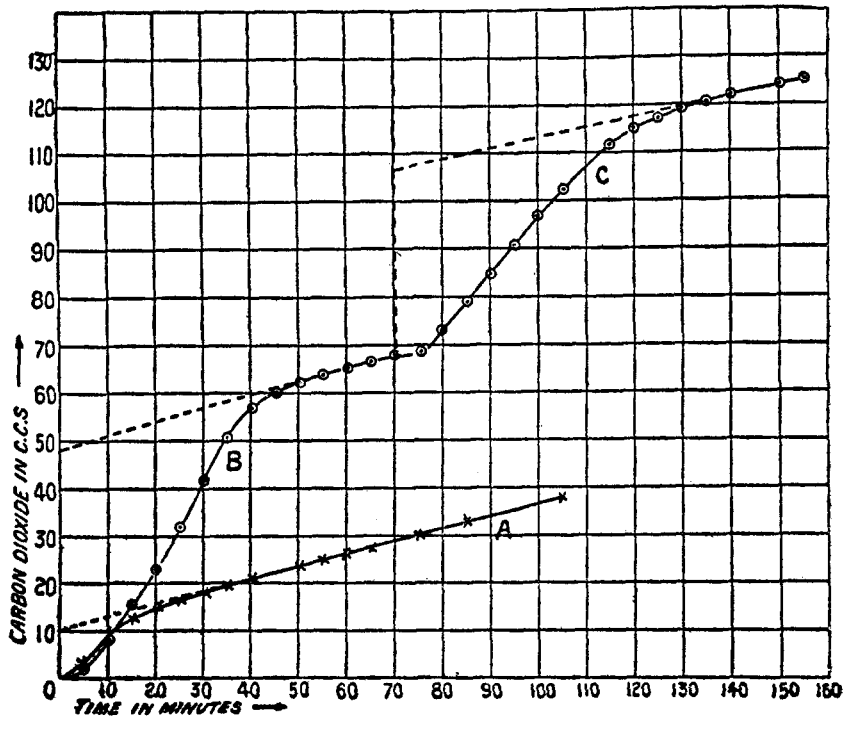


FIG. 1

ing the formula: $C_3H_5O_2 \cdot H_2PO_4$ and later showed that this change of inorganic phosphate to organic compounds could be brought about by many of the higher plants. Lebedew, assuming he was dealing with a hexose-mono-phosphoric acid, prepared a phenyl hydrazine derivative of the supposed formula $C_{24}H_{31}N_6O_7P$, but again Harden and Young were able to produce conclusive proof that the substance was the phenyl hydrazine salt of a hexosephosphoric acid osazone of the formula:

$$(C_6H_5NH\cdot NH_2)\ H_2PO_4\cdot C_4H_5(OH)_3\cdot \begin{matrix} C{:}N\cdot NHC_6H_5 \\ | \\ CH{:}N\cdot NHC_6H_5 \end{matrix}$$

That the carbohydrate phosphoric ester is, nevertheless, really a hexosediphosphate:

$$\begin{matrix} CH_2\cdot O\cdot PO_3H_2 \\ \cdot \\ C{:}O \\ \cdot \\ CH\cdot O\cdot H \\ \cdot \\ CH\cdot O\cdot H \\ \cdot \\ CH\cdot O\cdot H \\ \cdot \\ CH_2\cdot O\cdot H \end{matrix}$$

(where we leave open the question, which of the H-atoms is replaced by the second phosphoric acid radical) was established by Young when he showed that in the reaction with phenylhydrazine there was the separation of one mol of phosphoric acid. Also, the later degradation to the hexose-mono-phosphoric ester, $C_6H_{11}O_5\cdot H_2PO_4$ by Neuberg, can be viewed as further making clear the nature of the diphosphates of d-glucose, d-fructose, and d-mannose, which are corresponding "zymophosphates," but we do not know with much precision, as yet, the configuration of the hexose present in the ester. The fact that the γ-fructose present in cane sugar is partially enolized, suggests, by all means, the possibility of the enol formula:

$$\begin{matrix} CH\cdot OH \\ \| \\ C\cdot OH \\ \cdot \\ OH\cdot C\cdot H \\ \cdot \\ H\cdot C\cdot OH \\ \cdot \\ H\cdot C\cdot OH \\ \cdot \\ CH_2OH \end{matrix}$$

The possibility of a re-formation into fructose as shown by equation (3) forces us to consider, even if we accept the view of Harden and Young, that the hexose-diphosphate is no decomposition product but perhaps an especially reactive compound still retaining six carbon atoms. This conception is supported by the findings of L. Iwanow, v. Euler, and Johansson, who have shown that the formation of hexose-diphosphate can take place independently of fermentation. v. Euler, Kullberg, and Olsén, ascribed this effect to an enzyme which does not act reversibly. This enzyme may be identical with the synthease earlier assumed by Iwanow. As long as we are not authorized to see a conciliation of the contradictions in a manner analogous, perchance, to the findings according to which the fact of fermentation of *composite* sugars by the effect of specific zymases without preliminary hydrolysis is due to a considerably greater number of zymases as hitherto supposed—as long as that condition exists, there remain differences of great magnitude between the two conceptions and the significance of phosphate esters in the course of alcoholic fermentation, in contrast with the change of substances in muscles, is for the present not clear.

The grounds for this are as follows: It is taken as proved that zymophosphates are, in general, unfermentable by living yeast and also that the phosphoric esters can be obtained only by means of yeast juice or dried yeast. If, therefore, the formation of the phosphoric ester be possible without fermentation, it remains questionable whether the two phenomena are to be considered dependent upon each other respectively, whether it would be likely suitable conclusions would be drawn from the constant proportion between the fermented and phosphorated sugar.

If we further consider that an accumulation of hexose-diphosphate takes place only in the presence of an unusually large amount of salts of phosphoric-acid, as well as in the case of one of the normal alcoholic fermentations having an unfavorable hydrogen ion concentration (pH = 6.4),[3] then it was interesting to become acquainted with the opinion, that we perhaps have

[3] According to Haegglund and Augustson (Biochem. Z., **155**, 334, (1925)) the highest fermentative activity of living yeast is attained at pH = 4.5.

to do no more with the usual fermentation process but with an abnormal one which will be discussed later, with one similar to the peculiar general kind of forced glycerol fermentation.

But these considerations of Neuberg, Faerber, Levite and Schwenk repeatedly referred to since 1917 seem again to require revision since the results of recent experiments by Smedley Maclean, and Hoffert. The experiments of these British investigators indicate that the larger hexose phosphate molecule can not permeate the wall of the yeast cell, but that the sugar and phosphate enter the cell separately and then combine. Since yeast contains one of the enzymes which brings about the synthesis of hexosephosphate, and which does not pass through the cell wall, it seems to be easily intelligible that the hexosephosphates may be demonstrated only in cell-free fermentation after the enzyme is present in the fluid.

According to Paine, however, the sodium phosphate partially permeates the cell wall, and therefore in accordance with Emden's results it may also here be true that the permeability of a physiologic membrane varies as the hydrogen-ion concentration varies.

But of greatest importance for the interpretation of the introduction of the reactions in the three carbon chain series (see section 5), are the results of measurements made by v. Euler and Nilsson, who, in logical valuations of the fundamental investigations of Witzemann as well as of Spoehr, and in agreement with Kuhn and Jakob, apparently have established that in the case of non-enzymatic reactions the reactivity of the zymohexose molecule, especially in the case of fructose, is essentially raised.

