see. It therefore seemed desirable to present a preliminary report of our work at this time.

PPA has little or no growth-factor activity, either for phenylalanine auxotrophs or for mutants with earlier blocks.[2] Although PPA is stable in alkaline solution, it is very labile to acid; on incubation at 37° C it is converted within a few minutes at pH 2–3, or a few hours at 6.0–6.5, to an active growth factor. This conversion is accelerated by heat. PPA produces a bioautographic spot distinct from phenylalanine; it is ninhydrin-negative.

Because of the instability of PPA to acid, stable accumulations of it could be obtained only in media heavily buffered at pH 7.5–8.0. The usual medium, starting at pH 7.0, became acid enough during cultivation (pH *ca.* 6.5) to account for the conversion of the initially accumulated PPA into an active factor, which in turn could be converted by the cells into phenylalanine.

PPA has been partly purified by charcoal chromatography and its active product has been identified as phenylpyruvic acid. This identification is based on comparison with an authentic sample[3] with respect to the following properties: biological activity, lability to alkali, paper chromatography, $FeCl_3$ color test, ultraviolet absorption spectrum, dinitrophenylhydrazone of m.p. and mixture m.p. 161–163 uncorr. [lit. (*6*) 162–164 corr.]. Attempts to isolate PPA are in progress.

Since the reaction that is blocked in the mutant eventually takes place in the medium, an interesting autocatalytic phenomenon can be observed. When a small amount of phenylalanine is placed on the surface of solid minimal medium heavily seeded with this organism, a circle of growth of the expected diameter appears after one day of incubation. During further incubation, however, this circle spreads until it fills the Petri dish. The organisms growing at a distance from the initial stimulus are genetically unchanged; on reinoculation on minimal medium they fail to grow unless primed with a trace of phenylalanine. Furthermore, if the usual glucose in the medium is replaced by succinate the pH increases rather than decreases during growth, and the area of response to phenylalanine does not spread even though PPA can be shown to accumulate.

The accumulation of phenylalanine by phenylalanine auxotrophs now offers no obstacle to including these mutants in the large class of those that appear to lack a single enzyme.

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[2] These mutants with earlier blocks require a mixture of phenylalanine, tyrosine, tryptophan, *p*-aminobenzoic acid, and *p*-hydroxybenzoic acid (*5*). They were used in the presence of all the required factors other than phenylalanine.

[3] Kindly furnished by Dr. Alton Meister.

A Technique for Culturing *Opalina*[1]

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Efforts have been made in the past to culture the protociliates, *Opalina*[2] and *Cepedea*. Pütter (*1*), Konsuloff (*2*), Larson (*3*), Larson and Allen (*4*), and Kedrowsky (*5*) cultured opalinids in complex media with varied success. Recently, Lwoff and Valenteni (*6*) tried to culture *Cepedea* in a complex but bacteria-free medium. In some instances, they were able to continue the culture for 26 days, but they were not certain of the absence of bacteria.

The object of our experiments was to find a method by which opalinids could be maintained continuously in stock cultures. It was thought that the use of such cultures might facilitate life cycle studies and the search for a simplified medium.

A modified Boeck and Drbohlav medium (*7*) for the culture of *Entamoeba histolytica* was used. It was composed of two portions, a solid egg slant and a liquid overlay.

The solid egg slant. Two whole eggs were mixed with 25 ml of distilled water. Two-milliliter portions of this mixture were put into each of a series of test tubes (15 × 125 mm). With the tubes in a slanted position, the egg mixture was coagulated in a water bath at 80° C. *The overlay.* The solid egg slant was covered by a layer of buffered saline solution of the following composition: NaCl, 4 g; $Na_2HPO_4 \cdot 7H_2O$, 2 g; KH_2PO_4, 0.3 g; KCl, 0.2 g; $CaCl_2$, 0.02 g; $MgSO_4$, trace; $NaHCO_3$, 0.4 g; and enough distilled water to make 1 l. The overlay had a pH of approximately 7.8, apparently optimal for culturing these opalinids. Three milliliters of this solution were added to each test tube containing a coagulated egg slant, and the tubes were then plugged with cotton and autoclaved.

To get our initial inoculum, we removed the contents of the rectum of a toad, *Bufo valliceps*, and placed them in a Petri dish containing some of the buffered saline solution. This inoculum contained opalinids, bacteria, and a species of *Entamoeba*. To each culture tube, 0.2 ml inactivated human serum was added and thoroughly mixed with the liquid overlay. To each tube, bacitracin (5 γ), or penicillin G sodium (2000 units), or dihydrostreptomycin sulfate (20 mg) was also added. To each tube, 0.1 ml of the inoculum containing the opalinids and the accompanying organisms was then added and the tubes were kept at room temperature.

