Biochemistry. — *"The function of phosphates in the dissimilation of the hexoses."* By A. J. KLUYVER and A. P. STRUYK.

(Communicated at the meeting of January 30, 1926).

§ 1. *Introduction.*

An experimental investigation on the so-called co-enzyme of alcoholic fermentation led us to a closer study of the numerous researches which have been made on the first phases of the dissimilation of the hexoses. In this connection it was particularly necessary to pay attention to the part phosphates play in these conversions.

It is to the fundamental study of HARDEN and YOUNG 1906 [1]), that we owe the insight that in alcoholic fermentation a close relation exists between the decomposition of the hexose molecule and a conversion of the mineral phosphates present in the fermentation medium into organic compounds. They showed that by the addition of mineral phosphates to a fermenting mixture of free zymase and sugar the rate of fermentation is noticeably increased, namely to such a degree that for every molecule of phosphate that passes into an organic compound one molecule of carbon dioxide and one molecule of alcohol are produced in excess.

These observations have been fully confirmed by the later investigations and a series of publications on the biochemical phosphorylation has been issued. In recent years the interest in this process has considerably increased namely after it was found that the phenomenon just mentioned is not at all limited to alcoholic fermentation of sugars. It were EMBDEN and LAQUER [2]) who demonstrated the formation of a hexosephosphoric ester in the sugar dissimilation in the muscular tissue of animals, whereas recently VIRTANEN [3]) conclusively proved that also in the sugar dissimilation by true lactic acid bacteria and propionic acid bacteria the production of a phosphoric ester occurs.

Notwithstanding the considerable amount of information that has already been gained on the biochemical phosphorylation, we still grope in the dark as to the meaning of this, at first sight rather surprising, process. In support of this we may just quote NEUBERG and KOBEL [4])

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[1]) A. HARDEN and W. J. YOUNG, Proc. Royal Soc. Ser. B. Vol. 77, p. 405, (1906).

[2]) G. EMBDEN und F. LAQUER, Zeitschr. f. physiol. Chemie Bd. 93, p. 94, (1914); ibid. Bd. 98, p. 181, (1917); ibid. Bd. 113, p. 1, (1921); G. EMBDEN und M. ZIMMERMANN, ibid. Bd. 141, p. 225, (1924).

[3]) A. I. VIRTANEN, Zeitschr. f. physiol. Chemie Bd. 138, p. 136, (1924); idem, Societas Scientiarum Fennica, Comment. Phys.-Math. II, 20, (1925).

[4]) C. NEUBERG und M. KOBEL, Biochem. Zeitschr. Bd. 166, p. 488, (1925).

who wrote in their only one month old publication: „Die Bedeutung der Phosphorylierung ist bis zum heutigen Tage nicht durchsichtig."

A closer examination of the abundantly available experimental material, together with our desire to look also at this part of the sugar dissimilation in the light of the newer ideas about the dissimilation processes in general, enabled us to design a scheme into which the different data fit and that fulfills the requirement of elucidating the whole of the observations.

Before exposing this scheme we will briefly describe how HARDEN and YOUNG interpreted the observed facts, interpretation which is almost generally accepted up to this day. [1])

§ 2. *Critical consideration of the prevailing theory.*

The fact that both YOUNG [2]) and VON LEBEDEW [3]), and also later investigators have succeeded in isolating a hexosediphosphoric ester from the fermenting mixture of free zymase, hexose and phosphate, together with the above mentioned quantitative relation between the production of alcohol and carbon dioxide and the disappearance of the free phosphates, led HARDEN and YOUNG [4]) to give the following equations for the course of alcoholic fermentation:

$$2C_6H_{12}O_6 + 2PO_4HR_2 = 2CO_2 + 2C_2H_6O + 2H_2O + C_6H_{10}O_4(PO_4R_2)_2 \quad \text{(I)}$$

$$C_6H_{10}O_4(PO_4R_2)_2 + 2H_2O = C_6H_{12}O_6 + 2PO_4HR_2 \quad \text{(II)}$$

As appears from these equations the two English investigators have formed the idea that simultaneously with the phosphoric esterification of one molecule of hexose, a second molecule of hexose is decomposed into carbon dioxide and alcohol. The produced hexosephosphoric ester, however, is decomposed in its turn and consequently the hexose then formed can anew take part in the first reaction. They base this view on the observation, that on the one hand the rate of fermentation falls to its original value when the added phosphate is bound, whereas on the other hand it has appeared, that against the end of the fermentation the added phosphate entirely returns into the fermentation medium.