This fact agrees with the important observation of Warburg and Yabusoe. According to them there is indeed combustion of fructose in the presence of phosphate ions through molecular oxygen as opposed to glucose, which is not affected. It would be attractive, on the other hand, if we could possibly look on one ester of a γ-sugar, the "Transport form" of the dextrose of Hewitt and Pryde (the structure of which is still unknown) as a result of the effect of the Synthease (see section 2), instead of on the fructose-diphosphate. It will be especially necessary, agreeing with Irvine, to suppose that primary steps of isolated sugars

even in enzymatic processes are not chemical individuals, but labile forms.[4]

3. THE CO-ENZYME

For the initiation of fermentation or phosphoration, not only are enzymes and mineral phosphates necessary, but also auxiliary systems. Harden and Young (l. c.) in 1906 let yeast juice from top yeast pass through a Martin gelatin filter and thereby obtained an inert residue and an inert filtrate. Buchner and Antoni dialyzed the juice through parchment paper and obtained the same results. If residue and filtrate, each of which can no more decompose sugar, be united, then one obtains a mixture which, in its ability to bring about alcoholic fermentation, is equal to the dialyzed juice. Then besides the zymases there is necessary for the fermentation a substance which is dialyzable and, to a certain extent (according to Tholin until 80°) thermostable. Following the suggestion of Bertrand, Harden and Young called this system a co-enzyme. The inactive residue from the dialysis can be made active again also by the addition of boiled juice or by the addition of an inactive yeast extract termed also apozy-

[4] Note added at correction:

Raymond (Proc. Nat. Acad. Sci., **11**, 622, (1925)) has recently apparently also accepted this viewpoint, assuming that the hexose-mono-phosphoric acid of Robison (Biochem. Journ., **16**, 810, (1922)) (which occurs together with hexose-diphosphate but is not identical with the decomposition product of Neuberg) is the intermediate conversion to the ester of hexose-di-phosphoric acid. This takes place through the hexose-mono-phosphoric acid:

$$C_6H_{12}O_6 + R_2HPO_4 = C_6H_{11}O_5 \cdot R_2PO_4 + H_2O \qquad (\alpha)$$

which, splits into a phosphorus-containing and phosphorus-free triose:

$$C_6H_{11}O_5 \cdot R_2PO_4 = C_3H_5O_2 \cdot R_2PO_4 + (C_3H_6O_3) \qquad (\beta)$$

and by the condensation on the one hand to hexose-di-phosphoric acid,

$$2C_3H_5O_2 \cdot R_2PO_4 = C_6H_{10}O_4(R_2PO_4)_2 \qquad (\gamma)$$

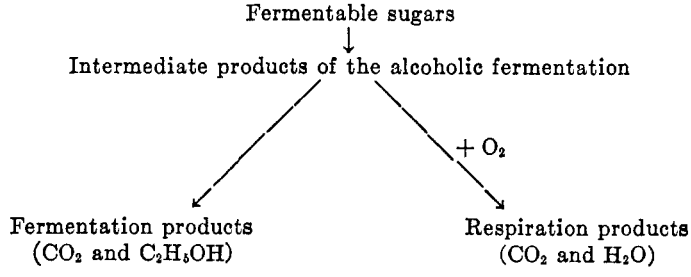
on the other hand by the breaking down of

$$(C_3H_6O_3) = CO_2 + C_2H_5OH \qquad (\delta)$$

to alcohol and carbonic acid. However, it is amazing that this particular reactive form is ascribed to the hypothetical triose although the process in the 3-carbon series seems explained and still more because the apparent and experimentally established interpretation of the transition to the 3-carbon series is lacking.

mase. The complex zymases capable of bringing about fermentation are composed, then, of the sum of the coferments plus apozymase, hence the former is one part of an activated system of ferments.

Of fundamental significance is Meyerhof's observation that the co-enzyme of alcoholic fermentations occurs in the muscles and organs of animals as well as in milk. He also showed that aqueous extracts of animal muscles and germinating plants strongly furthered alcoholic fermentation. This seems to be entirely in accord with the earlier observation of Kostytschew, Hübbenet, and Scheloumow that yeast extract increases the normal respiration of plants. Meyerhof, on this basis, suggested that the same co-ferments are necessary for the normal respiration of animal tissue as for yeast fermentation, i.e., it was supposed that hexosephosphate was just as indispensible for the fermentative oxygen respiration of animals as for the fermentative alcoholic fermentation. Although Scandinavian investigators have concentrated the coferment by precipitation on tannin, phosphotungstic, and silicotungstic acids, the character of the systems remains poorly known. Among all the different interpretations there is to be noted the result of Meyerhof, who suggested the probability that between fermentation and respiration there exists a connection. This view can be shown schematically, according to Kostytschew (l.c.) as follows:



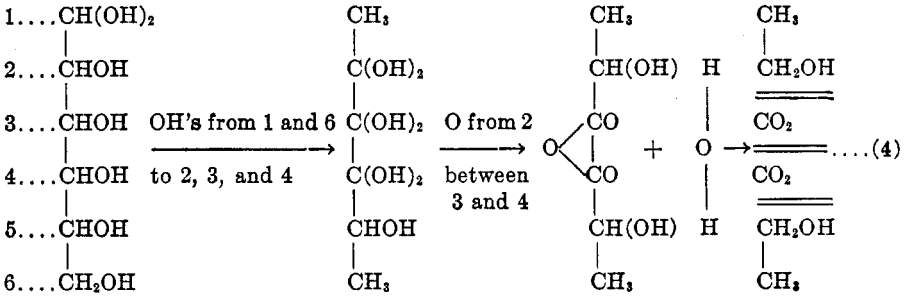
and it is finally based on the classical hypothesis of Palladin according to which the oxidation and reduction ferments of vegetable tissue transform the primary products of sugar split-

ting, which have been previously formed by the enzymes of fermentation, to the end-products.

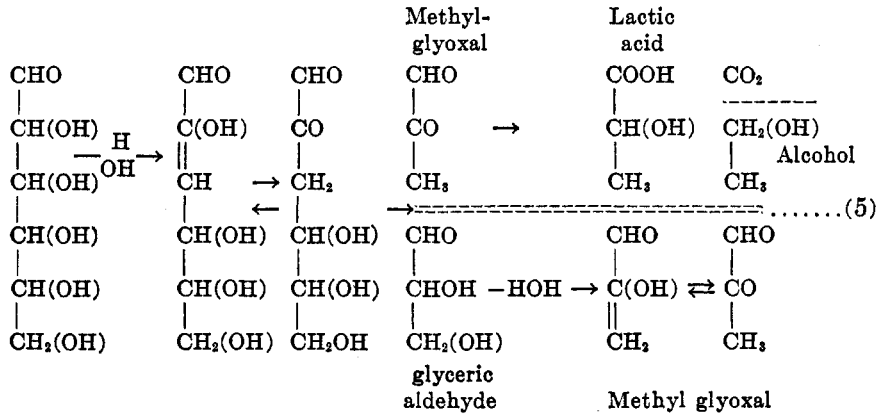
In view of the present confusion in the field of the "kinases" we are unable to state definitely whether the systems in question represent a real auxiliary catalyzer, although v. Szent-Gyoergyi, for example, has stated that he was able to replace co-ferments by definite substances (p-phenylenediamine). (Compare also page 57.)

4. THE TRANSFORMATION *into* THE THREE CARBON CHAIN SERIES

In 1870 v. Baeyer expressed the assumption that in the course of sugar decomposition the splitting out of water in one place in the sugar molecule and the addition of the same in another plays an important rôle. If one further assumes an oxygen migration from the end to the middle of the molecule, one can derive a schematic way of showing the formation of lactic acid as well as alcohol and CO_2:



From considerations based on investigations carried out by Wohl and Österlin, Baeyer's assumed displacement of oxygen from the end to the middle of the chain could no longer be considered the thing that takes place in fermentative reactions. The central idea of the scheme proposed by Wohl in 1904—in its characteristic features it is still considered valid—rested on the fact that in hydroxy compounds there can readily be a splitting out of water.



