Smithsonian Institution in 1925, Cushman dealt with the methods of study and other features of general interest in addition to presenting a bibliography of the most useful works and descriptions and illustrations of important genera. The present article, which in an outline preliminary to a larger treatise, aims to bring order out of the former classifications and to arrange the many genera in natural grouping. Cushman is particularly well fitted for this task, with his twenty-five years of experience in active work upon fossil and recent foraminifera from all parts of the world.

This is the first complete classification of the formaminifera based purely on the study of the ontogeny and phylogeny in conjunction with the geologic history. The form of the adult test has been used as the basis in most previous classifications, but this is not alone sufficient because it is only the earlier stages that give the true relationships. Even then these stages should be observed in the microspheric form, as this is retrospective, repeating in its young many of the ancestral stages. The megalospheric specimens skip many of these earlier stages, thus arriving earlier at adult development and even assuming later characters undeveloped in the microspheric form. These two forms of the same species, the microspheric, with the small initial chamber reproducing asexually, and the megalospheric, with large initial chamber producing zoospores which fuse as in sexual reproduction, have caused much confusion in earlier work.

Instead of the ten families used in recent years, Cushman employs forty-five, due to a stricter limitation resulting in more concise grouping. The number of genera recognized has also increased, but the closely defined genera will be more easily identified than the nine inclusive groups hitherto recognized, which often contain remotely related forms. The work of many writers on the group in the past twenty years has been adopted wherever possible and the author's own studies have been largely drawn upon. The development from simple undivided forms to chambered ones is followed and the chitinous and arenaceous species are recognized as primitive, as has been done by many writers in recent years. The paper is illustrated by a table and twenty-one plates of drawings showing the relationships of the families and genera.

The publication of similar outlines of classification in all branches of biology would be not only a great stimulus to the study of natural history but also a corresponding relief to the specialist burdened with nomenclatorial problems.

R. S. BASSLER

U. S. NATIONAL MUSEUM

SPECIAL ARTICLES

NOTES ON THE MECHANISM OF FERMENTATION[1]

INVESTIGATIONS in recent years have cleared up important parts of the chemical processes in which different fermentations are concerned. Without having found final explanations, Harden and Young or L. Iwanow, for instance, have proved the necessity of the presence of phosphoric salts in the case of alcoholic fermentation. On the other hand, in 1910 O. Neubauer was in a position to show, on the basis of experiments, that in a later phase of the breaking down of sugar, which is initiated by enzymes, pyruvic acid appears to be an intermediate product. However, several years earlier Magnus Levy indicated that acetaldehyde is a probable product of the disintegration of sugars. In fact, the acetaldehyde could be intercepted and fixed by the process of Connstein and Lüdecke,[2] furnishing in the following years, especially through the activities of several investigators,[3] and recently by Willaman and Letcher, significant analytical insight into the mechanism of the breaking down of carbohydrates by means of enzymes and different microorganisms.

However, none of these investigators have given an account of the processes which are doubtlessly indispensable in explaining the physico-chemical[4] mechanism which is involved. Probably assuming that the combination of the hexoses with inorganic salts, which is supposed to initiate the real decomposition of the sugar molecules, takes place outside of the cell, Paine, in the laboratory of Harden, investigated the permeability of yeast cells to hexosephosphates. These experiments have been interpreted very differently and very strangely by different workers. According to Harden, himself, "the yeast cell is at all

[1] From the Division of Agricultural Biochemistry, University of Minnesota, St. Paul, Minn. Presented before the meeting of the Minnesota Section, Society for Experimental Biology and Medicine, on December 15, 1926.

[2] German patent, No. 298593/12o (1915); German patent, No. 298594-6/12o (1916).

[3] Comp., F. F. Nord, *Die Naturw.*, *7*, 685 (1919).

[4] F. F. Nord, *Chem. Rev.*, *3*, 60, 76 (1926). (This paper on "Chemical Processes in Fermentations" contains a bibliography to which the reader is referred for many of the literature citations used in the present paper. There was expressed in the same paper (p. 69) the opinion that in view of the assumed relations between thyroxin and bios the reduction of carbonyl- and other compounds by means of fermenting yeast (Lintner, Lüers, Neuberg, Nord and others) could be explained by an intermittent interaction between bios and this compound. In the meantime the former formula of thyroxin and according to a private communication from Dr. Edward C. Kendall the analysis of bios were found to be in disagreement with the facts.

events partially permeable to the sodium salt." Höber draws the conclusion that the permeability to salts is small, but he regards it also as possible that the whole phenomenon may simulate superficial adsorption, and finally C. Neuberg understood from the description of the experiments that the cell is "throughout" permeable to hexose-di-phosphoric salts.