It might be superfluous te demonstrate that this conception, which indeed explains in an acceptable way the observed course of the rate of fermentation, at closer examination proves to be highly unsatisfactory, because it is quite inexplicable, that the esterification of one molecule should cause a second molecule of hexose, which has not the least part

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[1]) MEYERHOF too gives this interpretation in his monograph: Chemical Dynamics of Life Phaenomena. Chicago, 1924.

[2]) W. J. YOUNG. Proc. Royal Soc. Ser. B. Vol. 81, p. 528, (1909); Biochem. Zeitschr. Bd. 33, p. 178, (1911).

[3]) A. VON LEBEDEW, Biochem. Zeitschr. Bd. 20, p. 114, (1909).

[4]) See for instance: A. HARDEN, Alcoholic Fermentation, 3rd Edition, London, 1923.

in this reaction, to be decomposed into carbon dioxide and alcohol. [1]) Moreover it is very unsatisfactory, that in this way the phosphorylation does not concur to the elucidation of alcoholic fermentation, but on the contrary the last is postulated as a condition for the esterification. The signification of the esterification remains in this way wrapped in mist. Finally the fact, that as yet it has not been explained why the isolated hexosediphosphoric ester is fermented at a perceptibly slower rate by zymase than free hexose in the same concentration, is an other strong evidence against the prevailing theory [2]).

§ 3. *Hypothesis on the function of phosphates in the sugar dissimilation.*

In drawing up a new scheme concerning the function of phosphates in the dissimilation of the hexoses, we have in the first place been guided by our desire to make the formation of phosphoric esters a logical link in the whole chain of transformations of the hexose molecule. As will appear later on, this aim was highly favoured by the recent investigations on the configuration of the hexoses.

The starting point of our hypothesis further was the incontestable fact, that the fermentative dissimilation of the hexoses does in all cases lead to the production of compounds of the C_3-series.

Considering the possibility of reducing the succeeding stages of dissimilation processes to a catalytic transference of hydrogen, which DONKER and one of us (Kl) [3]) exposed in a previous communication, it is highly probable, that the transformations of the C_6 compounds into C_3 compounds will also consist in an intramolecular oxydoreduction.

At that time we have refrained from giving a further explanation of the fact why exactly a H-atom of the fourth C-atom was activated by the acting protoplasm. We will now, however, submit this point to a closer examination.

It is self-evident to suppose, that the stated formation of a phosphoric ester does not accomplish the decomposition of the hexose in the mysterious way pointed out by HARDEN and YOUNG, but on the contrary in a far more direct way, on the understanding, that it is the very hexose molecule that enters into reaction with the phosphate, which is decomposed. Now it will be clear, that at least in first instance undoubtedly a hexose *mono*phosphoric

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[1]) The fact, that biochemical hydrolyses and consequently also the esterifications are characterized by a very small energetical effect, shows the deficiency of the parallel which MEYERHOF draws between the connection of these two reactions and the connection he proved to exist between the combustion of a part of the lactate in muscular tissue and the simultaneously occurring synthesis to glycogen of an other part of the lactate. See: O. MEYERHOF, Chemical Dynamics of Life Phaenomena, 1924, p. 56.

[2]) See among others: C. NEUBERG und M. KOBEL l. c.

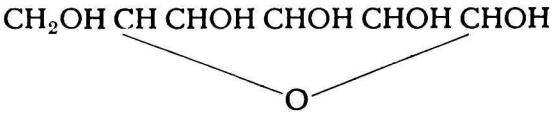
[3]) These Proceedings, Vol. 34, p. 237, (1925).

ester must be produced from this hexose molecule. In this connection we must immediately point to the fact, that the splendid investigation of ROBISON [1]) has shown that in a fermenting medium of free zymase, hexose and phosphate such an ester is actually produced.

Then, however, the question presents itself, at which of the 6 C-atoms such esterification will occur. To answer this question it is necessary to consider more closely the constitution of d-glucose.