(5)

The six carbon compound which is believed to be ready for decomposition in this scheme is the methyl glyoxal-glyceric aldehyde aldol, which would form, through an easy hydrolytic splitting, two fragments of three carbons each, viz., methyl glyoxal and glyceric aldehyde. As an illustration of the relationship it may be recalled that Pinkus observed the formation of methyl glyoxal when glucose was warmed with caustic potash, and Wohl (l. c.) obtained it from glyceric aldehyde itself.

v. Lebedew and Griaznoff made another deduction in which they take the stand that the hexose molecule decomposes into glyceric aldehyde and dihydroxyacetone. In the further course of the change, it is considered that the glyceric aldehyde forms the easily fermented pyruvic acid while the dihydroxy acetone forms a hexose diphosphate:

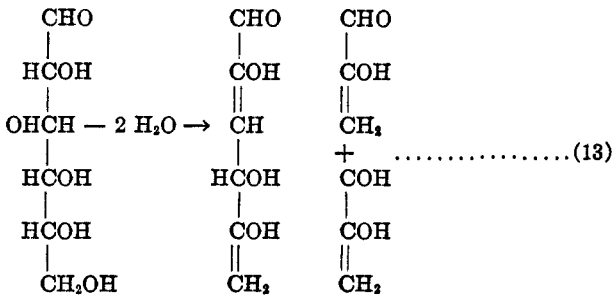
$$CH_2OH\cdot(CHOH)_4\cdot CHO = CH_2OH\cdot CHOH\cdot CHO + CH_2OH\cdot CO\cdot CH_2OH \quad (6)$$
$$CH_2OH\cdot CHOH\cdot CHO = CH_3\cdot CO\cdot COOH + H_2 \quad (7)$$
$$CH_3\cdot CO\cdot COOH = CH_3\cdot CHO + CO_2 \quad (8)$$
$$CH_3\cdot CHO + H_2 = CH_3CH_2OH \quad (9)$$
$$CH_2OH\cdot CO\cdot CH_2OH + RH_2PO_4 = CH_2OH\cdot CO\cdot CH_2\cdot O\cdot PHRO_3 + H_2O \quad (10)$$
$$2\ C_3H_5O_2\cdot O\cdot PHRO_3 = C_6H_{10}O_4(O\cdot PHRO_3)_2 \quad (11)$$
$$C_6H_{10}O_4(O\cdot PHRO_3)_2 + 2H_2O = C_6H_{12}O_6 + 2\ RHPO_4 \quad (12)$$

For the time, however, a real objection can be raised as to the correctness of both conceptions. One has a right to assume that if glyceric aldehyde, dihydroxyacetone, or methyl glyoxal are

really the intermediate products of alcoholic fermentation, at least one of these substances must be fermentable. In spite of the work of such well known investigators as Buchner, Emmerling, Färber, H. O. L. Fischer, Harden, Levite, Neuberg, Schwenk, Slator, Young and others, the uncertainty concerning this question is not removed and lacking clear, valid proof of their fermentability, we are forced to draw also on other compounds of the three carbon series to explain the intermediate steps in the fermentative decomposition of sugar.

Supported by the discovery of Fernbach which was later confirmed by Aubel, viz., that methyl glyoxal could be demonstrated in the carbohydrate splitting by bacteria, Neuberg and Kerb have recommended that the hypothetical formation of methylglyoxal-glyceric aldehyde aldol be replaced in the Wohl scheme by that of methylglyoxal-aldol. The split products of the former compound have been shown, as yet, through biological means, only partly to fit into the deductions of the fermentation reactions.

For avoidance of the glyceric aldehyde, this change makes possible, in the transformation into the three carbon chain series, the assumption of the formation of two mols of methyl glyoxal from each mol of glucose:



If we once more consider that the acid corresponding to methyl glyoxal is pyruvic acid,

$$CH_3COCOH \rightarrow CH_3COCOOH,$$

and that the relation of this acid to glucose, not only chemically

but also in its ease of fermentation, is established, then we are in a position, when we consider the combination of this assumption with the important observations by Battelli and Stern, Embden and Baldes, Trillat and Sauton, Wieland and Kostytschew (according to whom aldehydes can undergo fermentatively the Cannizzaro reaction), to find a way for explaining the formation of the end products of alcoholic fermentation.

5. THE ORIGIN OF THE END PRODUCTS

At the beginning of this century there was a hypothesis expressed and an investigation reported which have influenced up to the present and in undiminished strength the development of the chemistry of fermentation. The hypothesis and research developed entirely independently of each other. In the course of his fundamental researches concerning metabolism, Magnus-Levy expressed on March 14, 1902, the view that acetaldehyde is a split product of carbohydrate, and on November 1, 1920, O. Neubauer, in connection with the comprehensive investigations by Neubauer and Fromherz concerning the decomposition of amino acids by yeast fermentation, made in the form of an unpretentious note the following communication:

> Weiter ist zu schliessen, dass die hier als Zwischenprodukt auftretende Brenztraubensaeure durch gaerende Hefe unter Reduktion zu Kohlensaeure und Alkohol zersetzt wird, d. h. mit anderen Worten, dass sie leicht vergaerbar sein muss. Eigens angestellte Versuche, die noch nicht voellig abgeschlossen sind, haben die Richtigkeit dieses Schlusses bestaetigt. Damit ist nun ohne weiteres der Gedanke gegeben, die Brenztraubensaeure koennte ein Zwischenprodukt bei der alkoholischen Gaerung des Zuckers sein.

Later Neuberg and Karczag were able to prove the principal part of this communication by showing that the fermentation of pyruvic acid proceeded according to the simple equation:

$$CH_3COCOOH = CH_3COH + CO_2 \quad (14)$$

This reaction is brought about by the enzyme carboxylase which occurs in all zymases and takes place with no giving up of free

energy. It represents a typical case of an enzymatic breaking down of a carbon chain.

The zymases necessary for the carrying out of this splitting are no simple ferments but enzyme complexes in which the pyruvic acid splitting ferment, the carboxylase, occurs. The pyruvic acid, however, is, on the contrary, potentially represented in glucose or other fermentable carbohydrates. Through the important fact that carboxylase can break a carbon chain, this ferment assumes a special place among the known enzymes. In the fermentation of pyruvic acid, i.e., in the case of a non-sugar undergoing by means of yeast a characteristic change, we see the first case of a sugar-free yeast fermentation.

The essence of this process is that the carboxylase splits out CO_2 from α-keto acids and α-keto dicarboxyllic acids, leaving behind as a residue the aldehyde of the next lower series. This aldehyde is left in an unusually reactive form.

But through the circumstance that pyruvic acid not only stands in many close relationships to glucose (see page 54 and below), but also, for example, is the α-keto acid corresponding to alanine (as ketoglycerolacid to serine), there is some indication that carboxylase may have a predominant place among the proteolytic enzymes since we know from experiments of Neubauer and Fromherz mentioned above, that the amino-acids go over, in general, intermediately into the corresponding ketonic acids:

$$R \cdot CHNH_2 \cdot COOH \rightarrow R \cdot CO \cdot COOH \quad \ldots\ldots\ldots\ldots\ldots\ldots (15)$$

by splitting of the amino groups. The ketonic acids in turn by alcoholic fermentation liberate similar alcohols which according to Ehrlich are produced by the fermentation of amino-acids only by living yeast and in the presence of a large amount of sugar:

$$R \cdot CH(NH_2) \cdot COOH + H_2O = R \cdot CH_2(OH) + NH_3 + CO_2 \quad \ldots\ldots (16)$$

But it remains undecided, whether in this process the cleavage of the NH_2-group takes place first and then the α-Keto acid is formed, or whether in the sense of the models of Wieland and Bergel the amino group is converted to imine and later, having

lost carbondioxyde, the hydrolytic separation of the $<$NH-group

$$CH_3\cdot\underset{\displaystyle NH_2}{\underset{|}{CH}}\cdot COOH \rightarrow CH_3\cdot\underset{\displaystyle NH}{\underset{\|}{C}}\cdot COOH \rightarrow CH_3\cdot\underset{\displaystyle NH}{\underset{\|}{CH}} + CO_2 \rightarrow CH_3\cdot\underset{\displaystyle O}{\underset{\|}{CH}} + NH_3 \qquad (17)$$

follows.

