ausmünzen lassen. In dieser Beziehung muß an die Eiskeimtheorie von BERGERON — ausgebaut durch FINDEISEN[1] — für die Niederschlagsbildung erinnert werden, die eine der wichtigsten Bedingungen für die Niederschlagsbildung zu unserer Kenntnis gebracht und eine Erklärung dafür gegeben hat, warum einmal Regen fällt, während ein anderes Mal bei scheinbar gleicher Wetterlage Regen und Schnee ausbleiben. Noch läßt sich aber nicht absehen, wann diese Erkenntnisse zu einer wesentlichen Verbesserung unserer Niederschlagsvorhersagen im täglichen Dienst führen werden.

Wenn die letzten Jahrzehnte seit Ende des ersten Weltkrieges zu großen Fortschritten in der Prognostik geführt haben, so ist dieser Fortschritt einerseits dem gewaltig anschwellenden, täglich einlaufenden Beobachtungsmaterial, andererseits den Ergebnissen der wissenschaftlichen Forschung zuzuschreiben. Man kann dabei fragen, ob und wann denn die Meteorologen imstande sein werden, wenigstens die alltäglichen Kurzfristprognosen mit absoluter Sicherheit auszuarbeiten bzw. das künftige Wetter mit Sicherheit vorauszuberechnen, da ja die Theoretiker bereits ein Gleichungssystem von genügendem Umfang zur Durchführung derartiger Berechnungen ausgearbeitet haben. V. BJERKNES[2] zum Beispiel hat an die grundsätzliche Möglichkeit einer exakt rechnerischen Lösung des Problems in der Zukunft geglaubt, während SCHMAUSS[3] schon vor langer Zeit die Unmöglichkeit absolut richtiger Prognosen betont hat. Erst ERTEL[4] hat aber nachgewiesen, warum selbst bei streng kausaler Auffassung der meteorologischen Vorgänge eine eindeutige Wettervorhersage für Teilgebiete der Atmosphäre prinzipiell unmöglich sei, weil für die mathematische Behandlung des Problems natürliche Randbedingungen für die willkürlichen Grenzen der Teilgebiete vorhanden sein müßten, was nicht der Fall ist. Jede brauchbare Prognose kann daher grundsätzlich nur eine mit einer Unbestimmtheit behaftete Approximation sein, die natürlich trotzdem ein brauchbares Ergebnis liefert, sofern die Unbestimmtheit unterhalb der Erfordernisse des praktischen Lebens bleibt.

Die Arbeit des Prognostikers wird also nie ein ganz zweifelsfreies Ergebnis liefern können. Mit dieser Einschränkung werden sich die Meteorologen bescheiden und ihr Augenmerk darauf richten müssen, die Approximation immer genauer zu gestalten. Aber nicht nur die Meteorologen, sondern auch die Öffentlichkeit, die absolut richtige Prognosen wünscht, muß sich mit dieser Sachlage abfinden.

[1] FINDEISEN, Meteor. Z. *55*, 121 (1938); *56*, 365 (1939).
[2] V. BJERKNES, Metcor. Z. *36*, 68 (1919).
[3] A. SCHMAUSS, Das Problem der Wettervorhersage (Leipzig 1937).
[4] H. ERTEL, Methoden und Probleme der dynamischen Meteorologie (Berlin 1938).

Summary

Since the first world war the reliability of weather forecasts based on synoptic maps has been greatly enhanced, owing on the one hand to the extraordinarily increased number of wireless weather reports, on the other hand to new scientific insight into weather developments gained up to now. Of especial importance were the data from high levels of the free atmosphere obtained by radio sounding, by which the construction of weather maps with contours was made possible. Scientific research after the first world war was based on the polar front theory of BJERKNES and his followers and was greatly furthered in the course of time especially by the understanding of the great importance of the occurrences in the stratosphere for weather development.

New Investigations on Enzymatic Glycolysis and Phosphorylation

By OTTO MEYERHOF[1], Philadelphia, Pa.

The achievements of the last decades in the fields of enzymology, intermediary metabolism, hormones, and vitamins are so overwhelming that one could be tempted to quote the ironic lines of GOETHE, spoken by the famulus Wagner in *Faust II*, as a serious interpretation of these endeavours:

> Was man an der Natur Geheimnisvolles pries,
> Das wagen wir verständig zu probieren,
> Und was sie einst organisieren ließ,
> Das lassen wir kristallisieren[2].

However, simultaneously with this progress, with the elucidation of the self-sustaining cycles of intermediary enzymatic reactions, and with the purification and crystallization of many enzymes, hormones, vitamins, and viruses, much self-restraint and sobriety has returned. The biochemists and physiologists now repudiate the materialistic fantasies and boastings of their predecessors. In our view the organization of the cell, the living unity itself can not be explained by purely physical and chemical methods. We restrict ourselves, therefore, to the study of the physical and chemical reactions of these organized, self-perpetuating living units.

About 1900 BUCHNER was successful in separating from the living yeast cell the enzymatic system which

[1] Department of Physiological Chemistry, School of Medicine, University of Pennsylvania.

[2] GOETHE's *Faust II:*

> The mystery which for man in Nature lies
> We dare to test, by knowledge led,
> And that which she was wont to organize
> We crystallize instead.

converts sugar to alcohol and CO_2. The same separation was accomplished in 1926 for the glycolytic enzyme system of muscle[1]. Here glycogen is split into two mols of lactic acid per unit of hexose. Later on the same system was brought into solution from many other cells or organs, from brain, chicken embryo, malignant tumors, erythrocytes, and different kinds of bacteria.

