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.

References

1. PÜTTER, A. *Z. allgem. Physiol.*, **5**, 566 (1905).
2. KONSULOFF, S. *Arch. Protistenk.*, **44**, 285 (1922).
3. LARSON, M. *Trans. Am. Microscop. Soc.*, **47**, 1 (1928).
4. ———, and ALLEN, F. *Univ. Kansas Sci. Bull.*, **18**, 485 (1928).
5. KEDROWSKY, B. *Protoplasma*, **14**, 192 (1931).
6. LWOFF, A., and VALENTENI, S. *Ann. Inst. Pasteur.*, **75**, 1 (1948).
7. BOECK, W., and DRBOHLAV, J. *Am. J. Hyg.*, **5**, 371 (1925).

Manuscript received April 2, 1953.

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

T. Z. Csáky

Department of Pharmacology, University of North Carolina, Medical School, Chapel Hill

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*.

[1] Supported by a research grant from the U.S. Public Health Service.

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

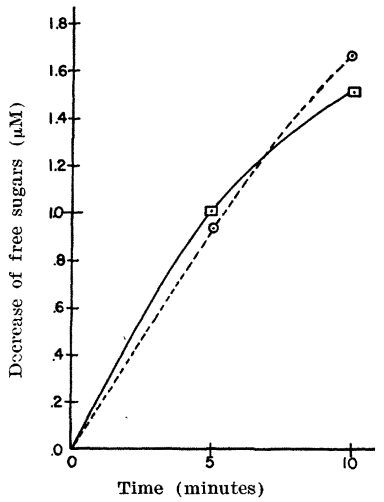

FIG. 1. Decrease of free glucose (interrupted line) and of 3-methylglucose (solid line) in 1-ml total samples in the presence of intestinal mucosa homogenate and ATP.

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

0.5 ml homogenate in total volume of 1.0 ml were incubated in a water bath at 30° C. Each tube contained besides the homogenate: tris(hydroxymethyl)-aminomethane-HCl buffer pH 8.0, 0.10 *M*; $MgCl_2$, 0.01 *M*; sugar, 0.01 *M*; and ATP, 0.03 *M*. Immediately after addition of homogenate and at short intervals thereafter 0.1-ml samples were taken and treated with $Ba(OH)_2$ and $ZnSO_4$ and centrifuged. In the supernatant the reducing capacity was measured according to the method of Somogyi (*9*). Figure 1 shows the rapid decrease of the reducing capacity in the samples containing glucose and 3-methylglucose in the presence of intestinal mucosa homogenate and ATP. The reducing capacity did not decrease in either of the sugars studied if the ATP was omitted from the experimental mixtures.

Similar results were obtained with soluble enzyme preparations from intestinal mucosa of rats and dogs. These preparations were made by scraping off the mucosa and freezing immediately with liquid air and pulverizing in a mortar. The powder while frozen was treated several times with cold (below −10° C) acetone, thereafter with ether, and dried in air. This acetone dry powder could be kept at temperatures below −10° C for 10–14 days without loss of activity. Soluble preparations were made by stirring the dry powder with ice-cold distilled water and centrifuging. The clear supernatant loses activity if stored more than 48 hr, even at temperatures below −10° C.

References

1. VERZÁR, F., and MCDOUGALL, E. F. *Absorption from the Intestine.* London: Longmans, Green (1936).
2. KJERULF-JERSEN, K. *Acta Physiol. Scand.*, **4**, 225 (1942).
3. HELE, M. P. *Nature*, **166**, 787 (1950).
4. CSÁKY, T. Z. *Ber. ges. Physiol.*, **94**, 662 (1936).
5. CSÁKY, T. Z., and SASS-KORTSÁK, E. *Ibid.*, **131**, 227 (1942).
6. CSÁKY, T. Z. *Z. physiol. Chem.*, **277**, 47 (1942).
7. CAMPBELL, P. N., and DAVSON, H. *Biochem. J.*, **43**, 426 (1948).
8. DAVSON, H. *A Textbook of General Physiology.* London: J. & A. Churchill, p. 303 (1951).
9. SOMOGYI, M. *J. Biol. Chem.*, **160**, 61 (1945).

Manuscript received March 27, 1953.

Comments and Communications

The Tension Concept in Aquatic Biology

STUDENTS of animal respiration have long used the tension concept to express the pressure of O_2 or CO_2 in body fluids. (Tension = partial pressure of O_2 or CO_2 in the gas phase at equilibrium between gas and liquid phases.) For terrestrial animals the concept is realistic and valuable because an exchange of gases between gas and liquid phases is essential to the respiration process. But in aquatic animals, where movement of CO_2 and O_2 is confined to the liquid phase, the tension concept is superfluous and even misleading. It is superfluous because the O_2 and CO_2 contents of the environment can be measured directly, and misleading because it leaves the erroneous impression that exchange of dissolved gases between animal and environment is similar to the exchange between gas and liquid phases. When respiration in aquatic environments is studied at constant temperature, the relation between tension and concentration is a constant one, and no serious ambiguity results; but when temperature varies, the tension-concentration relationship, of course, varies greatly as a result of changing solubilities of O_2 and CO_2.

These facts are well known, but their importance has not always been evaluated carefully. An example of such failure appears on pages 242–243 of *Comparative Animal Physiology* (Prosser, Brown, Bishop, Jahn and Wullf [Saunders, 1950]) where, in Fig. 53, a graph of Fry and Hart (The relation of temperature to oxygen consumption in the goldfish, *Biol. Bull.*, **94**, 66–77 [1948]) is reproduced. The graph shows the relation between oxygen tension and consumption in a goldfish exposed to temperatures varying between 5° and 35° C. From this graph Prosser *et al.* draw the conclusion: "At reduced tensions the oxygen consumption rapidly falls off, being affected at a higher oxygen tension for the higher metabolic rate, indicating the inability of the fish to maintain the specific activity at the designated temperature." But if this graph is reconstructed using concentrations, i.e., oxygen in solution, instead of tensions, the trend practically disappears, and the critical oxygen concentration proves to be about 60 μmol per liter regardless of temperature (or the fish's activity). The relative constancy of this critical oxygen concentration indicates that diffusion across the gill membranes was not an important limiting factor in O_2-uptake during those experiments—a significant implication of the data which Prosser *et al.* have missed while discussing a trend which was primarily an artifact of changing solubility.

The text goes on to say that one should not ignore the changing solubility of oxygen, and the increase in activity, with temperature. But no evaluation of these factors is made. One is left with the impression that these variables cannot be isolated. The authors could profitably have turned back to pages 210–211, where they reproduce Fick's law, and where they discuss the influence of temperature on the "diffusion constant." Fick's law employs concentrations, not tensions, thus the influence of solubility is taken into consideration simply by determining the concentration. The influence of temperature on the diffusivity or "diffusion constant" can also be estimated (about 1% per degree near 20° C) so the product Concentration × Diffusivity provides an expression of oxygen