b) Une diminution nette de la charge par unité de surface des mêmes bactéries irradiées, mais conservées dans leur milieu à l'abri de la lumière durant 24 h.

L'analyse spectrophotométrique des milieux tampons acétiques dans lesquels les bactéries, *Escherichia coli*, ont été soumises à l'action des rayons ultra-violets montre:

a) Un même niveau de substances absorbant la lumière dans la région du spectre correspondant à l'absorption des acides nucléiques, pour les liquides provenant des bacilles immédiatement après l'irradiation.

b) un enrichissement de ces milieux tampons en substances absorbant la lumière dans la région du spectre correspondant à l'absorption des acides nucléiques, des liquides dans lesquels les bacilles irradiés par les rayons UV. ont séjourné 24 h à l'abri de la lumière (Courbe 2).

L'analyse électrophorétique de la substance bactérienne met en relief le départ, après un certain temps de séjour à l'abri de la lumière, des substances qui étaient responsables de l'accroissement de la densité de charge par unité de surface dans les premiers moments qui suivent l'irradiation par rayons UV. L'analyse spectrophotométrique des liquides ayant contenus ces bacilles prouve en même temps la diffusion de ces substances de la cellule bactérienne vers la phase aqueuse et révèle leur nature. La photoréactivation possible dans les premiers moments qui suivent l'irradiation UV. des bacilles du fait de la présence dans la substance bactérienne des fragments de dépolymérisation de la macromolécule de l'acide nucléique, est rendu impossible lors du départ (diffusion) de ces fragments vers la phase aqueuse. Ces observations semblent indiquer que le phénomène de la photoréactivation serait lié à la répolymérisation de la macromolécule de l'acide désoxyribonucléique primitivement scindée.

I. Grundland,
H. Krzywicka et
M. Chojnacki

Institut de Biochimie et Biophysique P.A.N., Université de Varsovie, le 24 avril 1957.

Summary

Electrophoretic analysis of bacterial substance proves the departure, from UV irradiated microbes screened from light for a certain time, of substances which were responsible for increasing the charge density by surface unit during the first moments which follow the irradiation by UV rays. Spectrophotometric analysis of liquids having contained these bacilli proves concommitantly the diffusion of those substances from the bacterial substance to the aqueous phase and reveals their nature. The photoreactivity which is possible during the first moments following the UV irradiation of bacilli, due to the presence of the fragments of depolymerisation of nucleic acid macromolecule in the bacterial substance, becomes impossible after the departure (diffusion) of those fragments to the aqueous phase. These observations seem to show that the phenomenon of photoreactivity is connected with the repolymerisation of the macromolecule primitively divided of desoxyribonucleic acid.

Pro Memoria Carl Neuberg

Editorial Note. The Nestor of biochemistry, 14 days before his death, had worked out the following lecture experiments on the chemismus of certain fundamental biochemical processes of intermediary metabolism. We publish this original idea, which is of general and didactic interest, in memory of the great research worker and teacher, Carl Neuberg.

Lecture-Demonstration

by

Carl Neuberg*

Experimental Demonstration of some fundamental biochemical reactions**.

(1) Phosphorylation;
(2) Enzymatic dephosphorylation; simultaneous formation of insoluble bone salts;
(3) New form of trapping method for fixation of metabolic intermediates;
(4) Solubilization of the inorganic portion of bones and teeth in a neutral medium;
(5) Hydrotropic solubilization of insoluble matter;
(6) Instantaneous formation of free mineral acid by interaction of two neutral substances;
(7) Instantaneous occurrence of the carbamate reaction.

Ladies and Gentlemen,

You all know well the facts about which I will speak to you, but not all of you may have seen the corroborating experiments.

(1) Phosphorylation was discovered in 1905 by the famous Russian plant physiologist Leonid Iwanoff and described by him in French and German journals[1]. Iwanoff already showed that in many cases especially the biosynthetically famed phosphoorganic compounds consist of phosphoric acid esters of carbohydrates. Further work on this problem was done by Harden[2]. Iwanoff never received due credit for his important discovery since he did the worst thing that a researcher can do in such a situation: he died.

I want to show you the fundamental principle of the discovery in an extremely simple experiment as we can demonstrate it to-day based on the experience of 5 decades.