The oxidative system is less easily brought into solution, some of its enzymatic components strongly adhering to structural elements—but many enzymes responsible for individual oxidative steps of sugar metabolism were also obtained in extracts.

Besides the insolubility of some components of the oxidative system (cytochromes a and b and cytochrome oxidase) it differs also in another sense from the glycolytic system, *viz*: there seems to be no common pathway of sugar oxidation in all cells and tissues, although the so-called tricarboxylic acid cycle of KREBS is prevalent in many of them[2]. We restrict our discussion in the following to the anaerobic breakdown, to glycolysis in the stricter sense of this term. Here the pathway is uniform.

Even if the end products of various forms of sugar fermentation are different, and consist instead of lactic acid as in animal tissue, of alcohol and CO_2 as in yeast, of H_2, acetic acid, lactic acid and CO_2 in coli bacteria, or of propionic or butyric acid as in propionic or butyric acid bacteria, all the steps leading from sugar to pyruvate are none the less identical, and only the fate of the pyruvate differs. Moreover, if the pyruvate remains as an end product, glycerol is formed simultaneously as a reduction product[3]. In the case of glycolysis of animal tissues (and also in lactic acid bacteria) the pyruvate reaction is a single reduction step:

$$\underset{\text{(pyruvic acid)}}{CH_3 \cdot CO \cdot COOH} + H_2 \rightarrow \underset{\text{(lactic acid)}}{CH_3 \cdot CHOH \cdot COOH}$$

Furthermore, the steps leading from sugar to pyruvic acid are also the initial steps of the prevalent form of sugar oxidation. (The oxidation of sugar to hexonic acids or of phosphohexoses to phosphohexonic acid is apparently of minor biological importance.) Since pyruvic acid can also be reversibly aminated, forming alanine, and also plays a role in the turnover of fat, it occupies a central position in intermediary cell metabolism.

I shall discuss the subject under three headings:

I. The scheme of intermediaries of glycolysis.
II. The phosphorylating mechanisms.
III. The isolated enzymes.

Our attention will be devoted mainly to the progress in the last eight years, before the Nazi regime in Germany and the second World War disrupted international science and scientific intercourse[1].

I. *The Scheme of Intermediaries of Glycolysis*

This scheme was fully developed between 1932–1939 and has undergone no further change since. All intermediaries from glucose-1-phosphate to phosphopyruvic acid are phosphorylated compounds. Most of them are true phosphoric acid esters, formed by condensation of an alcoholic (OH) group with the third valency H of phosphoric acid; some of them have the phosphate group linked to a carboxyl or carbonyl group. During the years of the development of this scheme some investigators and critics obstinately clung to the idea that this scheme was not ubiquitous, but that some organs were able to glycolyze in a different way by a so-called non-phosphorylating glycolysis. Even now the ghost of this non-phosphorylating glycolysis is still haunting some biochemical laboratories and the desks of some scientific writers. Actually, it never existed. It could be refuted in every instance where an experimental approach was feasible. Mostly the arguments in favor of it were fallacies in themselves: in many enzymatic systems, separated from the cell, the reactivity of glucose and hexosediphosphate (HDP) is altered. Often hexosediphosphate reacts with a much lower speed than sugar or not at all. This was used as an argument against this diphosphate as a common thoroughfare of sugar breakdown. But because of the transphosphorylation mechanism, which will be discussed in the next section, the speed of the global reaction can be much higher than the isolated single reaction steps; hexosediphosphate decomposes in such a system in the presence of free sugar much faster than in the absence of it[2]. The opposite can also be observed; viz.: that hexosediphosphate forms lactic acid under conditions where free sugar does not. Here the very sensitive enzyme which phosphorylates sugar (hexokinase) is destroyed by the extracting procedure.

There is also a positive argument: methyl glyoxal ($CH_3 \cdot CO \cdot COH$) is transformed in the presence of glutathione to lactic acid by a very widespread enzyme[3]. But methyl glyoxal itself, contrary to earlier assumptions, is not a physiological intermediary product of sugar breakdown. It arises from triosephosphate by a non-enzymatic decomposition[4]. In the living cell this reaction surely plays a subordinate role, if a role at all.