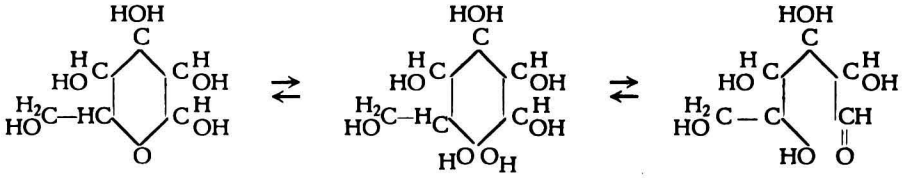
In recent years already several times it had been supposed, that d-glucose might have the configuration of an amylene oxide ring [2]). By the recent investigations of HAWORTH [3]) it has now been proved that this supposition was right.

So d-glucose has the following ringstructure :



As appears from the reducing properties of d-glucose this ring-structure will, in aqueous solution, have a strong tendency to pass by ring-opening, that is to say by hydrolysis, into a chain-structure.

With ARMSTRONG [4]) we may now conclude, that in this ring-opening intermediate formation of an aldehyde hydrate occurs, which may be rendered in a scheme as follows :



In realizing that reversely ring closure comes to a liberation of water by the reaction of two OH-groups which are bound to the first and fifth C-atom respectively, we may conclude that it must be one of these two apparently reactive OH-groups, which in enzymatical phosphorylation will bind the phosphate rest. That the phosphate rest should be bound to the first C-atom would be inconsistent with the fact, that the hexose *mono*-phosphoric ester, prepared by ROBISON, has strongly reducing properties.

Considering all this it seems probable to us, that the primarily formed

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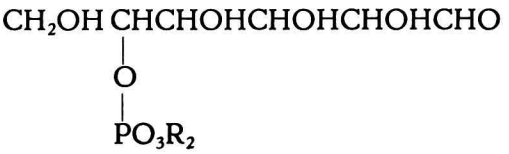
[1]) R. ROBISON, Biochem. Journ. Vol. 16, p. 809, (1922).

[2]) See for instance: J. BÖESEKEN, The Configuration of the Saccharides, Part. II, p. 28, Leyden.

[3]) W. N. HAWORTH, Nature, Vol. 116, p. 430, (1925).

[4]) E. F. ARMSTRONG, The Carbohydrates and the Glucosides. London, 1924, p. 8. It is true that ARMSTRONG here still gives the formerly accepted butylene oxide ring structure, but there seems to be no reason to suppose that this should cause any alteration in the above consideration.

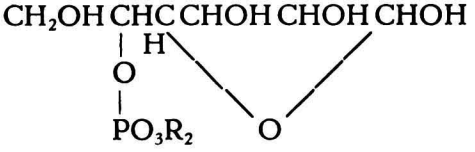
hexose monophosphoric ester will in first instance have the following formula :



The fact, that by the usual, in purely chemical way performed esterification, for instance of d-glucose by acetic acid, a penta-acetyl compound is produced, in which the acetyl-groups are bound to the OH-groups of the carbon atoms 1, 2, 3, 4 and 6, seems to us not at all inconsistent with our view. This is easily understood in realizing that this purely chemical esterification always occurs in an anhydrous medium and under addition of strong dehydrating agents (anhydrous sodium acetate, concentrated sulphuric acid, etc.). Contrary to the enzymatical phosphorylation which takes place in a diluted aqueous solution, in this case there can be no question of ringopening, that is to say of hydrolysis.

Meanwhile the above indicated ester will not be stable and in its turn will tend to pass into a ringstructure. As, however, the OH-group of the 5th C-atom is blockaded, it is self-evident that now the OH-group of the 4th C-atom will enter into reaction, in consequence of which a phosphoric ester of the butylene oxide ring is produced. This is the more readily acceptable since HAWORTH considers the butylene oxide structure to be the configuration of IRVINE's γ-glucose, which of late is also often mentioned in connection with biochemical transformations.

The ester which has now been formed will consequently have the structure :



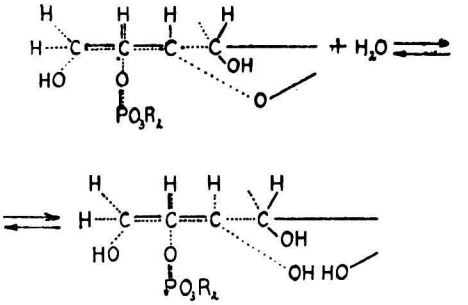
In our train of thought the transformation of the normal glucose into the last mentioned ester should now cause the H-atom at the fourth C-atom, under the influence of the hydrogen activating action of the protoplasm, to be activated to such a degree, that transference to the third C-atom, under simultaneous breaking up of the bond between these two C-atoms, takes place.