We must further consider that the transformation of pyruvic acid to lactic acid produces an increased hydrogen ion concentration (since the dissociation of pyruvic acid is 3.6×10^{-13}, that of lactic acids 1.38×10^{-4}). The fermentation of salts of pyruvic acid, on the other hand, brings about a change of the hydroxyl ion concentration, so, according to Neuberg, there can be no exclusion for the assumption that the carboxylase, through the separation of CO_2, executes the function of regulating the reaction of the fermentation medium.

The connecting rôle of α-keto acids, which represent common ground between proteins and sugar, manifests itself also in the phenomenon that these acids serve as stimulators in alcoholic fermentation. This is believed to have been proved also by experiments which aroused the impression that the co-enzyme of yeast, in the presence of potassium phosphate, can be replaced by a mixture of different α-keto acids such as occur in yeast protein. Meyerhof was not able to confirm this statement using pyruvic acid. (Compare also v. Szent-Gyoergyi, l. c.)

The fermentability of pyruvic acid conditioned by its relative nonpoisonous nature, or the properties of the above mentioned decomposition products, constitutes the unrestrained confirmation of Neubauer's assumption and experiments that this acid is an intermediate product in alcoholic fermentation, or a split product of the sugar molecule, for, in contrast to its ability to withstand the action of a temperature up to 165° and of concentrated sulfuric acid up to 150°, in the presence of various yeasts, it is, in a few minutes, split (70 to 80 per cent) into CO_2 and acetaldehyde.

But apart from the biological and earlier (page 53) discussed chemical connections, there is also a hint of the genetic connection

to the glucose in that it may be formed endothermically by the oxidation of lactic acid,

$$CH_3CHOHCOOH \rightarrow CH_3COCOOH \quad \ldots\ldots\ldots\ldots\ldots\ldots (18)$$

or by the dehydration of glyceric acid

$$CH_2OHCHOHCOOH \rightarrow CH_2{:}C(OH)\cdot COOH \quad \ldots\ldots\ldots\ldots\ldots (19)$$

In the above given scheme of decomposition we became acquainted with the compounds in which carbonyl and ethylidene radicals are represented so that their origin is withdrawn from "paper chemistry" considerations, and is placed within the range of experimental proof.

The connection between the assumption of Magnus-Levy and the discovery by Neubauer, or Neuberg and Karczag, is therefore clear at first sight, and in the case of the fermentation process it can be established that the proof of the origin of CO_2 is given. Biologically speaking, it is the same whether acetaldehyde and CO_2 occur as such or are held fast in the form of their combination, i.e., pyruvic acid. (Compare section 6.)

Not as simple is the explanation of the formation of alcohol. It has been shown by Battelli and Stern (l. c.) and by Parnas that there occurs in animal tissues a ferment which acts upon two mols of an aldehyde with the taking up of water to give a Cannizzaro reaction, i.e., the aldehyde is rearranged to equal molecular amounts of alcohol and acid. An analogous observation was later made, also, by Kostytschew (l. c.) in the case of the action of yeast on acetaldehyde. But a Cannizzaro reaction on the acetaldehyde formed in the decomposition of pyruvic acid gives ethyl alcohol itself.

By means of a suitable arrangement of the above mentioned discoveries, and taking the sugar from the decomposition of hexose diphosphate to be in an especially reactive form (see page 49) the earlier discussed scheme of Wohl (see page 53) could be completed in the following way:

$$C_6H_{12}O_6 - 2\ H_2O = C_6H_8O_4 \text{ (methylglyoxalaldol)} \quad \ldots\ldots\ldots\ldots (20)$$

$$C_6H_8O_4 = 2\ CH_2{:}C(OH)\cdot CHO \text{ or } 2\ CH_3\cdot CO\cdot COH \text{ (methylglyoxal)} \quad \ldots (21)$$

$$CH_2{:}C(OH)COH + \begin{matrix} H_2 \\ | \\ O \end{matrix} + H_2O = CH_2OHCHOHCH_2OH \text{ (glycerol)} + CH_2{:}C(OH)\cdot COOH \text{ (pyruvic acid)} \quad (22)(23)$$

$$CH_3COCOOH = CO_2 + CH_3COH \text{ (acetaldehyde)} \quad (24)$$

$$\begin{matrix} CH_3COCOH \\ + \\ CH_3COH \end{matrix} \begin{matrix} O \\ | \\ H_2 \end{matrix} = \begin{matrix} CH_3COCOOH \text{ (pyruvic acid)} \\ + \\ CH_3CH_2OH \text{ (ethylalcohol)} \end{matrix} \quad (25)$$

Omitting processes experimentally not clear at present, processes which are concerned in the formation of the three carbon chain series, methylglyoxal is considered the first product having three carbon atoms in the molecule. It may be formed after the splitting out of two molecules of water from the hexose molecule, which requires perhaps the intermediary existence of methyl glyoxal aldol. All further processes then are brought about through repeated Cannizzaro rearrangements on methyl glyoxal. In case of the pyruvic acid first formed, the addition of water gives glycerin. If we assume as isochronous the formation of acetaldehyde and CO_2 by the action of carboxylase on the pyruvic acid, then a Cannizzaro reaction must take place between two *different* aldehydes. The result of this phenomenon is the formation of ethyl alcohol on the one hand and the re-formation of pyruvic acid on the other. From the latter the carboxylase always produces new CO_2 and acetaldehyde. We see, therefore, an uninterrupted formation and decomposition of pyruvic acid, which, just as methyl glyoxal, can not then be accumulated. After this restless conversion naturally some acetaldehyde would be left over. This assumption is in harmony with the fact that in fermentations there are always detectable traces of acetaldehyde equivalent to some 0.15 per cent of the raw materials used.

One has the impression that the above mentioned facts may also be supported by the important findings of Henri and Fromageot. These investigators have shown, by measuring the absorption spectra of pyruvic acid that the quantitative relations of the keto and enol forms of this acid, under conditions of concentration that approach closely to those of biological processes are strongly dependent upon the hydrogen ion concentration.

The much more reactive enol-form

$$\begin{array}{l} H_2C{:}C\cdot COOH \\ \quad\quad | \\ \quad\quad OH \end{array}$$

could thus also be responsible for the fermentability of the acid in normal fermentation media.

But, when one considers the above statements in connection with the repeated assertion that the velocity of fermentation of pyruvic acid, at ordinary temperature can several times exceed that of glucose, which has been deduced from experiments carried out mainly in the presence of salts of sulphurous acid, in which the hydrogen ion concentration was not measured, one will not be able to rid himself of a certain feeling of uncertainty as to the actual rôle of pyruvic acid in these processes.

Before we enter into the discussion of the evidences upon which the experimental basis of this scheme rests (see section 6), let us first inspect the instances in which critical consideration could see further difficulties.