After 48, 72, or 96 hr, 0.2 ml of the culture from the bottom of the tube was transferred with a sterile pipet to a new culture tube, similar in all respects

[1] The authors wish to acknowledge valuable suggestions from T. T. Chen and J. L. Mohr of the Zoology Department and Miss F. Hallman of the Department of Microbiology, School of Medicine, University of Southern California.

[2] The *Opalina* used in the experiments was obtained from the rectum of *Bufo valliceps* collected in Louisiana. The species has not been identified.

to the initial culture tubes. The period at which the transfer was made depended upon the abundance of opalinids in the culture. Subsequent transfers were made as required to keep the culture going. If bacterial growth was excessive, a combination of antibiotics (dihydrostreptomycin sulfate and penicillin G sodium) was added at the time of transfer.

Opalinids isolated from a single toad have been cultured continuously since Dec. 10, 1952. At the time of writing, March 30, 1953, they were still in abundant culture. The number and uniform size of the opalinids in all cultures indicated active multiplication and growth. Dividing forms have been observed. Attempts to start a culture with one or a few opalinids have not been successful. In some tubes, entamoebae were rather abundant. Control of bacterial growth with the above-mentioned antibiotics was, in most cases, satisfactory. If bacterial growth was very heavy, simultaneous or alternate use of two antibiotics was more satisfactory than the use of one alone.

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Phosphorylation of 3-Methylglucose by Hexokinase from Rat's Intestinal Mucosa[1, 2]

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It is now a well-established fact that galactose, glucose, and fructose are more rapidly absorbed from the intestine than are other monosaccharides and that in the selective absorption of these sugars some enzymic processes must be involved besides the simple physical process of diffusion. Verzár and his colleagues (*1*) proposed the theory that the selective absorption is connected with phosphorylation of the particular sugars in the intestinal mucosa. This theory was supported by the finding that the amount of hexose phosphate esters in the mucosal cells increases considerably during the absorption of galactose, glucose, and fructose (*2*). It was found, moreover, that homogenates of rat intestinal mucosa phosphorylate galactose, glucose, and fructose *in vitro* in the presence of adenosinetriphosphate (ATP) (*3*). In the latter experiments there was a fair agreement between the rate of phosphorylation of these sugars *in vitro* and the rate of absorption *in vivo*.

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[2] The valuable technical assistance of Jane H. Humm is gratefully acknowledged.

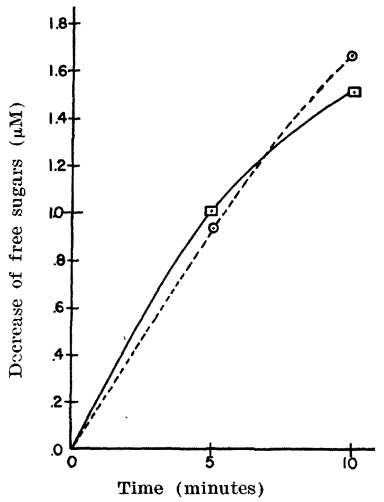

FIG. 1. Decrease of free glucose (interrupted line) and of 3-methylglucose (solid line) in 1-ml total samples in the presence of intestinal mucosa homogenate and ATP.

The use of the unnatural yet stable methyl ethers of glucose in the study of different aspects of carbohydrate metabolism was suggested several years ago by this author (*4, 5*) and the absorption of 2-, 3-, 5-, and 6-methyl derivatives of glucose from the rat's intestine was studied (*6*). Only 3-methylglucose is absorbed at about the same rate as glucose, whereas the blocking of the carbon atoms 2, 5, or 6 completely abolishes the selective absorption. This finding seemed to support the phosphorylation theory and emphasized the possibility of an aldose-ketose transformation during the absorption to yield phosphofructofuranoses.

The absorption of 3-methylglucose from the intestine of rats and cats was studied later in another laboratory with similar results (*7*). From the latter experiments the conclusion was drawn that phosphorylation cannot play an important role in the selective absorption of glucose. This conclusion was based on the finding that 3-methylglucose is not glycogenic in rats and thus "almost certainly not phosphorylated *in vivo*" (*8*). No direct experimental evidence was presented, however, to demonstrate the failure of the intestinal mucosa to phosphorylate 3-methylglucose.

In the course of further study of the metabolism of glucose methyl ethers, it was found recently in this laboratory that the amount of free reducing sugars decreases in the case of both glucose and 3-methylglucose if incubated with rat's intestinal mucosa homogenate and ATP *in vitro*. No decrease of such nature is noticeable if ATP is omitted from the mixture. This is strong presumptive evidence that the disappearance of free reducing sugars is due to phosphorylation by hexokinase. The following experimental procedure was used. White rats were sacrificed by decapitation and the intestinal mucosa was scraped off and homogenized with 2 ml of ice-cold distilled water. In small open Pyrex test tubes, mixtures containing