In other words, under the influence of the entered phosphate rest a dislocation in the molecule will have happened, which is indispensable for the fission of the C_6-compounds under the influence of the hydrogen activating protoplasm, so that, whereas the hydrogen activation by this last agent as such is not sufficient to achieve this effect in

normal glucose, this would only become possible by the additional effect of the action of the phosphate rest.

Perhaps the subjoined schematic representation of the action of the phosphate rest may serve to elucidate our view to some extent, although we fully recognize the relativity of its value.

If we simply reproduce by ..... a weakened, and by ——— a strengthened bond between the atoms, then it is not excluded that the dislocation in the hereby more directly concerned part of the glucose molecule, effected by the phosphate rest, might be rendered as follows :



From the readiness, with which the phosphate rest can be split off, follows that the fifth C-atom in the ester is unsaturated to a certain degree, which will among others cause an increased occupation of the affinities of the 4th C-atom. This must lead to an activation of the H-atom, bound to this last C-atom, and a weakening of the bond between the fourth and the third C-atom ; consequently this last becomes unsaturated and is so to say prepared to act as a hydrogen acceptor. If now to this the hydrogen activating action of the protoplasm is added, this will transfer the hydrogen atom from the fourth to the third C-atom, under simultaneous breaking up of the bond between the two C-atoms. The fourth C-atom, which has now become unsaturated will consequently also break up the C-O-C bond under hydration and thereby at both sides give rise to the formation of aldehyde groups.

Whereas we are fully aware of the fact, that hitherto we have entirely moved on hypothetical field, we will now trace, how far the available extensive experimental material agrees with our postulation.

§ 4. *Testing of our hypothesis to the available experimental material.*

From the foregoing section it will be clear that we have formed the idea, that in the dissimilation of glucose in first instance a glucose *mono*-phosphoric ester is produced, which then is decomposed into one molecule of glycerine aldehyde and one molecule of glycerine aldehyde phosphoric ester.

The whole course of the alcoholic fermentation might now be represented as follows :

$$C_6H_{12}O_6 + PO_4R_2H = C_6H_{11}O_5(PO_4R_2) + H_2O. \quad (I)$$

$$C_6H_{11}O_5(PO_4R_2) = C_3H_6O_3 + C_3H_5O_2(PO_4R_2). \quad (II)$$

$$C_3H_6O_3 = CO_2 + C_2H_6O. \quad (IIIa)$$

$$C_3H_5O_2(PO_4R_2) + H_2O = C_3H_6O_3 + PO_4R_2H. \quad (IIIb)$$

The $C_3H_6O_3$ produced in equation IIIb is afterwards also split up into carbon dioxide and alcohol according to equation IIIa. The course of the reaction IIIa is here left out of consideration; for this we may refer to the before quoted publication of DONKER and one of us [1]).

Now our scheme does as well lend itself to the explanation of the course of the rate of alcoholic fermentation when phosphates are added to a sugar solution containing free zymase, as that proposed by HARDEN and YOUNG. Whereas in the scheme of HARDEN and YOUNG at the moment when all the phosphate is bound the rate of fermentation is controlled by the velocity of the hydrolysis of the hexose *di*phosphoric ester — this being the reaction which proceeds at the slowest rate — in our way of explanation the first mentioned rate is in a similar way controlled by the velocity of the hydrolysis of the triose phosphoric ester. Meanwhile from our scheme, as much as from that of the English investigators follows that by the addition of free mineral phosphates, according to our equations I, II and IIIa, in a certain period an extra amount of carbon dioxide and alcohol is formed, which is equivalent to the phosphate bound in the same period.

Consequently in the above discussed regard both ways of explanation are perfectly equivalent.

The scheme of HARDEN and YOUNG, however, fails to give an explanation of the convincingly proved fact, that *during the initial period of fermentation* the ratio of the esterified phosphate and the carbon dioxide produced is greater than 1 and not before the end of the phosphate period the ratio 1, observed by the said investigators, is reached [2]). As in our scheme the esterification precedes the fermentation and consequently a certain accumulation of hexose *mono*phosphate may occur before the production of carbon dioxide begins, the above mentioned phenomenon obtains an unconstrained explanation.