Smedley, MacLean and Hoffert[5] in 1924 expressed the opinion "that the hexosephosphate molecules are not able to pass through the wall of the yeast cell, but that glucose and phosphate molecules pass separately into the cell, and are there combined." This assertion might be regarded as an unconscious application of the proposition of Ruhland and Hoffman[6] according to which the smaller the volume of the molecules, the faster is supposed to be their penetration into plant cells. In spite of the fact that this is contradicted by the rule of Overton, the assertion possesses a certain probability.

We must remember that the almost impossible detection of hexosephosphates in fermentations by means of yeast cells is in good harmony with the abundant formation of hexosephosphates by fermentations which are free of cells. The membrane of the cell is scarcely permeable to the synthease of Iwanow. In the case of uninjured yeast cells, there is in the outer medium only a very small quantity of hexose-di-phosphate, which might partially penetrate into the cell. If we henceforth assume, especially in accordance with the considerations and experiments of Witzemann, Gurchot and others, that also the membrane of the yeast cell represents a dynamic system which might be compared to a copper-ferrocyanide membrane, and therefore can be acted upon by intermittent coagulation and peptization, then the whole process will become readily intelligible.

The externally produced hexose-di-phosphates penetrate into the interior of the cell until a suitable salt concentration is reached which brings about the coagulation of the membrane. Through the fissures of this now "crystalline" membrane, uncombined sugar—which is typically non-diffusible—can now penetrate into the cell where it will be esterified by means of the synthease there present. The alteration (not fermentation) of the hexosephosphates into the "transportation form" of the sugar which takes place isochronously, and which is again subject to the direct splitting into the compounds of the 3-carbon chain, changes the internal concentration of the salts in such a manner that a repeptization takes place, the influx of the sugar ceases, and the cycle may be started again.[7]

The chief characteristic of the process would be, under these conditions, an intermittent coagulation-peptization of the membrane, as well as endeavoring to maintain a membrane equilibrium in the sense of Clowes (1916). No acceptance is expressed, herewith, of his or v. Möllendorff's (1918) opinion that the membrane is comparable to an emulsion, which, of course, would make it impossible to understand the osmotic activities of the cell.

It is probable to a high degree that the greater part of the sugars is esterified within the cells where the enzymes exerting fermentation are located and where they will be liberated. It is, therefore, very important to possess a conception of the mechanism of the admittance. In contrast to this, it is only of secondary significance as to whether the hexose-di-phosphates originate through an intermediate hexose-mono-phosphate (compare, for instance, Komatsu and Nodzu, 1924). In any case, they are disintegrated and leave behind the sugar in the transportation form, which is readily cleavable and which does not need to be re-esterified.[8] Isochronous rearrangements of intermittent processes exclude, of course, the accumulation of any intermediate products in the case of a normal method of fermentation.

The chief characteristic of the transportation form of a compound or system may be regarded as its capability either to mediate in intermittent actions, or to enable irreversible reactions to proceed, especially in cases where the use of a potentially higher energy content is involved.[9] It is not supposed to exist in a form which can be investigated successfully by means of our present tools as a chemical entity. Its capacity to promote the aforementioned types of biological reactions is probably due in the main to an electron transfer caused by the ionic antagonism within the cell. Ionic antagonism, we know, also exerts a great influence upon enzyme action and there are certain reasons for assuming that the same is also true for the influence of adsorption. The reasons why it is improbable that we deal in the latter case with "molecular compounds" in the sense of P. Pfeiffer will be presented in a later paper.

At this point of the considerations, attempts were made to ferment the readily accessible hydroxy-pyruvic aldehyde in its mono-molecular or trimeric form,

[5] It is probably through an error that M. Schoen (Monogr. de l'Institut Pasteur, No. 3, p. 128: 1926) recently ascribed this suggestion to other authors.

[6] W. Ruhland and E. Hoffman, *Arch. wiss. Bot.*, 1, p. 1, (1925).

[7] Compare G. Bredig and M. Minaeff, Festschrift Z. Hundertjf, d. Technischen Hochschule Karlsruhe, 1925.

[8] O. Meyerhof, *Die Naturw., 41,* 757 (1926).