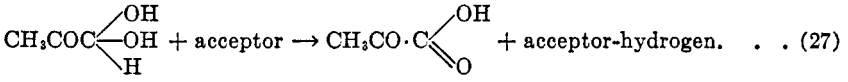
It is quite true that the fermentability of methylglyoxal is not as yet demonstrated, but nevertheless this circumstance can not speak conclusively against the assumption that the compound can be an intermediate in alcoholic fermentation, since Neuberg and Hirsch suppose that it shows a surprising number of different isomeric forms, which might have a different behavior when acted upon by yeast. It should, moreover, be mentioned that Dakin and Dudley, even as Neuberg, state that they have found in yeast, muscle and other animal organs, an enzyme which brings about an internal Cannizzaro reaction:

$$CH_3COCOH + HOH \rightleftharpoons CH_3CHOHCOOH \quad \ldots\ldots\ldots\ldots\ldots (26)$$

thereby forming lactic acid, which appears, as is known, in the case of fermentation of sugar by yeast juice. Therefore, there are chemical (see page 54) and biological grounds which do not exclude the right for assuming the intermediate appearance of methylglyoxal.

In some communications worthy of note but not yet adequately backed by experiment, Kluyver and Donker have recently turned

against the idea of a mixed Cannizzaro reaction. The essential thing in their theory, in which they attempted to apply Wieland's theory of dehydrogenation in processes of aerobic respiration to anaerobic dissimilation is that all parts in the process of sugar decomposition are none other than coupled dehydrogenation-hydrogenation reactions. Correspondingly, alcohol would be formed from a dehydrogenation of methylglyoxal hydrate through passage of the hydrogen to the acetaldehyde.[5]



They state: "On the basis of these facts it is evident that there exists no reason whatever to have recourse in certain cases to the narrower explanation offerred by a Cannizzaro transformation."

We shall see later that these explanations are automatically weakened by experiment (section 6, paragraph 2).

6. EVIDENCES

Dumas early showed that alcoholic fermentation can proceed in the presence of various alkali salts, and in 1874 he hinted the correctness of the possibility of influencing the alcoholic sugar decomposition through the presence of alkali sulfites. Later Mueller-Thurgau and Osterwalder observed that in the case of fruit juices or fermenting sugar solutions, added sulfurous acid instantly combined with the—as they correctly assumed—acetaldehyde present. It was obvious that this compound might be the acetaldehyde-sulfurous acid of Ripper (1892), of which the sodium salt, known since the time of Bunte (1873), is so important for our following discussion.

In the course of extensive investigations concerning the hydrolytic splitting of bound sulfurous acid, Kerp and Laudon have

[5] Compare also A. Lebedew, Bull. soc. chim. France, [4] **11/12**, 1040, (1912).

established that the dissociation constant of acetaldehyde sodium bisulfite (2.84×10^{-6}) to the corresponding glucose compound (311×10^{-3}) was an average of about 1:90,000.

Proceeding from the knowledge of the above mentioned facts and from the desire to increase as much as possible the yield of glycerol in alcoholic fermentation (see page 59), Connstein and Luedecke began pertinent experiments in 1914. The ordinary fermentation of sugar takes place always either in neutral or slightly acid solution. Should, however, the possibility exist of removing the acetaldehyde formed (equation 24) as Bunte's compound, it was to be expected that in the case of an appropriate method of fermentation or experimental conditions, not inconsiderable amounts of glycerol could be obtained.

The first investigations were made with various alkaline substances as disodium phosphate, sodium acetate, sodium bicarbonate, ammonium carbonate, etc. However, the alkaline mashes brought to light a disagreeable characteristic in a short time, viz., these alkaline mashes formed an excellent nutrient medium for all possible acid forming bacteria, especially lactic acid bacteria. These acid forming bacteria consumed not only the greater part of the sugar, but also made the glycerin so impure that it was very difficult to purify. Then were begun the investigations of the manufacture on a large scale with disodium sulfite, which salt the yeast tolerated in increased quantities, and, when added in larger amounts to goods to be fermented, exerted a pronounced antiseptic action.

A. With just a hint as to the complete explanation found elsewhere, the mention of technical investigations in this case has significance. Here is displayed a not frequent case where the practical workers have very fruitfully and stimulatingly influenced theoretical research in this sphere. Chemists in the United States (Eoff, Lindner and Bayer), Germany, England (Cocking and Lilly), the former Austria-Hungary, Switzerland (Schweizer), and Japan (Tomoda), vied with each other in the endeavor to solve the problem, and it remains to the merit of Faerber, Hirsch, Neuberg, Reinfurth and Ursum on the one

side and Pollak, Reik, and Zerner, on the other, to have produced a correct interpretation and a part of the proof for the scheme given on page 58.

Starting out from the long opposed observation of Fernbach and Schoen, that pyruvic acid occurs among the products of alcoholic sugar decomposition, it could be proved that if one carries out the fermentation in the presence of the weakly alkaline alkali sulfites, the theoretically possible amounts of acetaldehyde and glycerin are obtained. The already mentioned aldehyde-bisulfite addition compound, $CH_3 \cdot CHOH \cdot O \cdot SO_2Na$, concentrates itself in the mashes and can be separated. The corresponding hexose compound in the presence of water is practically completely dissociated. Compared with ethyl alcohol, acetaldehyde is considered as an oxidation product, and hence the presence of a reducing compound is to be expected. This assumption really proved correct, since the hydrogen atoms, which can not act upon the acetaldehyde because of its formation of a compound with Na_2SO_3, make possible the formation of glycerol by acting upon another half molecule of sugar:

$$2\,C_6H_{12}O_6 + Na_2SO_3 + H_2O = CH_3CHOH \cdot O \cdot SO_2Na + NaHCO_3 + C_3H_8O_3 \quad (29)$$

Detached from the binding to the alkali salt, the equation takes the form:

$$C_6H_{12}O_6 = CH_3CHO + CO_2 + CH_2OH \cdot CHOH \cdot CH_2OH \quad (30)$$

and according to the curve in figure 2 (Biochem. Z. **98**, 153) the ratio between glycerin and acetaldehyde at any given time a constant.

It is quite worthy of note that the blocking of the acetaldehyde can be brought about not only in a chemical way but also, as Abderhalden, Glaubach and Stix showed, through adsorption. They have proven that the above reactions can be made to proceed in an approximate manner in the presence of animal charcoal.

If the fermentation be carried out in the presence of simple alkali salts which, in contrast to the bisulfites, have no specifi-

cation on the acetaldehyde formed, only traces of the latter can be found. It undergoes a Cannizzaro transformation and can be found again also in molecular proportion to glycerol, but as acetic acid and ethyl alcohol. The proportions can be expressed in the following equation:

$$2\,C_6H_{12}O_6 + H_2O = C_2H_5OH + CH_3COOH + 2\,CO_2 + 2\,CH_2OHCHOHCH_2OH\,. \quad (31)$$

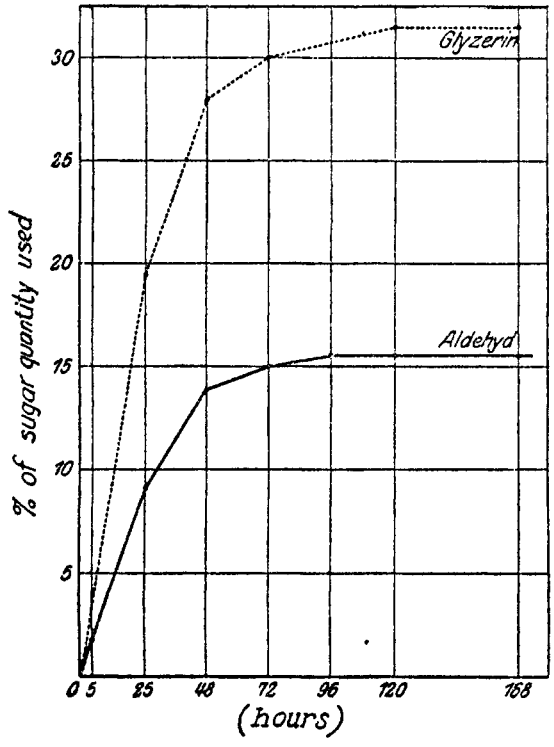


FIG. 2

and show that for every mol of acetic acid there are always two mols of glycerol.