Further we have already brought into relief, that the production of the hexose *mono*phosphoric ester, inserted in our scheme, in the fermentation of glucose by free zymase in the presence of mineral phosphates, has been

[1]) With NEUBERG, who makes the same remark in connection with methylglyoxal, we are of opinion that the fact, that the rate of fermentation of the triosepreparations, which have up to now been examined, is smaller than that of the fermentable hexoses, need not in the least plead against the conception that trioses should intermediately occur in the fermentation of the hexoses. The phenomenon just mentioned may very probably be ascribed to the strong tendency of trioses to pass by hydration, polymerisation, or possibly formation of ring structures, into more stable compounds.

[2]) See the investigation of H. EULER und D. JOHANSSON, Zeitschr. f. physiol. Chemie. Bd. 85, p. 192, (1913). Compare also: O. MEYERHOF, ibid. Bd. 102, p. 204, (1918).

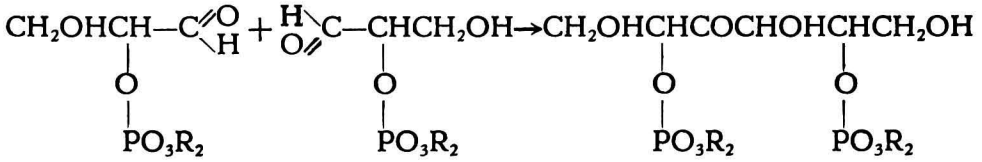
proved experimentally by ROBISON [1]). More important, however, is the fact, that this hexose *mono*phosphoric — contrary to the before mentioned hexose *di*phosphoric — ester is well fermentable and that with a velocity, which in accordance with our scheme practically agrees with the rate of the glucose fermentation, *enhanced* by the addition of phosphate.

Meanwhile the objection will be made against our above exposed view, that we have left out of account the already mentioned formation of hexose *di*phosphoric ester, conclusively proved by the investigations of YOUNG, VON LEBEDEW, NEUBERG a.o..

For the formation of this ester under the conditions chosen by YOUNG and ROBISON, whereby the fermentation proceeds in the presence of large quantities of mineral phosphates, we think however to be able to give an unconstrained explanation. We have seen, how in our way of representation the rate of fermentation is, on the basis of the results obtained by HARDEN and YOUNG, conditioned by the rate of the decomposition of the triose phosphoric ester, in last instance by the maximal capacity of the enzyme effecting the hydrolysis (the phosphatase). The remaining partial reactions must consequently proceed at a higher rate. Under these circumstances it is inevitable that the excessive addition of phosphates applied by YOUNG and ROBISON must lead to an accumulation of triose phosphoric ester.

Considering now the fact, that by the investigation of DONKER, VISSER T HOOFT and one of us (KI) [2]), it was convincingly proved, that the forced accumulation of acetaldehyde during the alcoholic fermentation causes a condensation of this substance to acetylmethylcarbinol (the carboligase action of NEUBERG) it is self-evident to suppose that the triose phosphoric ester accumulated under abnormal conditions is in quite a similar way condensed to hexose *di*phosphoric ester.

This transformation should then have to take place as follows:



Now in this connection we will not neglect to mention that already in 1907 the formation of a triose phosphoric ester in the considered fermentation medium was defended on experimental grounds by IWANOFF [3]). It is true that IWANOFF's observation has never been confirmed, but

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[1]) l. c.

[2]) A. J. KLUYVER, H. J. L. DONKER und F. VISSER 'T HOOFT, Biochem. Zeitschr. Bd. 161, p. 361, (1925).

[3]) L. IWANOFF, Zeitschr. f. physiol. Chemie. Bd. 50, p. 281, (1907); Centr. f. Bakter. IIe Abt. Bd. 24, p. 1, (1909).

apparently his adversaries have thereby lost out of sight, that IWANOFF isolated his ester in a fermentation mixture with far smaller phosphate concentration and which moreover was placed at a lower temperature. Under these circumstances it need not in the least be considered improbable that IWANOFF has indeed obtained a triose phosphoric ester.