We start with a 20% solution of glucose, fructose or sucrose. To 100 ml of this sugar solution we add a freshly prepared, therefore well buffered solution of 4·2 g sodium dihydrogen phosphate dihydrate and 1·1 g of sodium bicarbonate in 25 ml water. The resulting 125 ml of liquid are shaken at 37° with 10 g of one of the dried yeasts now commercially available plus 20 ml carbon-

* Two weeks before he died Prof. Neuberg gave this lecture-demonstration at the New York Medical College. He spoke the introductory and the closing remarks, his daughter I. S. Forrest read the lecture, his co-worker A. L. Grauer carried out the experiments which she had adapted for lecture demonstration. After the lecture, tributes to Dr. Neuberg were made by Dr. F. F. Nord of Fordham University and Dr. Severo Ochoa of New York University.

** Supported by a contract of the US Atomic Energy Commission with the New York Medical College.

[1] I. Iwanoff, Trav. Soc. Nat. St.-Pétersb. (Léningr.) *34* (1905); Ber. dtsch. bot. Ges. *20*, 366 (1902); Z. physiol. Chem. *39*, 31 (1903); *42*, 464 (1904).

[2] A. Harden, *Alcoholic fermentation* (Longmans, Green & Co., London-New York 1932).

tetrachloride to effect plasmolysis. After 20 min the clear filtrate no longer reacts with magnesia mixture, showing the absence of inorganic phosphate, while the control sample to which the phosphorylating agent, dried yeast, has not been added still gives a strong Mg-reaction for inorganic phosphate.

(2) In 1911, there worked in my laboratory in Dahlem, KEMAL DJENAB[3], the brother of ATA TURK, the first president of Turkey. Together we published that the enzyme phosphatase which is present in all cells, in particular in microorganisms, precipitates insoluble inorganic calcium phosphate from solutions of Ca-salts of sugar phosphates.

Many authors soon confirmed this finding. It formed the basis of a theory of calcification of cartilage and bone formulated a few years later by ROBISON[4], and also for the application of this reaction to the histological determination of phosphatase recommended in 1938 by TAKAMATSU[5] and in 1939 by GOMORI[6]. Without discussing the question of the existence of various phosphatases with different pH optima in the acid, neutral or alkaline range and different types of phosphorylation, I wish to show you the basic experiment with an enzyme preparation from *Aspergillus oryzae*, the so-called Taka-phosphatase. This preparation has various applications in industry and medicine. It contains phosphatases, active at various pH values. As substrate we use calcium glycerophosphate, which is also commercially available. A suitable solution is prepared by dissolving 4·8 g calcium glycerophosphate and 3 g ammonium chloride in 100 ml water. On shaking with 3 g of the enzyme at room temperature, most of the enzyme goes into solution. At 40°, solid calcium phosphate separates from the clear filtrates in a few minutes. At room temperature, the same phenomenon can be observed after somewhat longer standing[7].

While we run this experiment, I shall show you the effect in tests which we set up a few hours ago.

Instead of calcium glycerophosphate, soluble calcium salts of many other organic phosphoric acid esters can be used. Calcium can be replaced by any cation which forms insoluble inorganic phosphates. I show you here the precipitation of ferric phosphate as the result of an analogous experiment set up this morning with pharmaceutically important ferric glycerophosphate.

(3) For all branches of experimental biology, knowledge of intermediary metabolism is of utmost importance. To establish the various steps involved, it is usually necessary to deduce the function of added compounds by the fact that they produce the same metabolic end products as would be expected under normal conditions. A procedure by which insight into metabolism is gained by trapping presumptive intermediary products with suitable reagents, was first applied in 1918 and has since been utilized in many cases[8]. These so-called trapping methods were especially valuable in the elucidation of intermediates in glycolytic processes. As a demonstration I have selected the classical example of the trapping of acetaldehyde which occurs as an intermediary product in alcoholic fermentation of sugar.

200 ml of a 5% sugar solution are fermented with 15 g dried yeast or the equivalent amount of fresh yeast. Another sample is treated in the same way but a neutral sulfite is added as trapping agent. As soon as fermentation starts an aliquot is withdrawn from both tests and a few drops of a sodium nitroprusside solution plus a few ml of a 5% diethylamine solution added. Without filtering or distilling, it can be seen that the sample containing sulfite becomes deep blue while the control solution remains colourless. To allow us to carry out the experiment in this simple manner, we use calcium sulfite as trapping agent. It is more stable than the sodium salt and no adjustment of pH is needed in this case. Diethylamine, like any secondary aliphatic amine, decomposes the aldehyde disulfite compound. The sugar is split according to the equation:

$$C_6H_{12}O_6 \rightarrow CH_3 \cdot CHO + CO_2 + CH_2\,OH \cdot CHOH \cdot CH_2OH$$

and in the presence of sulfite the intermediary acetaldehyde is accumulated:

$$C_6H_{12}O_6 + Na_2SO_3 + H_2O \rightarrow CH_3 \cdot CHOH \cdot SO_3Na + NaHCO_3 + CH_2OH \cdot CHOH \cdot CH_2OH.$$

Incidentally this is the process by which, during the first World War, glycerol was produced biosynthetically in considerable amounts in industry.