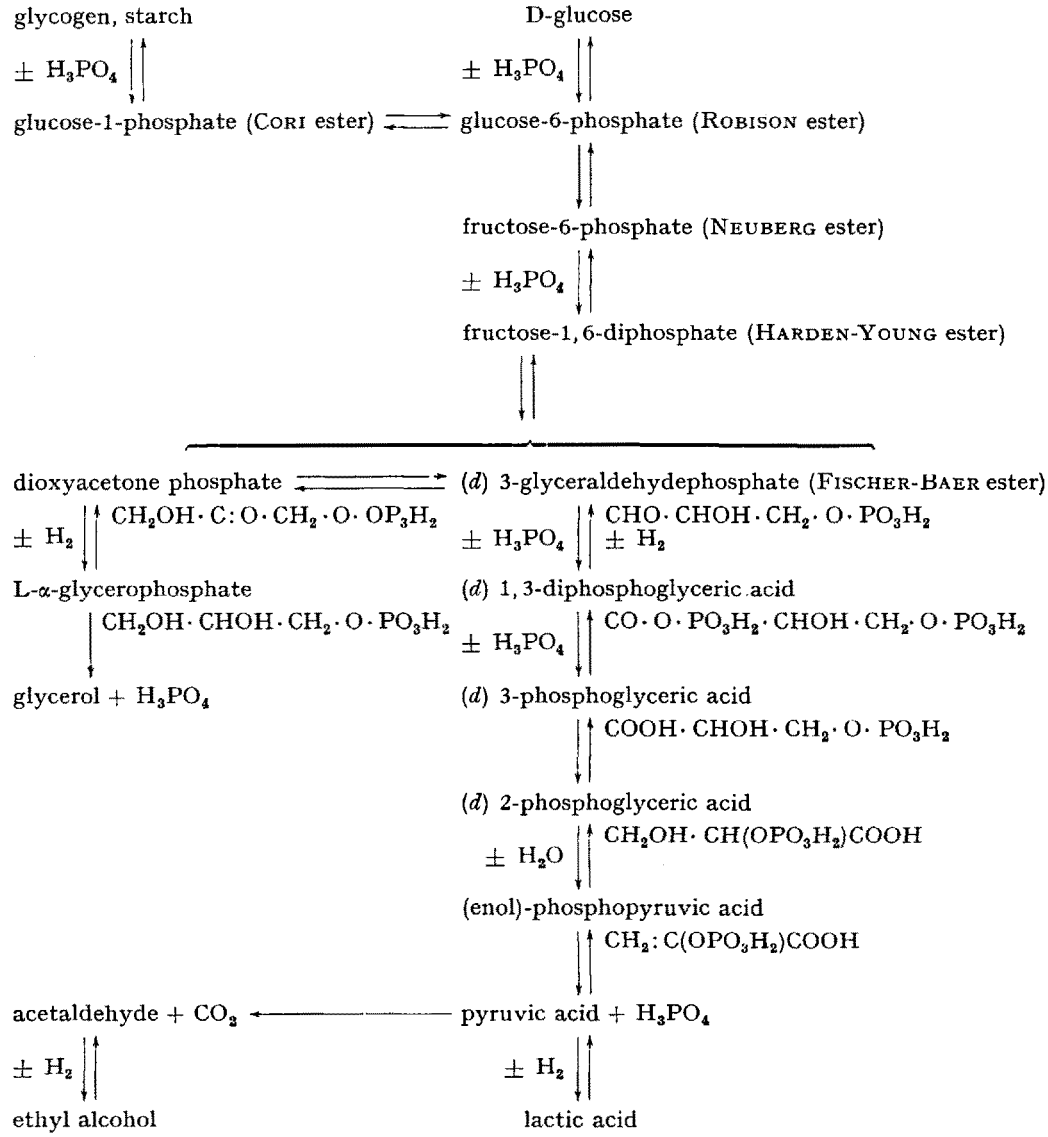
Several hexosemonophosphates other than those given in the scheme are synthesized and metabolized by the living cell. Mannose-6-phosphate is formed from

[1] O. MEYERHOF, Naturwiss. *14*, 1175 (1926).
[2] H. A. KREBS, Advances in Enzymology *3*, 191 (1943).
[3] C. NEUBERG and E. REINFURTH, Biochem. Z. *92*, 234 (1918).

[1] Former reviews: O. MEYERHOF, Erg. Physiol. *39*, 10 (1937). – J. K. PARNAS, Erg. Enzymforschung *6*, 57 (1937).
[2] O. MEYERHOF, W. KIESSLING, and W. SCHULZ, Biochem. Z. *292*, 25 (1937).
[3] K. LOHMANN, Biochem. Z. *254*, 332 (1932); *262*, 152 (1933).
[4] O. MEYERHOF and K. LOHMANN, Biochem. Z. *271*, 89 (1934).

Scheme

Two changes are introduced into the scheme as given in my publications of 1941 and 1942[1]. 1,3-diphosphoglyceraldehyde, supposed to be an intermediary by O. WARBURG and W. CHRISTIAN[2], is left out, because no such substance is formed[3]. The reaction between phosphopyruvic acid and pyruvic acid + phosphate, which was supposed to be irreversible[4], was proved to be reversible[5] like all the other intermediary steps and is now designated by a double arrow.



mannose as glucose- and fructose-phosphates from the respective sugars[6]. Galactose-1-phosphate[7], the analogue of glucose-1-phosphate, is formed in the liver during the assimilation of galactose and is probably also an intermediary in yeast if the latter is adapted to the fermentation of galactose. Fructose-1-phosphate, another analogue of glucose-1-phosphate, was first obtained by splitting off one phosphate group from hexosediphosphate[1]. It was also obtained by enzymatic synthesis from *d*-glyceraldehyde and dioxyacetonephosphate. The enzyme responsible for this synthesis was called aldolase, because this is an aldol-condensation; moreover, the exact chemical nature of the reversible splitting and synthesis of hexosediphosphate to and from triosephosphates was proved in this way[2]. Quite recently it was shown that fructose-1-phosphate may be formed by enzymatic phosphorylation of fructose by means of ATP[3].

[1] O. MEYERHOF, Biol. Symposia *5*, 143 (1941); Symposium on Respiratory Enzymes (Univ. of Wisconsin Press, Madison, 1942), p. 3.