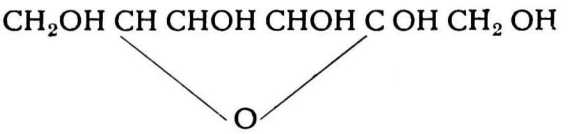
We will now examine more closely the consequences of our interpretation of the occurrence of the hexose *di*phosphoric ester.

Let us therefore consider how in our train of thought d-fructose will be fermented.

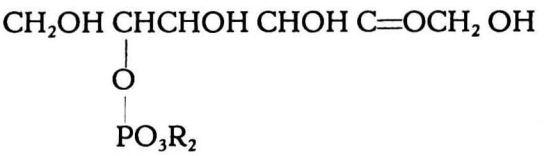
This hexose, with the chain structure

$$CH_2OH\ CHOH\ CHOH\ CHOH\ C{=}OCH_2OH$$

has the following ring structure according to the insight of the chemists :

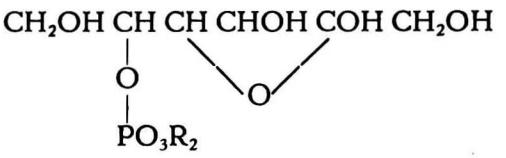


In analogy with what has been remarked in this respect for d-glucose we must take for granted that in the biochemical phosphorylation

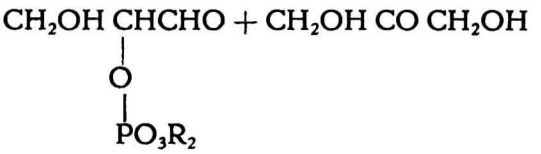


will be formed.

This ester however will only be an intermediate product, as by renewed ring closure it will pass into :



This is the monophosphoric ester of the so reactive γ-fructose of IRVINE. Now the formation of this ring explains unconstrainedly why it is exactly the H-atom of the fourth C-atom which after ring opening will by preference be activated by the acting protoplasm. This again will cause quite similarly the intramolecular transference of hydrogen from the fourth to the third C-atom by which will be produced :



Besides dioxyacetone the same glycerine aldehyde phosphoric ester will

be produced from d-fructose as well as from d-glucose, and from this ester under the indicated conditions again the same hexose *di*phosphoric ester will be formed.

Also for d-mannose we must accept a quite analogic course as for d-glucose, whereby again the same hexose *di*phosphoric ester must occur.

This inevitable consequence of our view now is perfectly in keeping with what already long ago has been experimentally ascertained by HARDEN and YOUNG [1]), but which hitherto had not been satisfactorily explained.

Resuming we may shortly state that the significance of the biochemical phosphorylation of the hexoses is to be found in the conversion of sugars into the so reactive γ-modifications.

Furthermore the given way of representation affords an unsought explanation of the contrast which in alcoholic fermentation in several respects appears to exist between glucose and mannose on the one side and fructose on the other side [2]). We need only consider that in reaction II the two first mentioned sugars produce glycerine aldehyde, fructose on the other hand produces dioxyacetone.

Meanwhile there is still another consequence of our view to be examined. We must namely consider the question, which sugar will be produced when phosphoric acid is split off from the hexose *di*phosphoric ester. Considering the constitution of the hexose *di*phosphoric ester as following from our theory it will be obvious, that a hexose will be formed, which will have the chain structure: CH_2OH CHOH CHOH CO CHOH CH_2OH, consequently a 3-ketohexose. What do now the experiments show? NEUBERG, FÄRBER, LEVITE and SCHWENK [3]) have convincingly proved that in the hydrolysis of the hexose *di*phosphoric ester with oxalic acid d-fructose is formed. Considering the inconsistency of the 3-ketohexoses and the readiness with which the C=O group by enolisation transmigrates in the molecule, it may not be bold to suppose that only during the preparation fructose is formed from the just mentioned 3-ketohexose. And we feel the sooner entitled to express this opinion since YOUNG has already stated long ago that the *solution* of the sugar split off from the ester has a ratio of reduction and optical rotation which does not agree with that of d-fructose.

Consequently neither in this respect does our conception appear to encounter difficulties. On the contrary it gives a ready explanation of the fact that also the hexose diphosphoric esters prepared from glucose and mannose respectively, on hydrolysis produce d-fructose.