(4) Next I want to show you the solubilization of insoluble substances in aqueous solutions at neutral or weakly alkaline pH[9]. I make a fresh precipitate of inorganic calcium phosphate and you will see that it dissolves in a 5% solution of sodium nucleate while it obviously remains insoluble in water. In an article in the *Advances in Enzymology*, I have described 9 different mechanisms by which various naturally occurring substances can be brought into solution at neutral or weakly alkaline pH[10]. The theoretical basis for these solubilization phenomena is not quite simple. In general terms, it is a question of formation of molecular complexes of higher order but not necessarily always chelates.

To 30 ml of a 0·1 *M* solution of $CaCl_2$, I add 20 ml 0·1 *M* disodiumhydrogen phosphate. To one half of the resulting suspension of tricalcium phosphate, I add 75 ml water and to the other half 75 ml of 5% sodium nucleate solution. The sample which contains the nucleate becomes perfectly clear. Salts of ATP and other nucleotides show the same behaviour. The number of possible 'solvents' which effect the solubilization of such precipitates is enormous.

(5) Although hydrotropy may be considered as one special phenomenon of the general problem of solubilization of insoluble matter in nature, I still want to explain the reaction by showing you an example. When aqueous solutions of alkali salts of many aliphatic, aromatic and hydroaromatic acids are added to water, insoluble substances are brought into the aqueous phase. This group of solubilizing agents includes the nitrogen free acids formed on putrefaction of amino acids. Synthetic compounds in which the carboxylic acid group has been replaced by a sulfonic acid group show the same effect.

[3] K. DJENAB and C. NEUBERG, Biochem. Z. *82*, 391 (1917).

[4] R. ROBISON, *Significance of phosphoric esters in metabolism* (The New York University Press, 1932).

[5] H. TAKAMATSU, J. orient. Med. *31*, 34 (1938); Trans. Soc. path. Japan *29*, 492 (1939).

[6] G. GOMORI, Proc. Soc. exp. Biol. Med. N. Y. *42*, 23 (1939).

[7] I. S. FORREST, A. GRAUER, M. KREIDL, and C. NEUBERG, Enzymologia *17*, 97 (1954).

[8] C. NEUBERG and E. REINFURTH, Biochem. Z. *89*, 365 (1918). – C. NEUBERG and J. HIRSCH, Biochem. Z. *98*, 141 (1919). – C. NEUBERG, Amer. Brewer *75*, 22 (1942).

[9] C. NEUBERG and I. ROBERTS, Arch. Biochem. *20*, 185 (1949). – I. MANDL, A. GRAUER, and C. NEUBERG, Biochim. biophys. Acta *8*, 654 (1952) and *10*, 540 (1953).

[10] I. MANDL and C. NEUBERG, Advanc. Enzymol. *17*, 135 (1956).

This property described in detail in 1916[11] led to the development of modern detergents when the technical production of higher aliphatic alcohols was perfected in the United States.

For the demonstration I chose instead of Na-benzoate, which is a common product of metabolism, the related sodium salt of the medically important ortho-hydroxybenzoic acid, sodium salicylate. To 10 ml isoamyl alcohol, I add 10 ml of a 25% sodium salicylate solution. As you see, a clear solution is obtained immediately. The same is true when aniline and sodium salicylate are mixed. If I now add water to these clear solutions, separation into the components takes place.

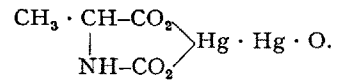
(6) Back in 1825, WÖHLER[12] made a fundamental discovery. He showed that in living organisms the neutral alkali salts of hydroxy acids ingested with vegetable intake are converted into alkali carbonates. Interpreted on the basis of modern concepts, this would mean that successively liberated CO_2, first emerging in the form of alkalibicarbonate, is converted into carbonate by enzymatic action of carbonic anhydrase. Through this conversion of salts of hydroxy acids, such as lactic, citric, tartaric, malic acids etc. into alkalicarbonates, the organism has a means of regulating the pH in the cells. On the other hand, hardly any reactions in which neutral substances are converted into acidic ones have been known. Recently we described a large number of mechanisms for the simple formation of strong mineral acids on mixing two neutral substances. This can be demonstrated for all thiol compounds, dicarbonyl compounds, salts of keto acids etc.[13].