[2] O. WARBURG and W. CHRISTIAN, Biochem. Z. *301*, 221 (1939); *303*, 40 (1939).

[3] O. MEYERHOF and P. OESPER, J. Biol. Chem. *170*, 1 (1947).

[4] O. MEYERHOF, P. OHLMEYER, W. GENTNER, and M. MEIER-LEIBNITZ, Biochem. Z. *298*, 396 (1938).

[5] H. A. LARDY and J. A. ZIEGLER, J. Biol. Chem. *159*, 343 (1945). O. MEYERHOF and P. OESPER, Federation Proc. *7*, 174 (1948).

[6] C. M. JEPHCOTT and R. ROBISON, Biochem. J. *28*, 1844 (1934).

[7] H. W. KOSTERLITZ, Biochem. J. *37*, 319 (1943).

[1] B. TANKO and R. ROBISON, Biochem. J. *29*, 961 (1935).

[2] O. MEYERHOF, K. LOHMANN, and P. SCHUSTER, Biochem. Z. *286*, 301 (1936).

[3] G. T. CORI and M. W. SLEIN, Federation Proc. *6*, 246 (1947).

The last compound discovered in the scheme, 1,3-diphosphoglyceric acid (or phosphoglycerylphosphate) was found by NEGELEIN and BRÖMEL in 1939[1]. A similar compound, acetylphosphate,

$$\begin{array}{l} CH_3CO \\ | \\ OH_2^{\bullet}PO_3 \end{array}$$

where the phosphate is likewise bound to a carboxyl group, was discovered by LIPMANN in 1940[2]. It is formed by lactic acid bacteria in the oxidation of pyruvate and was synthesized in the laboratory. Similar acylphosphates are probably also formed from fatty acids and may play a role in the oxidation of fat[3].

II. *Phosphorylating mechanisms*

The special meaning of the phosphorylation of the intermediaries is revealed by their interaction with the coenzyme systems. The coferment of fermentation, discovered by HARDEN in 1900, consists actually of three different coenzymes (besides inorganic ions like Mg^{++}):

(1) the hydrogen transferring cozymase, chemically, diphosphopyridinenucleotide (DPN);

(2) the phosphorylating coenzyme or adenylic system containing the three stages adenosintriphosphate (or adenylpyrophosphate or ATP), adenosindiphosphate (ADP), and adenylic acid (or adenosinmonophosphate or AA);

(3) the cocarboxylase, diphosphothiamine.

We will concern ourselves mainly with the second system. Phosphate is taken up or received from the intermediaries by transphosphorylation with the adenylic system. With the help of radioactive inorganic phosphate it was shown definitely that this phosphate transfer occurred without intermediary liberation of inorganic phosphate[4]. Phosphorylation of glucose, therefore, in the presence of the specific enzyme "hexokinase", goes on according to the equation:

$$\text{glucose} + \text{ATP} \rightarrow \text{glucose-6-phosphate} + \text{ADP}$$

Dephosphorylation of phosphopyruvic acid, accordingly, obeys the equation:

$$\text{phosphopyruvic acid} + \text{ADP} \rightarrow \text{pyruvate} + \text{ATP}$$

Taking both equations together we have the phosphate transfer:

$$\text{phosphopyruvate} + \text{glucose} \xrightarrow{\text{ATP}} \text{pyruvate} + \text{glucose-6-phosphate.}$$

This transfer in the stationary state of glycolysis therefore goes on with minute amounts of ATP, which acts as a true catalyst in the presence of a specific transphosphorylase.

The phosphate bond in the adenylic system is "energy rich"; this fact is of primary importance. With the splitting of ordinary ester-phosphate linkages, 1,200 to 1,500 cal heat per mol are liberated; with the splitting of each single P group of the labile phosphate of ATP, 12,000 cal are liberated[1]. The free energy change is similar. In the phosphorylation process of glycolysis, phosphate is first taken up in an "energy poor" bond of the hexosephosphate, and by the transformation of the molecule, especially with the help of the oxidative step, it is changed into an energy rich bond. This high energy phosphate is transferred as such to the adenylic system and eventually to other substrates, which preserve the energy rich bond. The phosphorylating mechanism, therefore, serves for the generation and storage of energy rich phosphate bonds. How does the oxidative step of glycolysis generate such a bond? Glyceraldehyde-3-phosphate by itself does not react with inorganic phosphate. But if both are brought together with the purified oxidizing enzyme of O. WARBURG and DPN, a reaction takes place in which DPN is reduced and 1,3-diphosphoglyceric acid originates[2]. Apparently a loose addition product forms between glyceraldehyde-phosphate and phosphate on the surface of the enzyme with the expenditure of an exceedingly small amount of energy[3]. But when by reaction with DPN the carbonyl group is oxidized to the carboxyl group the acylphosphate now has a bond with the free energy of nearly 15,000 cal. Thus the energy of oxidation of the aldehyde to carboxyl is captured in this phosphate group. By transphosphorylation with the adenylic system it is stored in ATP:

$$\text{1,3-diphosphoglyceric} + \text{ADP} \rightleftarrows \text{3-phosphoglyceric} + \text{ATP.}$$

In consequence of the internal rearrangement of 3-phosphoglyceric acid by way of 2-phosphoglyceric acid to enol-phosphopyruvate, a second energy-rich phosphate bond is created (enol-phosphate), which likewise transphosphorylates with ATP[4].