[9] It is under these circumstances misleading when *e.g.*, the hexose-mono-phosphoric acids are recently designated to be "active" (compare O. Meyerhof, *Die Naturwissenschaften* 14, 1179 (1926)).

with the purpose of obtaining a better insight into the processes governing the decomposition of the 3-carbon chain compounds. No significant fermentability of a 2 per cent. solution could be observed by means of the American top or bottom yeasts which were at my disposal, in the presence or absence of mono-potassium phosphate, not even after digestion for several days at 34° C. This observation does not justify at present the drawing of definite conclusions regarding the behavior of this compound toward yeasts especially in comparing its behavior with the simultaneously effected control fermentation of d-glucose.[10] In any case the substances are not biological equivalents.

Under these conditions, we could be inclined to doubt[11] the view of Neubauer concerning the occurrence as an intermediate product of pyruvic acid in the course of alcoholic fermentation which requires as a first step methylglyoxal. C. Neuberg, Hildesheimer and Karczag in 1911 reinvestigated this question on a macro-chemical scale and, after an uncertain interpretation in a brilliant manner confirmed the previous statement concerning the transient existence of this acid. Fortunately it does not seem necessary to question these observations when we take into consideration that the experiments of Neubauer or the last-named authors are not depending on each other, viewed from a biological standpoint. In contradiction to this, the assertion that pyruvic acid is acted upon faster by fermentation than is sugar could not be confirmed by the exact investigations of Lebedew (1917, 1924), Hägglund and Augustson (1925) and others, all the more, as the control experiments on the fermentability of the pyruvic acid were carried out under unphysiological conditions. The authentic measurements of the absorption spectra by Henri and Fromageot, to which we referred already in 1925, show that under conditions of biochemically permissible concentrations, the acid is only present in the readily fermentable enol form. On the other hand, we know that the pyruvic acid is a very strong acid (K = 0.56) and since it is so highly dissociated, in accordance with the observations of Brenner,[12], Brooks,[13] and others, it may only enter uninjured cells or reach the place of enzymatic activity with great difficulty if at all. The connection of these observations is clear! The acid which is originated in a biochemical process, that is to say, within the cell, is present in the enol form which is readily fermentable. It will be isochronously decarboxylated with the same speed as the transportation form of the sugar is formed. In contra-distinction to this, when pyruvic acid is added to the mash itself, it is in an uncomparable degree more highly concentrated and will be fermented only in such proportion as the enol form is present and ready to undergo disintegration. This again is dependent on its ability to penetrate into the cell. We see, therefore, in conformity with earlier results first the outstanding significance of the transportation form of a compound which is indispensable to the initiation of a biochemical reaction.[14] There belong probably in this small group also some sulfur containing compounds newly described and investigated which were supposed to have a decisive rôle in reversible physiological processes and certain compounds of the bile promoting the hydrodiffusion of the cell.[15] The same importance also attaches to the "isochronic rearrangement" of this form, to which we referred recently[16] in connection with certain cases. It may, therefore, be regarded as certain that "unphysiologic" pyruvic acid is fermented slower than sugar in contra-distinction to the "biologic" acid which ferments practically with the same speed as sugar. It appears equally probable that considerations based on structural organic chemistry alone are hardly suitable to justify positive or negative conclusions which may be drawn from the macrochemical behavior of methylglyoxal, hydroxypyruvic aldehyde or related compounds concerning their behavior under biochemical conditions. Accordingly in the case of intracellular reactions there does not appear to be any logical basis for the calculation of a quotient based upon the rate of the fermentation of glucose as compared to that of pyruvic acid.

The more we increase our knowledge concerning the marvelous functions of the cell, the more we appear to be justified in explaining chemical reactions

[10] This observation is in contrast to yet unpublished results obtained with Miss Mollie G. White, when this compound is acted upon by *fusarium lini B.* In this case it was possible to show that this fungus may utilize this compound as a sole carbon source.

[11] Compare also H. v. Euler, *Samml. chem. u. chem. techn. Vortr., 28,* No. 6/7, p. 60 (1926).

[12] W. Brenner, *Öfvers. Finska Vetensk.-Soc. Forhandl.,* 60, A, No. 4 (1917–1918).

[13] M. M. Brooks, Public Health Reports, No. 845 (1923).

[14] It does not seem desirable at present to complicate the conception by analyzing the highly probable influence that the transient acid must have on the interfacial tension of the membrane.

[15] Edward C. Kendall and F. F. Nord, *Journal Biol. Chem.,* 69, 295 (1926). F. F. Nord, *Die Naturwissenschaften,* 15, 356 (1927).