On the basis of the above facts, v. Grab working with C. Neuberg, could confirm the statements of Fernbach and Schoen by carrying out a Doebner synthesis of α-methyl-β-naphto-cinchonic

acid in a cell-free fermentation of sugar. This was done by the reaction of β-naphthylamine with pyruvic acid:

$$CH_3 \cdot CO \cdot COOH + CH_3 \cdot CHO + C_{10}H_7NH_2 = C_{15}H_{11}N + 2\ H_2O + H_2 \quad (32)$$

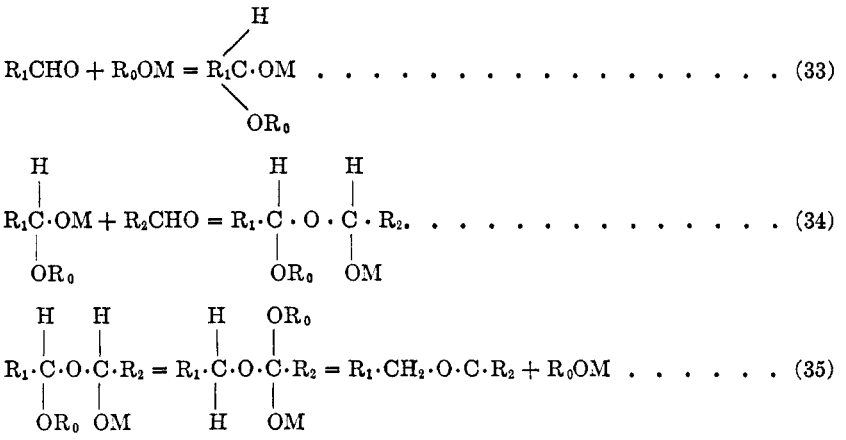
On the other hand Aubel and Salabartan showed that pyruvic acid was an intermediate product in the Coli fermentation of glucose (see below). When we take all these things into consideration this part of the demonstration may be considered closed although Lebedew expressed doubt about the data produced by v. Grab.

B. After the above discussion (not mentioning equations 20 and 21 on page 58) there remains in doubt only the correctness of the assumption of a Cannizzaro reaction between two *different* aldehydes. Several investigators, indeed, have pointed out the necessity of proof to strengthen the assumptions involved.

During the last years extensive investigations for showing this reaction were taken up by Endoh, Nakai, Nord, and others, and in 1921 v. Grab, stating (l. c., page 71): " . . . . durch den von Nord erbrachten Nachweis, dass in der Tat eine gemischte Dismutation zwischen ungleichen Aldehyden der aliphatischen Reihe moeglich ist," was able to rely upon the feasibility, as founded on certain principles, of the assumption of a mixed Cannizzaro reaction; afterward the preliminary work of the Lieben school did not clear up the question.

Meanwhile these catalytically influenced model investigations have been completed, and we now know the following examples: acetaldehyde plus isovaleric aldehyde, isovaleric aldehyde plus benzaldehyde, acetaldehyde plus benzaldehyde, acetaldehyde plus furfural, furfural plus isobutyric aldehyde, acetaldehyde plus chloral, acetaldehyde plus bromal, acetaldehyde plus cinnamic aldehyde. Indeed, the reaction can take place with the most different kinds of aldehydes and we were authorized already by the indications of Verley to assume that this reaction might also occur with mixtures of aldehydes *and* ketones.

Simultaneously, the investigations have brought two other conclusions to maturity. (*a*) Through the formulation:



which is based upon the work of Wieland or Lachmann, it is probable that an *isochronic* rearrangement of dialkyl ethers precedes the formation of mixed esters. (*b*) By the application of the method to single aldehydes, several alcohols of importance in physiological investigations could readily be made accessible.

7. REDUCTIONS AND SYNTHESES IN THE COURSE OF FERMENTATIONS

A. Of the biochemical processes found in nature, the reduction processes are of greatest interest because they are the most difficult to interpret. We see, that beside the deoxidation of CO_2 in assimilation processes, plants bring about the greatest hydrogenation performance in the synthesis of protein from sugar and ammonia, as well as in many other processes. One has an object worthy of study in the reductions done by yeast cells. Since these reductions, because of lack of disposable hydrogen, can be brought about only in roundabout ways—one often thinks of so-called coupled or induced reactions—the question is often asked, from where does the energy for the reduction processes come? A whole series of biological experiences assign a connection between reductions and the oxidative decomposition of car-

bohydrates, and especially the processes of fermentation which have been discussed appear as an alternate oxidation and reduction of the sugar molecule.

In continuation of the basic observation of Lintner and v. Liebig concerning the reduction of furfural by yeasts in alcoholic fermentation, the reductive action of yeast on other aldehydes was made the object of further work and it could be shown that this characteristic is so marked that the yeast could even act upon substances foreign to their organism. Experiments of "phytochemical" reductions made with various nitro compound showed the formation of the amino compounds by way of the corresponding intermediate compounds and in the case of o-nitrobenzaldehyde, for example, it could be shown that the yeast could also act selectively. This is in harmony with the experiences of Nord on the catalytic hydrogenation in steps.

Several years ago it was stated in a lecture that the reducing power of yeast could also act on ketones. The reaction takes place as with methylheptenone in both the aliphatic and aromatic series, and as it is an assymetric one, it furnishes a method of obtaining optically active secondary alcohols which would otherwise be difficult to obtain. Diketones undergo the same change and give glycols. Examples of this class which have been studied are diacetyl and benzil. It is definitely proven that these reductions are not purely chemical in nature, but are catalyzed by ferments, by the fact that if one uses a racemic aldehyde, e.g., racemic valeric aldehyde, one gets optically active amylalcohol, or, if one begins with diacetyl, one gets optically active butylene glycol.

The alcohols in nature result from the corresponding carbonyl compounds, i.e., from aldehydes and ketones. As for the thioalcohols, the mercaptans, the possibility of their origin from the thioaldehydes could also be substantiated. For this purpose one uses in the phytochemical reduction of the thioaldehydes their ammonia derivatives, the thialdines. In this case it must be evident that the origin of these compounds in nature appears entirely possible, as ammonium salts are available everywhere.

In the case of ethyl mercaptan, however, it could be shown that the formation of the mercaptan is purely an enzymatic process, as it can be brought about in cell-free fermentations by use of yeast juice.

In the course of these investigations there is acquiescence to the idea that the reduction of various compounds has a relation to the simultaneous course of the processes of alcoholic sugar decomposition, pointing to the possibility that in ultimate analysis the "hydrogen" used in reductions is fermentation "hydrogen," which, naturally, is not evolved in the free state and therefore requires an acceptor.[6]

Since the course of sugar decomposition seems to involve pyruvic acid and acetaldehyde as intermediate steps, and since the former is an oxidation product of half a sugar molecule, it is necessary, if there is to be any change in this oxidation product, for an equivalent reduction process to occur simultaneously.

Normally the acceptor necessary is formed through the further splitting of the pyruvic acid, occurring in the form of acetaldehyde which takes up the "hydrogen" and passes over to alcohol. If this representation is correct, other hydrogen acceptors, i.e., phytochemically reducible substances, must work in a manner analogous to acetaldehyde.