The free energy (ΔF), as distinct from the total energy or heat (ΔH), amounts to 11,000 cal for each of the labile P groups of ATP[5]. This available store of phosphate bond energy can be used either for biochemical synthesis or for the transformation into mechanical or electrical energy. Indeed, the new work of SZENT-GYÖRGYI gives us every reason to believe that ATP reacts directly with the contractile protein

[1] O. WARBURG and W. CHRISTIAN, Biochem. Z. *301*, 221 (1939); *303*, 40 (1939).
[2] F. LIPMANN, J. Biol. Chem. *134*, 463 (1940).
[3] A. LEHNINGER, J. Biol. Chem. *161*, 432 (1945); *162*, 333 (1946). – H. J. KOEPSELL, M. J. JOHNSON, and J. S. MEEK, J. Biol. Chem. *154*, 535 (1944).
[4] O. MEYERHOF, P. OHLMEYER, W. GENTNER, and M. MEIER-LEIBNITZ, Biochem. Z. *298*, 396 (1938).

[1] O. MEYERHOF and K. LOHMANN, Biochem. Z. *253*, 431 (1932).
[2] O. WARBURG and W. CHRISTIAN, Biochem. Z. *301*, 221 (1939); *303*, 40 (1939).
[3] O. MEYERHOF and P. OESPER, J. Biol. Chem. *170*, 1 (1947).
[4] J. K. PARNAS, P. OSTERN, and T. MANN, Biochem. Z. *272*, 64 (1934).
[5] O. MEYERHOF, Ann. New York Acad. Sci. *45*, 9, 377 (1944). – F. LIPMANN, Advances in Enzymology *1*, 99 (1941).

of muscle ("actomyosin") and causes such modifications in its physical structure that it can contract or relax, depending on slight changes in concentration of K^+ and Mg^{++} ions[1].

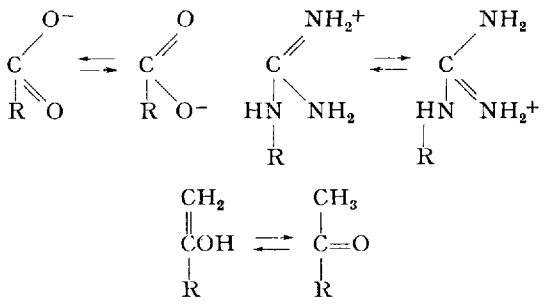
There exists another large store of high phosphate bond energy in those organs and cells which need this energy for transformation (by way of ATP) into mechanical or electrical energy. These cells are: voluntary muscle, heart, spermatozoa, the electric organs of electric fishes, and the central nervous system. All these cells and tissues are rich in phosphocreatine (vertebrates) or phosphoarginine (invertebrates). The phosphate bond energy is stored in these compounds without loss by means of the reversible reaction:

$$\text{ATP} + 2\ \text{creatine} \rightleftarrows \text{AA} + 2\ \text{phosphocreatine}.$$

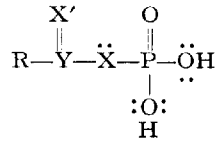
The same reaction applies to phosphoarginine.

The phosphate bond energy of ATP can also be used for endothermic (and endergonic[2]) syntheses, i.e., syntheses which need an outside source of free energy in order to occur. Especially interesting in this respect is the finding of VOGLER and UMBREIT that the energy of an inorganic chemical reaction, the oxidation of sulfur, serving as a source of energy for autotrophic Thiobacteria, *Thiobacillus thio-oxidans*, can be stored in the energy rich phosphate bond of ATP and used afterwards for the reduction of CO_2[3]. Less substantiated seems the hypothesis that a similar mechanism may be involved in the CO_2 assimilation of the green plants.

The question may be asked: what are the characteristics of an energy rich phosphate bond as distinguished from an ordinary ester bond? All the energy rich phosphates, such as acylphosphates, guanidinophosphates, enolphosphates, and pyrophosphates are formed with organic groups of high resonance. The resonance in such groups as carboxyl or guanidyl or enol (in equilibrium with keto) is produced by the oscillation of two valency electrons (or a hydrogen atom) between two otherwise identical configurations:

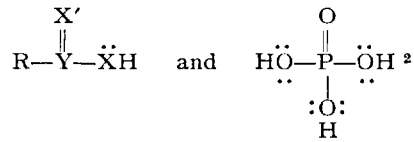


Moreover, because the phosphate molecule exhibits resonance between its different hydroxyl groups, the bridge of the ester linkage $-O-$ or $-N-$ is influenced by "opposing resonance" of the organic group and the phosphate group[1]. This makes such a structure unstable:



(X is O or N)

It leads to a great liberation of free energy when it is hydrolyzed to



If no endothermic syntheses occur, as for instance in the enzymatic extract of yeast, the labile phosphate of ATP, regenerated by the two steps of glycolysis already mentioned (the acylphosphate in 1,3-phosphoglyceric acid and the enolphosphate of phosphopyruvate) is transferred to new hexose molecules by the reactions:

$$\text{hexose} + \text{ATP} \rightarrow \text{hexosemonophosphate} + \text{ADP}$$

$$\text{hexosemonophosphate} + \text{ATP} \rightarrow \text{hexosediphosphate} + \text{ADP}$$

Every mol of HDP formed in this way gives rise to 2 mols of triosephosphate, each of which again produces 2 energy rich phosphate bonds, or together four. On the other hand, only two are needed to form hexosediphosphate. This leads to the autocatalytic increase of hexosediphosphate, or "HARDEN-YOUNG ester", in yeast extract, a reaction which was originally discovered (although misinterpreted) by HARDEN and YOUNG in 1905. In the living cell a very active ATP-ase counteracts this increase by decomposing the excess of ATP. But this enzyme is strongly bound to the structural elements of the cell and only present in traces in the enzymatic extracts[3,4]. In the living cell phosphorylation and dephosphorylation are kept in step by the ATP-ase.