[16] F. F. Nord, Germ. Patent No. 434728/12o (1924); F. F. Nord, *Beitr. z. Physiologie,* 2, 301 (1924); C. Endoh, Rec. trav. chim. 44, 866 (1925).

on the generalization of a concept of transportation forms in contra-distinction to forms of a compound which can only be described by formulas represented in the usual manner of structural chemistry. It would also be necessary, before we can wholly explain the reactivity of the transportation form as contrasted to the ordinary form in its relation to the various variables, to know the arrangement of the electrons within the compound and the rôle which the electrical forces[17] play in the activity of the transportation form.

It is intended to report later in greater detail the experiments which are now in progress.

F. F. NORD

UNIVERSITY FARM,
ST. PAUL, MINNESOTA
November 30, 1926

PROTEUS HENRICENSIS NOV. SPEC.

A MICROORGANISM believed not to have been previously described has recently been isolated from putrefying material. The characteristics of this bacillus are such that it may be placed in the classification of Castellani and Chalmers, but not in the classification of Bergey, *et al.*

The genus Proteus Hauser[1] is defined as "highly pleomorphic rods." Filamentous and curved rods are common as involution forms. Gram-negative. Actively motile, possessing peritrichous flagella. Produce characteristic amoeboid colonies on moist media and decompose proteins. Ferment dextrose and sucrose but not lactose. Do not produce acetyl-methyl-carbinol.

The tribe Proteae Castellani and Chalmers, 1918,[2] is defined as "Bacillaceae growing well on ordinary laboratory media, not forming endospores, aerobic, without fluorescence or pigmentation, but liquefying gelatin." The tribe may be divided into genera as follows:

(A) Rapid gelatin liquefaction; do not ferment lactose; mostly Gram positive—Proteus.

(B) Slow gelatin liquefaction; ferment lactose; Gram negative—Cloaca.

The isolated microorganism has the following characteristics:

Rods: 0.5 to 0.7 by 1.0 to 3.0 microns, occurring singly and in pairs. Actively motile. Gram-negative. No spores.

Aerobic and facultative anaerobic.

Gelatin colonies: Irregular, spreading, rapidly liquefying.

Gelatin stab: Rapid, stratiform liquefaction.

Agar colonies: Opaque, gray, spreading.

Agar slant: Thin, bluish-gray, spreading.

Broth: Great turbidity, with thin, bluish pellicle.

Milk: Slightly acid, becoming markedly alkaline in forty-eight hours. Quick peptonization.

Indol formation abundant.

Acetyl-methyl-carbinol not formed.

Nitrates not reduced.

H_2S formed. Lead acetate turned brown.

Acid and gas in dextrose, xylose, trehalose and galactose.

Acid in glycerol.

No acid or gas in lactose, sucrose, mannitol, dulcitol, raffinose, levulose, arabinose, inositol, maltose, dextrin, salicin or sorbitol.

Not pathogenic for guinea pigs or rabbits.

This organism appears to be closely related to Proteus diffluens Castellani, 1915. It differs from diffluens in that it peptonizes milk, produces indol and does not liquefy coagulated blood serum. No information is at hand as to the action of P. diffluens on xylose and trehalose.

The name Proteus henricensis is suggested.

FREDERICK W. SHAW

MEDICAL COLLEGE OF VIRGINIA,
RICHMOND

THE RELATION OF TEMPERATURE TO HYDROGEN-ION CONCENTRATION OF BUFFER SOLUTIONS

THE influence of temperature on optimum hydrogen-ion concentration for diastatic activity of malt has been discussed by Olsen and Fine.[1] The experimental evidence submitted showed that with "water suspensions of a mixture of wheat and malted barley flours . . . containing different amounts of dilute acid and alkali [HCl and NaOH were used] . . . the optimum pH changes from about 4.3 at 25° C. to beyond 6.0 at 69° C." These authors "suggested that this change is due to the increased activity of the hydrogen-ions present and that these apparently different pH measurements would represent approximately equal hydrogen-ion activities if measured at the temperature of the reaction."

Similar observations have been made for other enzymes. More than thirty-five years ago O'Sullivan and Tompson[2] observed for invertase that "the most

[17] Compare J. N. Mukherjee und B. N. Ghosh, *J. Ind. Chem. Soc. 1*, 213 (1924).

[1] Bergey, *et al.*, "Manual of Determinative Bacteriology," 1923, page 209.

[2] Castellani and Chalmers, "Manual of Tropical Medicine," third edition, 1919, page 943.

[1] Olsen, Aksel G., and Morris S. Fine. *Cereal Chem.*, Vol. I (1924), pp. 215–221.

[2] O'Sullivan, C., and F. W. Tompson. *Jour. Chem. Soc.*, Vol. 57 (1890), 834–931.