But the fact of the reduction of many compounds in itself says nothing concerning the course of the process. The yield of the reduction product only teaches that no process like a Cannizzaro rearrangement can come into play, not mentioning that simple ketones and nitro compounds are not in general capable of a transmutation. We are inclined, then, to draw the fact into consideration that it has been possible to demonstrate acetaldehyde in such biochemical fermentations. In singular cases the acetaldehyde has been found in amounts almost equivalent to the reduction product, so, one receives the conception that here indeed the added reducible compound has become the acceptor

[6] Compare in the case of sulfur the interesting statement of A. Hottinger (Schweiz. med. Wchnschr., **53**, 430, (1923)).

for the fermentation hydrogen instead of the acetaldehyde and, in agreement with that, has pushed aside out of the reaction an equivalent amount of acetaldehyde.

It is interesting to note in this connection the fine demonstration of the origin of acetylmethyl carbinol and 2, 3-butylene glycol, corresponding to the earlier results of Harden, Walpole, and Norris, by Kluyver and Donker, in the fermentation of glucose in the presence of methylene-blue or sulfur. The products of decomposition occurring in the fermentation of fructose have, in this sense, also the effect of a hydrogen acceptor.

If with these authors, we include oxygen in this sphere, the aforementioned oxidation phenomena would in addition to this take on a very original and attractive interpretation (see page 43).

But we must remember also that yeast co-ferment can pretendedly be replaced by a mixture of various α-keto acids (see page 57) (but apparently not by insulin). According to Kendall, thyroxin acts as a catalyst which exercises its action by being alternately oxidized and reduced; that is, it acts simultaneously as donator and acceptor.[7] Should it be found that thyroxin and the bios isolated from autolized yeast by Eddy, Kerr, and Williams, are joined in relationship on the basis suggested by Kendall that the active group in both these (and other) compounds is $\overset{\ulcorner\urcorner}{\text{C-C-N-}}$ then perhaps further investigations may put us in a position to seek in this direction for an explanation of the reductive processes in yeasts.

B. Reference has already been made (see page 56) to the fact that zymases are not uniform, but constitutes enzyme complexes in which various tasks fall to the different parts. It is interesting to note, as ways of checking up this opinion increase in number, the things that can be drawn up as confirmation for this assumption. In the fundamental investigations of Lintner and v. Liebig the observation was made that a considerable part of the furfural added to a fermenting sugar solution was not reduced

[7] Compare also F. Knoop and H. Oesterlin, Z. physiol. Chem., **148**, 301, 302, (1925).

in the way described above, but apparently underwent a condensation by the acetaldehyde which appeared, as an intermediary. In continued investigations they showed that the by-product was in reality the result of a direct carbon chain linkage, C-C, and, since the compound called by them furyltrimethylene-glycol, $C_4H_3O \cdot CHOH \cdot CH_2CH_2OH$, was optically active, there could be no doubt that it was the result of fermentative action. In contrast to the ordinary process of biochemical breaking down of molecules, which we regard as the function of fermentation, here we see established the first case of a carbon chain synthesis or the carboligation action of a ferment, hitherto recognized only in its performances.

If one replaces the furfural with benzaldehyde, optically active phenyl acetyl carbinol, (α-Phenyl-pryuvic alcohol) $C_6H_5C^*HOH \cdot COCH_3$, is formed, and, in the fermentation of pyruvic acid alone there occurs according to Hirsch, in addition to acetaldehyde, methylacetyl carbinol (Acetoin)

$$CH_3 \cdot CHO + OHC \cdot CH_3 = CH_3C^*HOHCOCH_3 \ldots\ldots\ldots\ldots (36)$$

All these reactions indicate that they can take place only with nascent acetaldehyde. Neuberg and v. May could also show this by fixing the acetaldehyde by sulfite whereby they obtained a total suppression of the carboligation action. But the process of carboligation itself is not connected with the aldol reaction and it is indicated in comparison to the Benzoin condensation as "Acyloinreaction."

There occurs therefore the conversion of an oxidative splitting process under raising of the potential into the direction of a fermentative resynthesis of carbohydrate, for in all these cases of transformations there occurs sugar or pyruvic acid which is being decomposed to give the necessary acetaldehyde for the carboligation effect.

But also, the view of Haehn that acetaldehyde forms also a fundamental ingredient of fats, is confirmed through the origin of higher fatty acids, when the organism, *endomyces vernalis* is used. In opposition to Smedley Maclean and Hoffert, Haehn and Kintoff have proved that the building up of the fatty acid

* C indicates an assymetric C-Atom.

molecule is accomplished in two steps, the first by the decomposition of glucose to acetaldehyde through the action of the zymases, the second by the action of the synthesizing enzymes on this aldehyde. It is impossible to state whether the pyruvic acid or lactic acid precedes acetaldehyde. The above mentioned fungus has shown itself able to assimilate both acids as well as the aldehyde.

8. BACTERIAL FERMENTATION

Not only in the case of alcoholic fermentation does acetaldehyde, as shown above, play an important rôle (by being one step in alcohol production) but also in cases of important bacterial fermentations in nature, the same is equally true. In order to show this, one permits the bacterial process concerned to proceed in the presence of a substance which will fix the expected aldehyde. For this purpose it has been shown that, in the case of bacterial fermentations, the neutral sulfites, as Na_2SO_3 and $CaSO_3$ can be used. They, in contrast with most of the bisulfites required for the fixation of aldehydes, cause little injury to the enzymatic and life processes, or in any case, do not stop them. The whole difficulty of this problem consists in this, that the expected intermediate products which are formed during the disintegration of sugar to its split products, acetaldehyde and alcohol, all show labile characteristics, and, collectively, must contain the carbonyl (-CO-) group. Because of that, the choice of appropriate means of intercepting the reaction is extraordinarily limited. The compounds used must combine already with the sugar to form a substance not readily acted upon biologically or else, under the conditions of the experiment, give no condensation product at all. This obstacle blocked all of the earlier steps attempted in this direction. The way out of this difficulty consists in selecting a compound which will intercept the reaction, one which has a fine gradation in its affinity for sugar and its different transformation products so that the least affinity is manifested for the initial material and the maximum for one of the later products of this change. (Compare Kerp and Laudon, page 61.) Such substances, as mentioned above, are the normal

sulfites. Sulfites give with sugar an addition product in which the components are exceedingly loosely bound, and which, in water solution are highly dissociated. On the other hand, the stability of the sulfite complex increases, down to acetaldehyde. As normal sulfites (M_2SO_3) are not poisonous for microörganisms, it is possible to carry out fermentation in their presence. This is different from free sulfurous acid (H_2SO_3) and bisulfites ($MHSO_3$) which, because of their disinfecting qualities, are used in the different industries based on fermentation.

By this method Neuberg and Nord were able to show that acetaldehyde occurred as an intermediate product in the fermentation of sugar, mannite, and glycerol, by means of the widespread kinds of coli and various pathogenic microörganisms.

The exchange of carbohydrates through means of these agents is, in nature, very great in scope. Their action extends not only to the true sugars but also to related substances as mannite and glycerol. The yeasts are relatively strongly constituted organisms which will stand the addition of a substance which takes up some substance formed in the course of the reaction. In the case of bacteria it is necessary to make a modification, which consists of using an alkali-earth sulfite (as $CaSO_3$) which gives a neutral reaction instead of the alkali metal sulfites which have a basic reaction. The former not only have the advantage of being neutral in reaction, but also are relatively insoluble in water. Because of that, one can exclude, in the case of sensitive microörganisms, detrimental osmotic processes. On the other hand, one obtains only a diminished sulfite-ion concentration which might be adjusted to a certain degree, through mechanical means. The presence of a sulfite is the only essential.

The aldehyde clearly is produced at the expense of the reaction which normally leads to ethyl alcohol or acetic acid production. Harden's investigation of the coli fermentation gave as the normal reaction:

$$2\,C_6H_{12}O_6 + H_2O = 2\,CH_3CHOHCOOH + 2\,CO_2 + 2\,H_2 + CH_3COOH + C_2H_5OH \quad (37)$$

In the presence of sulfite, aldehyde in the appreciable amount of

40 to 45 per cent of the quantity of alcohol concerned, was obtained.