Another group-transferring enzymatic mechanism may be briefly described: the "transglucosidation", which is also involved in the remarkable reversible phosphorolysis of glycogen. CORI[5], who had discovered the splitting of glycogen to glucose-1-phosphate, showed later on that the reversal of this reaction (observed by KIESSLING[6] as well as by the school of CORI) does not start with completely pure (or crystallized) en-

[1] A. SZENT-GYÖRGYI, Muscular Contraction (Acad. Press, New York, 1947).

[2] C. D. CORYELL, Science *92*, 380 (1940).

[3] K. G. VOGLER and W. UMBREIT, J. Gen. Physiol. *26*, 157 (1942).

[1] M. KALCKAR, Chem. Rev. *28*, 72 (1941).

[2] For organic pyrophosphate a similar reasoning applies.

[3] O. MEYERHOF, J. Biol. Chem. *157*, 105 (1945).

[4] This is the origin of the somewhat puzzling "HARDEN-YOUNG equation", stating that for the fermentation of one mol of hexose a second mol of hexose is esterified to hexosediphosphate.

[5] C. F. CORI, G. T. CORI, and S. P. COLOWICK, J. Biol. Chem. *121*, 465 (1937).

[6] W. KIESSLING, Naturwiss. *27*, 129 (1939).

zyme, from the side of glucose-1-phosphate, unless some glycogen or soluble starch is already present as a "primer". The reaction, therefore, must be formulated[1]:

glucose-1-phosphate + glucosidic linkage ⇄ glucose-glucosidic linkage + phosphate

Because adenylic acid is a necessary part of this enzymatic mechanism (either firmly bound to the protein or as a dissociable coenzyme[2]) the uptake of phosphate occurs probably by way of intermediate phosphorylation of adenylic acid. On the other hand the main reaction here is not a transphosphorylation but a "transglucosidation". This was recently proved by a group of California workers[3]. E.g., in *Pseudomonas sacharophila*, an enzyme exists which catalyzes the reaction:

glucose-1-phosphate + fructose → glucosefructoside (sucrose) + phosphate.

Different ketoses and also aldopentoses can replace fructose, while a glucose-1-linkage (carbonyl bond) is always needed to synthesize a disaccharide. That the glucose unit is transferred in these cases, is neatly demonstrated when the phosphorylase is added to a solution of glucose-1-phosphate in the presence of inorganic phosphate, which contains radioactive P, but in the absence of any other sugar, which could act as "acceptor". Then a free exchange of radioactive P takes place between inorganic phosphate and glucose-1-phosphate without any glucose appearing. The reaction is[4]:

glucose-1-phosphate + enzyme → glucose--enzyme + phosphate.

The glucose-enzyme compound retains the free energy of about 2,000 cal of the carbonyl phosphate and can use it, if a ketose is added to it, for the formation of a disaccharide. Such a transglucosidation, moreover, can take place in the absence of bound and free phosphate, when a mixture of glucose-1-fructose (sucrose) and a second ketose is present. Here, reversible exchange reactions of the following type occur:

glucose-1-fructoside + sorbose ⇄ glucose-1-sorboside + fructose.

Still another type of enzymatic phosphorolysis, very similar to those described, was recently discovered by KALCKAR[5]. Here ribose, the aldopentose which is a constituent of the nucleotides, takes the place of glucose in forming the carbonylphosphate, ribose-1-phosphate. This reaction may be formulated:

ribose-1-purine + phosphate ⇄ ribose-1-phosphate + purine.

The purine may be hypoxanthine or guanine. This phosphorolysis, which replaces the glucosidic bond with the imidazol nitrogen, is probably an important step in the metabolism of the nucleic acids.

III. *Studies of individual enzymes*

From the host of intermediary reactions, which are connected either with the transformation of the intermediaries themselves or with their interaction with the various coenzymes, many pure enzymes were isolated, and in some cases crystallized. Of these purified enzymes, I shall discuss three where especially interesting information was gained in the last years.

A. *Enolase*

It was found in the Heidelberg laboratory (LOHMANN and MEYERHOF, and KIESSLING[1]) that enolphosphopyruvate was formed from 2-phosphoglyceric acid by an enzyme called enolase. This enzyme was also responsible for the inhibition of glycolysis by NaF. Although many dephosphorylating enzymes are also sensitive to fluoride, they need much higher concentrations for the same inhibition. In consequence, phosphoglyceric acid accumulates in glycolyzing enzyme extracts in the presence of NaF. By blocking the enolase, fluoride inhibition acts as a barrier behind which the last intermediary piles up because it cannot be further transformed. But this barrier remains tight only if enough inorganic phosphate is present; phosphate is therefore also a component of the inhibiting system[2]. This situation was further cleared up in 1941 by WARBURG and CHRISTIAN[3]. Enolase, obtained in pure form from the crystallized Hg salt, needed a bivalent cation for its action, preferentially Mg or Mn. If Mg is present (this must be regarded as the physiological cation of the reaction) and inorganic phosphate, a Mg—F-phosphate complex forms, which deprives the enzyme protein of its necessary Mg. This explains the fluoride inhibition. Probably the same explanation holds for the inhibition of ATP-ase by fluoride, since this enzyme likewise needs Mg. The fact that Mg is a necessary coenzyme factor of glycolysis and fermentation was already discovered in 1931 by LOHMANN[4].