Since further according to our view in the three hexoses mentioned the esterification attacks the molecules in those parts which are identical, as far as the position of the H-atoms and the OH groups in space is concerned, the produced triose phosphoric esters also must in stereo-

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[1]) A. HARDEN und W. J. YOUNG, Proc. Royal Soc. Ser. B. Vol. 81, p. 336, (1909).

[2]) See: A. HARDEN, Alcoholic Fermentation, p. 120.

[3]) C. NEUBERG, E. FÄRBER, A. LEVITE und E. SCHWENK, Biochem. Zeitschr. Bd. 83, p. 244, (1917).

chemical respect be quite identical. Of course the same holds good for the hexose *di*phosphoric ester formed bij the condensation.

Finally we want to remark that our view also implies the fact, stated by ROBISON [1]), that the well fermentable hexose *mono*phosphoric ester he isolated is in no respect identical with the hexose *mono*phosphoric ester prepared by NEUBERG [2]) by partial hydrolysis of the hexose *di*-phosphoric ester. Perfectly in accordance with the anticipations of our view ROBISON obtained in chemical and enzymatical hydrolysis of his hexose *mono*phosphoric ester a dextro-rotatory reducing solution, from which he prepared glucosazone.

Also the divergent behaviour of the hexose-*di*phosphoric ester and of ROBISON's *mono*phosphoric ester towards phenylhydrazine is fully explained by the constitution of these esters accepted by us. The hexose-*mono*phosphoric acid osazone prepared from hexose-*di*phosphoric acid is, as may be expected from our scheme, *not* identical with the hexose-*mono*-phosphoric acid osazone from ROBISON's *mono*-compound.

§ 5. *Conclusion.*

In the foregoing we have developed a new theory on the function of phosphates in the dissimilation of the hexoses. This theory is, as far as the general course of the rate of alcoholic fermentation in presence of phosphates is concerned, equivalent to the current mode of explanation as formulated by HARDEN and YOUNG.

The remarkable situation now occurs, that the experiments one would be inclined to carry out in the first place in verification of our hypothesis have already been described in the extensive literature.

The following points may illustrate this:

1st. Our theory implies that in dissimilation the hexoses are in first instance transformed into a hexose *mono*phosphoric ester. This ester was indeed isolated by ROBISON.

2nd. Our theory implies that this hexose monophosphoric ester is fermented at a rate which agrees with the rate of fermentation of glucose, enhanced by the addition of phosphates. This was actually stated by ROBISON.

3rd. Our theory affords an explanation of the fact that during the initial period of fermentation the ratio of the esterified phosphate to the carbon dioxide produced is greater than 1, as has been proved by the experiments of EULER and JOHANSSON.

4th. Our theory concerning the way the hexose *di*phosphoric ester is formed implies that from d-glucose, d-fructose and d-mannose quite identical, also with regard to the stereochemical configuration, esters are produced. This was conclusively proved by HARDEN and YOUNG.

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[1]) l. c.

[2]) C. NEUBERG, Biochem. Zeitschr. Bd. 88, p. 432, (1918).

5th. Our theory implies that the hexose *mono*phosphoric ester isolated by ROBISON is not identical with the hexose *mono*phosphoric ester prepared by NEUBERG from the before mentioned hexose *di*phosphoric ester. This appears from the investigation of ROBISON. Also the behaviour of hexose-*di*- and of ROBISON's hexose-*mono*-phosphoric acid towards phenylhydrazine agrees very well with our view.

6th. Our theory implies that by hydrolysis of the hexose *di*phosphoric ester obtained from d-fructose and also from d-mannose a same hexose is split off, this being a 3-ketohexose, which we may suppose to pass easily into fructose. The isolation of d-fructose after hydrolysis of this ester by NEUBERG and his collaborators concurs with this view.

We wish to state expressly that the current view absolutely fails to elucidate these experimentally established facts, which on the other hand are logical consequences of our theory.

To this we may add, that the theory set forth in the above affords also an unconstrained explanation of the fact, stated by HARDEN and YOUNG, that the fermentation of d-glucose and d-mannose on the one hand and d-fructose on the other differ in many respects.

Finally in our theory the formation of phosphoric esters is for the first time made into a logical element in the whole of the dissimilation of the hexoses, because phosphates are the agent which converses these sugars into the so reactive γ-form.

Delft, January 1926.