But the aforementioned authors believe that the acetic acid and the alcohol are derived by a Cannizzaro reaction from acetaldehyde. If this is true the equation given above for the breaking up of the glucose by colon bacilli may be interpreted to mean that pyruvic acid and acetaldehyde come next. This gives the following equations:

$$C_6H_{12}O_6 = CH_3 \cdot CHOH \cdot COOH + CH_3 \cdot CO \cdot COOH + H_2 \quad (38)$$

$$CH_3CO \cdot COOH = CH_3CHO + CO_2 \quad (39)$$

$$2\,CH_3 \cdot CHO + H_2O = CH_3 \cdot CH_2OH + CH_3COOH \quad (40)$$

The similarity which has often been noted between fermentation of yeast and colon bacilli would be based on the fact that in the latter, also, that part of the sugar which does not change into lactic acid is decomposed by way of pyruvic acid and acetaldehyde. The reduction of acetaldehyde in the last phase does not take place in fermentation by colon bacilli because the hydrogenizing hydrogen is developed freely in the molecular state while at the same time the acetaldehyde primarily produced becomes a simple transformation into alcohol and acetic acid.

The recent results of de Graaff and LeFevre seem to coincide with this viewpoint. According to them the methylglyoxal is not decomposed by the bacteria of the colon-typhoid bacillus group by way of acetaldehyde. On the other hand, Neuberg and Gorr noted the effect of lactic acid bacteria (*B. coli*) on methylglyoxal and found that this unstable compound was stabilized to lactic acid, just as Aubel and Salabartan (l. c.) had noted in their observations.

The same information obtained with the coli bacteria was also obtained for the metabolic processes of two pathogenic microorganisms, viz., the dysentry bacillus and the gas gangrene bacteria. The known kinds of dysentry bacilli, the Flexner, Y, and Shiga-Kruse, all give acetaldehyde in cultivation in solutions of maltose, glycerol, and mannite. The causal organism of gas gangrene, which in the late world-war was so feared in wound infection, is likewise a powerful carbohydrate consumer. This

anaërobic organism related to the butyric acid bacteria grows in sugar solutions with the production of acetaldehyde. Since acetaldehyde is a violent poison for higher organisms, its occurrence amongst the products of metabolism of pathogenic microorganisms may also be of interest for the study of infectious diseases.

It can be shown without objection that the formation of acetaldehyde is an intermediate product also in the case of butyric fermentation by *B. butyricus* (Fitz) when the fermentation is carried out in the presence of Na_2SO_3. In place of butyl alcohol or butyric acid there is here formed ethyl alcohol and acetic acid in connection with the splitting processes, which, beside the gaseous decomposition products of sugar, could only lead to acetaldehyde production.

But it seems remarkable that also the fermentation of alcohol to acetic acid, which is technically used for acetic acid production, passes through the acetaldehyde stage:

$$CH_3 \cdot CH_2OH \xrightarrow{O} CH_3COH \xrightarrow{O} CH_3COOH \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (41)$$

If the fermentations by yeast and bacteria discussed up to this point deal with intramolecular processes, with the splitting of compounds in which the inner shifting of oxygen plays a part, then in the acetic fermentation there is displayed an oxidative process in which, beside the Cannizzaro rearrangement, atmospheric oxygen also takes part. But the acetic fermentation is closely related to sugar splitting not only through the fact that many organisms can form acetic acid from carbohydrates but also in this, that in the oxidation of alcohol the substance which is formed by a definite agency is acted upon by another organism. Exactly as in the case of the previously described fixation of acetaldehyde as an intermediate product, so also in the case of acetic fermentation, especially by *B. ascendens* and *B. pasteurianum*, aldehyde, as the sulfite complex, accumulates in quite appreciable quantities. From one-third to three-fourths of the weight of acetic acid which is formed under the circumstances of the experiment, was obtained as acetaldehyde.

It was experienced that the fixation of acetaldehyde results much more satisfactorily in the presence of $CaSO_3$ than in the presence of Na_2SO_3. The reason for this might be that the strongly alkaline alkali sulfite acts as a check on the agent causing the fermentation. Since the fixation of acetaldehyde can result only in accordance with an equilibrium—that in the case of acetic fermentation is displaced toward the side of dissociation—a part of the acetaldehyde is available for the normal process, i.e., for the further transformations. This can be obtained, however, according to the most recent results, not directly but under certain circumstances in the manner of a Cannizzaro reaction on the acetaldehyde. If acetic acid bacteria are given pure acetaldehyde or pyruvic acid they always produce from it equal molecular amounts of acetic acid and ethyl alcohol and this fact establishes a still closer parallelism between acetic fermentation and the alcoholic fermentation.

The appearance of acetaldehyde in the fermentation of sugar by *B. lactis aerogenes* or of pentoses by *B. acetoaethylicus* and others, likewise can also be demonstrated.

In this connection the recent work of Speakman is of interest. The work which he began in 1914–1915 interprets in a convincing manner the mechanism of the acetone fermentation. In using *B. acetoaethylicus* he was able to demonstrate that in the fermentation of glucose, maltose, or glycerol, pyruvic acid was the intermediate product which, by the way of acetaldehyde is the parent substance of the ethyl alcohol, as well as of the acetone.[8]

At the onset of the fermentation when practically no acetone is formed, the following formulas,

$$\begin{array}{ccccc} & CH_3\cdot CHOH\cdot COOH & & & \\ & \uparrow & & & \\ & +H_2 & & & \\ C_6H_{12}O_6 \rightarrow & CH_3\cdot CO\cdot COOH + H_2 & \rightarrow & CH_3\cdot CHO + CO_2 & \ldots\ldots\ldots (42) \\ & \downarrow & & \downarrow & \\ & +H_2O & & +H_2 & \\ & \downarrow & & \downarrow & \\ & CH_3\cdot COOH + H\cdot COOH & & CH_3\cdot CH_2OH & \end{array}$$

[8] Compare also Freiberg, G. W., Proc. Soc. Exp. Biol. and Med., **23**, 72 (1925).

and later when rapidity of the hydrogenation of the acetaldehyde is decreased in relation to its formation, the following scheme:

$$2\ CH_3\cdot CHO = CH_3\cdot CHOH\cdot CH_2\cdot CHO \quad \text{(aldol)} \quad (43)$$

$$CH_3\cdot CHOH\cdot CH_2\cdot CHO + \left|\begin{matrix} O \\ = \\ H_2 \end{matrix}\right. + CH_3\cdot CHOH\cdot CH_2COOH + CH_3\cdot CH_2OH \quad (44)$$

$$CH_3\cdot CHOH\cdot CH_2\cdot COOH + \left|\begin{matrix} O \\ = \\ H_2 \end{matrix}\right. + CH_3\cdot CO\cdot CH_2\cdot COOH + CH_3\cdot CH_3OH \quad (45)$$

$$CH_3\cdot CO\cdot CH_2\cdot COOH = CH_3\cdot CO\cdot CH_3 + CO_2 \quad (46)$$

explain the situation, as the alcohol: acetone proportion of 2.2 is established, beginning with about the fifth day. The same explanation, however, is attempted, as in the fermentation by yeast, to explain the processes through a joint reaction of different carbonyl compounds (see page 59).

This review might give the impression that a central position may be ascribed to acetaldehyde in the processes summarized above. This view is not in general contradicted by the present state of our conceptions, which are supported in part by experimental data. Nevertheless it must not be overlooked that the final answer concerning the rôle of the parent substance of this aldehyde—pyruvic acid—in the course of alcoholic fermentation itself can only be obtained if in further investigations additional attention is given to the influence of factors that can be determined by physico-chemical methods.

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