B. *Hexokinase*

Hexokinase, the enzyme necessary for initiating the turnover of glucose or fructose, was separated from yeast and partially purified in 1927[5]. In 1935 it was demonstrated that this initiating reaction was the

[1] C. F. CORI, G. T. CORI, and A. A. GREEN, J. Biol. Chem. *151*, 39 (1943); Federation Proc. *4*, 234 (1945).
[2] G. T. CORI and A. A. GREEN, J. Biol. Chem. *151*, 31 (1943). – G. T. CORI and C. F. CORI, J. Biol. Chem. *158*, 321 (1945).
[3] M. DOUDOROFF, J. Biol. Chem. *151*, 351 (1943).
[4] W. Z. HASSID, M. DOUDOROFF, and H. A. BAKER, Arch. Biochem. *14*, 29 (1947).
[5] H. M. KALCKAR, J. Biol. Chem. *167*, 477 (1947).

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phosphorylation of the hexoses by ATP[1]. KALCKAR proved in 1942 that the total reaction induced by hexokinase in a muscle extract:

2 glucose + ATP = 2 glucose-6-phosphate + AA (adenylic acid)

is the combined effect of two different enzymes[2]. Pure hexokinase is able to catalyze only the reaction:

glucose + ATP = glucosemonophosphate + ADP

A heat stable enzyme in muscle, called myokinase, completes the reaction by dismutating the ADP:

$$2\ ADP \rightleftarrows ATP + AA.$$

In this way ATP is formed anew and reacts again with a sugar molecule, if hexokinase is present, until practically all is finally transformed into adenylic acid.

Hexokinase was recently brought into the focus of interest on account of its specific sensitivity in the animal body. During the war the high sensitivity of this enzyme to vesicants, like mustard gas and similar substances, was discovered by English investigators[3]. Since the blistering effect on the skin was exactly parallel to the inhibition of the hexokinase in the skin, they assumed this enzyme to be the locus of attack of these poisons. Although this interpretation is not generally accepted, it stimulated the study of this enzyme and led to its crystallization from yeast.

The physiological importance of hexokinase was still more underscored by recent work in CORI's laboratory on the insulin effect[4]. The known antagonism in the living animal between the hyperglycemic principle in the anterior pituitary gland (HOUSSAY factor) and the adrenal cortical hormone on the one side and insulin on the other, can be duplicated with purified hexokinase from muscle. The activity of purified hexokinase of an alloxan diabetic rat or from the normal rat treated with anterior pituitary hormone, is lower than from the controls and still more so if adrenal cortical extract is added to the hexokinase. This inhibition is relieved by insulin, which has no effect in the absence of the antagonists. This finding undoubtedly has an important bearing on the role of insulin in the normal and diabetic organism, even if this were not the only enzymatic mechanism influenced by insulin[5].

Finally, the different reactivity of glucose and fructose in biological systems is caused by their affinity to hexokinase. Whether we have to assume generally the presence of two hexokinases, glucokinase and fructokinase, is still not definitely known[6]. Independently of this problem a puzzling discrepancy was observed between glycolysis in the intact tissue, in tissue slices, in homogenates (disintegrated cells suspended in Ringer solution) and glycolysis in extracts free of structures[1]. In the first named types of tissue preparations from brain or malignant tumor glucose is much more quickly metabolized than fructose but the difference disappears in extracts from the same tissues; here, the total reactivity of both sugars is much greater and almost equal. The distinguishing factor is, as has recently been shown, the different concentration of ATP. The ATP splitting enzyme, the ATP-ase, is strongly adsorbed on the cell structures and so long as these are present, ATP is kept down by the activity of this enzyme. In this low range of ATP the affinity of fructose is much less than that of glucose, but in a higher range their affinity is equal. If these structures are removed, the same difference can be obtained in the extract with concentrations of added ATP much lower than those found by analysis in the living cell. Incidentally, it follows from these results that the *active* concentration of ATP in the living cell where the difference in the rates of fructose and glucose turnover is very conspicuous must be a small fraction of the total ATP found by analysis. The bulk of it is either separated by cell structures from the enzymes or otherwise bound to inert proteins.