Mercapto compounds, such as cysteine or glutathione in the form of their neutral salts, when mixed with cupric chloride at almost neutral pH immediately produce free HCl. Without presuming that this might explain the mechanism of HCl production in the stomach, we believe that this reaction provides the cell with a universal means of procuring H-ions instantaneously.

In the experiment that I shall show you, sodium ascorbate is mixed with $CuCl_2$. Free HCl and cuprous chloride form according to the equation: $2\ CuCl_2 + H_2$ $2\ CuCl + 2\ HCl$. Cuprous chloride as such is insoluble in water. If sodium chloride is added a water-soluble $NaCl \cdot CuCl$ complex forms and the mixture remains clear. The reaction mixture contains free HCl which can be determined electrometrically or simply with congo paper.

A mixture of 20 ml 0·1 *M* Na-ascorbate, 10 ml saturated sodium chloride solution and 40 ml 0·1 *M* cupric chloride solution immediately changes congo paper to a deep blue. It is well known that cupric ions are cell constituents of biological significance. Actually the salt of any metal with various states of oxidation can be used. Since in air the cuprous salt becomes reoxidized this reaction represents a continuous system. By the same principal all acids of physiological significance, hydrochloric, sulfuric, nitric, phosphoric, thiocyanic, may become available to the cell by this instantaneous reaction.

(7) ERLENMEYER[14] in 1875 made an observation the importance of which was not recognized by his contemporaries or later workers in this field. ERLENMEYER found that alkalicarbonate can add on to the α-amino group of synthetic α-amino caprylic acid forming the salt of a carbamino carboxylic acid. Later workers prepared alkaline earth salts of N-carboxy amino acids by bubbling CO_2 for hours or days into mixtures of amino acids and alkaline earth hydroxides at pH 13·5 with thorough ice cooling. For analytical purposes, NEUBERG and ISHIDA[15] had published in 1911 that instantaneous precipitation of α-amino acids at room temperature can be accomplished with a mixture of mercuric acetate and sodium carbonate. The reaction results in the formation of a basic mercuric carbamate, which, in the case of alanine, may be formulated



With the aid of this reaction, we can show the instantaneous precipitation of carbamates which are present in urine and blood. The reaction takes place at neutral pH under physiological conditions at room temperature.

I shall show you the experiment. To 10 ml of *M* alanine solution we add 10 ml potassium carbonate solution. If we now add 30 ml of a solution containing 160 g mercuric acetate per liter of methanol, a precipitate immediately separates. The amino acid itself is not precipitated by the reagent. If, however, any soluble carbonate is added, a dense precipitate of pure white flakes immediately separates. In the absence of the amino acid, the alkalicarbonate solution forms a yellowish-red basic mercuric acetate precipitate[16].

Ladies and Gentlemen—this is the end. I hope to have demonstrated to you that important biochemical reactions take place at great speed. Under physiological conditions, such reactions undoubtedly proceed momentarily. This statement by LIEBIG always remains valid: 'In a comprehensive view of the phenomenon of nature, we have no scale for that which we are accustomed to name small or great... That which is scarcely observable in a confined district appears inconceivably large when regarded in its extension through unlimited space'. The sentence refers to the continuous solution of phosphates by the cataracts of the River Nile to which the almost legendary fertility of the Nile Valley is ascribed.

Zusammenfassung

Es werden einfache Vorlesungsversuche beschrieben für Phosphorylierung, Dephosphorylierung, Abfangmethode, Auflösung des anorganischen Bestandteils von Knochen und Zähnen in neutralem Medium, Hydrotropie, momentane Bildung freier Säure beim Zusammenbringen von zwei neutralen Substanzen, momentanen Eintritt der Carbamatreaktion. Die einzelnen Reaktionen wurden derart modifiziert, dass sie in einer etwa einstündigen Vorlesung vorgeführt werden können.

[11] C. NEUBERG, Biochem. Z. *76*, 107 (1916).
[12] F. WÖHLER, Z. Physiol. *1*, 305 (1825).
[13] I. S. FORREST, A. GRAUER, M. KREIDL, and C. NEUBERG, Arch. Biochem. Biophys. *55*, 555 (1955).
[14] E. ERLENMEYER and O. SIEGEL, Liebigs Ann. *177*, 128 (1875).
[15] C. NEUBERG and M. ISHIDA, Biochem. Z. *37*, 142 (1911).
[16] C. NEUBERG, A. GRAUER, and M. KREIDL, Arch. Biochem. Biophys. *58*, 169 (1955).