C. *Carboxylase*

K. LOHMANN showed in 1937 that cocarboxylase, separable from the enzyme carboxylase by washing yeast with slightly alkaline solutions, is nothing else but diphosphothiamine, the diphosphoric ester of vitamin B_1[2]. The carboxylase reaction of yeast:

$$\text{pyruvic acid} \rightarrow \text{acetaldehyde} + CO_2$$

does not occur in the animal. But the important observation of PETERS[3], that in vitamin B_1 deficient animals pyruvate metabolism is inhibited and pyruvate concentration in the blood is strongly increased, proves that here also the breakdown of pyruvate needs cocarboxylase. The effectiveness of free thiamin in relieving the symptoms of B_1 deficiency in animals is easily explained by the reversible reaction[4]:

$$\text{thiamin} + 2\ ATP \rightleftarrows \text{thiaminediphosphate} + 2\ ADP.$$

The difference of the reaction of pyruvate in yeast and in the animal must be attributed to the specific enzyme proteins[5]. In the latter case the decarboxylation is always coupled with oxidation:

$$\text{pyruvate} + O = \text{acetate} + CO_2.$$

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F. LIPMANN discovered[1] that as intermediate acetylphosphate was formed, the phosphate group was taken up from inorganic phosphate and attached to the carboxyl group in the same way as in 1,3-phosphoglyceric acid. Because this enzyme has not yet been obtained in pure state and flavine protein and possibly diphosphopyridinenucleotide are also components of the system, it cannot be stated definitely whether these oxidations are one step reactions or not. Even in the case where the final decomposition of pyruvate is achieved by the way of synthetic reactions involving the tricarboxylic acid cycle of KREBS, cocarboxylase is a necessary component of the system.

The characteristic feature which we have already encountered with the other coenzymes, the adenylic system and cozymase, is likewise true for cocarboxylase: viz. the exact specifities of the reactions are bound to the enzyme proteins while the coenzymes exhibit only a sort of class specifity, as H_2 transporting, phosphate transferring, or decarboxylating agents. This is also true for other coenzymes like riboflavin or pyridoxal, etc.

Conclusions

Carbohydrate metabolism furnishes some very striking examples of the biological function of vitamins and hormones. As is now more and more revealed by the studies of intermediary metabolism, vitamins must be generally regarded as constituents of the effective groups of enzymes or coenzymes. Nicotinamide, the pellagra vitamin, is the reacting group of the cozymase, thiamin of the cocarboxylase, and adenylic acid plays also some role as a sort of vitamin. Quite recently it was found by LIPMANN and his group[2] that pantothenic acid (dihydroxydimethylbutyryl-β-alanide), another B-vitamin, is a constituent of the coenzyme of acetylation and acetate oxidation. In this way a great part of the acetic acid arising during the oxidation of carbohydrate and fat is disposed of.

Hormones on the other hand combine with enzymes, forming compounds of an increased or diminished activity, thereby controlling or regulating the speed of turnover of the metabolites. This is exemplified by the role of the cortical hormone and insulin on hexokinase.

It should therefore be acknowledged that the approach to intermediary metabolism described here, which tries to separate the global metabolic reaction into its constituting elements and to study the behavior and kinetics of the individual enzymatic steps, is not only of theoretical or academic interest but has yielded also results of great practical importance for general medicine, agriculture, and nutrition.

Résumé

Le schéma des étapes du dédoublement des glucosides dans la glycolyse des tissus animaux est exposé ici conformément aux résultats obtenus de 1932 à 1939. Toutes les substances intermédiaires sont phosphorylées. La transphosphorylation s'accomplit selon le système adénylique (acides adénosinetri-, di- et monophosphoriques). La grande énergie libre de ce groupe phosphorique n'est pas perdue, mais transmise aux intermédiaires de la glycolyse et s'accumule dans la phosphocréatine. Un autre type de phosphorylation est représenté par la phosphorolyse réversible du glycogène, avec formation du glucose-1-phosphate. Ce type est en réalité une «transglucosidation» comme on peut le démontrer dans la formation semblable du disaccharide à partir du glucose-1-phosphate et de la lévulose. En l'absence de la lévulose, le glucose-1-phosphate réagit avec l'enzyme en proportion stœchiométrique, et conserve l'énergie du groupe phosphorique dans la combinaison du glucose avec l'enzyme.

L'article traite des nombreuses réactions enzymatiques des enzymes purifiées et cristallisées et discute plus en détail la fonction des trois enzymes, soit: la transformation de l'acide 2-phosphoglycérique en phosphoénolpyruvique par l'énolase, la phosphorylation de la glucose par l'adénosinetriphosphate en présence de l'hexokinase, et le dédoublement de l'acide pyruvique par la carboxylase. Le rôle des vitamines comme groupes essentiels des coenzymes, et celui des hormones comme activateurs et inhibiteurs des enzymes sont mis en évidence dans les réactions métaboliques des glucosides.

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La biologie des hématinoprotéides oxygénables[1]

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On trouve dans toutes les cellules aérobies une série de biocatalyseurs de la famille des hématinoprotéides: les trois cytochromes, la peroxydase, la catalase. Souvent aussi, on y trouve la substance que KEILIN appelle «unspecific cell hematin». Selon STERN et MELNICK[3] les cellules aérobies contiennent un autre enzyme hématinoprotéidique, «l'enzyme de la réaction de Pasteur» qui catalyse l'action inhibitrice de l'oxygène sur la fermentation et la glycolyse.

Ces différentes substances fonctionnelles interviennent dans des processus biochimiques mettant primordialement en jeu la liaison chimique existant entre deux atomes d'oxygène, que ces atomes soient liés entre eux comme dans la molécule d'oxygène, ou

1 Conférence principale, présentée à la Société suisse de biologie médicale lors de la 127e Assemblée générale de la Société helvétique des sciences naturelles à Genève, le 31 août 1947.

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