liver glycogen level in fasting animals after administration of Carbutamide and Tolbutamide (*3, 15*), point toward mechanisms that are independent of the stimulating effect of these oral antidiabetics on insulin output.

C. v. HOLT, L. v. HOLT, J. KRACHT, B. KRÖNER, J. KÜHNAU
Department of Biochemistry and *Department of Pathology, University of Hamburg, Hamburg, Germany*

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17. We wish to acknowledge the technical assistance given by I. Hallmann, J. v. Kistowski, L. Klaue and J. Voss.

22 January 1957

Transport of Glucose by the Renal Tubule Cells of Anesthetized Dogs

Under ordinary circumstances the removal of glucose from the urine in the proximal tubules of the kidney is sufficiently complete to prevent the escape into the bladder of any significant fraction of the glucose crossing the glomerular barrier. The glucose moiety absorbed by the proximal tubule is usually considered to combine reversibly with a postulated intracellular carrier and to be returned to the peritubular fluid and thence to the peritubular capillaries and the renal vein. It is generally tacitly assumed that the transport process does not involve breakdown and resynthesis of the six-carbon chain of glucose. It has been suggested as an alternate hypothesis that the transport might involve a three-carbon unit (*1*). Evidence is presented here to substantiate the assumption that the six-carbon chain remains intact during the transport process (*2*).

The procedure is similar to that described in an earlier paper (*3*). Injection of a solution containing the blue dye, T-1824, glucose labeled with carbon-14 in the 1 position, and creatinine is made nearly instantaneously into the renal artery of an anaesthetized dog. Thirty successive samples of renal venous blood are obtained by means of a polyethylene catheter introduced into the left renal vein by way of the inferior vena cava. The concentration of each injected substance, determined in each blood sample, is divided by its concentration in the mass injected. The resultant concentration ratios are plotted as ordinates against sample number or time after injection as abscissas. The results of a representative experiment are shown in Fig. 1 (top).

That the radioactivity in the venous blood samples was derived from glucose rather than from other nonvolatile carbon compounds was established as follows. Paper chromatograms were prepared of desalted perchloric acid filtrates of the blood samples. Glucose was identified by means of aniline phthalate. Radioautograms and strip scanning for radioactivity indicated that glucose was the only substance present that possessed significant radioactivity.

The difference between the glucose and creatinine curves is assumed to represent the glucose absorbed from tubular urine by the proximal tubule cells and delivered to the peritubular capillaries. A necessary corollary of this assumption is that phlorhizin should, by blocking tubular transport of glucose, obliterate the differences between the glucose and creatinine curves. That it does this is shown in Fig. 1 (bottom). The broken line in Fig. 1 (top), relating the differences between the glucose and creatinine ratios, may therefore be taken to represent glucose returned to the circulation by the tubule cell.

Degradation, by a microbiological procedure (*4*), of the glucose in the venous blood samples containing this returned glucose showed that essentially all the recovered radioactivity resided in the 1 position. This absence of randomization establishes that there is no breakdown and resynthesis of the six-carbon chain of glucose in its transport across the tubule cell.

The mean transit time (*5*) from the glomerulus to the peritubular fluid cell border of glucose transported by the tubule cells is calculated as the difference between the mean transit times, from renal artery to catheter tip, of the transported glucose and of the creatinine; the assumption is made that creatinine does not enter these cells from either cell border. The average of the values found in eight experiments is approximately 10 sec. If it is assumed that the mean transit time of glucose from the glomerular barrier to the lumen border of the proximal tubule cells is of the order of 5 sec, then the mean transit time of glucose across the tubular cells is approximately 5 sec. The assignment of the value of 15 μ to the thickness of the cells and the assumption that diffusion is the mechanism of the passage of the complex across the cell permit calculation (*6*) of a value for the diffusion coefficient in the cytoplasm of the postulated glucose-carrier complex. This value is 0.2×10^{-6} cm²/sec. If it is now assumed, as was previously done for *p*-aminohippurate transport (*6*), that

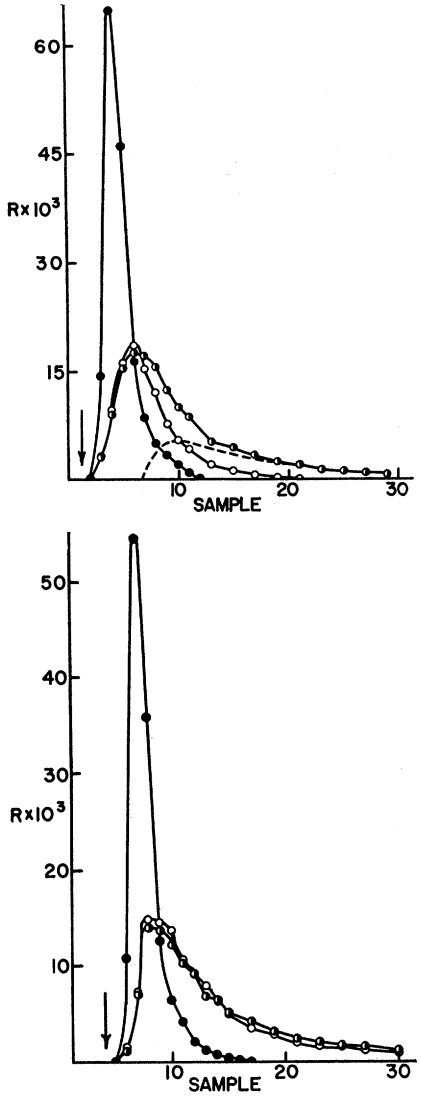

Fig. 1. Ordinates represent concentration of substance in sample divided by concentration in injection mass. Abscissas are the sample number. Filled circles indicate T-1824, open circles creatinine, half-filled circles glucose-1-C^{14}. Arrows denote the moments of injection. (Top) No phlorhizin; broken line indicates glucose returned from tubule urine; collection time, 1.32 sec per sample; (bottom), after phlorhizin; collection time, 1.40 sec per sample.

the viscosity of the cytoplasm is 10 times that of water, the particle weight of the complex is calculated to be of the order of 2000 g/mole. This calculation is presented as the basis for the suggestions that the glucose-carrier complex, if such exists, is not a macromolecule and that the transport does not directly involve organized intracellular particles, such as mitochondria.

In another series of experiments, collection of urine samples was made after injection into the renal artery of glucose uniformly labeled with carbon-14 and of ordinary creatinine; the excretion patterns (*6*) were then determined. After a load of ordinary glucose to produce glucosuria, the excretion patterns of C^{14}-labeled glucose and of creatinine were symmetrical. No displacement of the patterns, such as has been found with urea relative to creatinine (*7*), was noted. This finding suggests that glucose entering the tubule cells does not return to the lumen. After the administration of phlorhizin, the excretion patterns of C^{14}-labeled glucose and of creatinine were essentially identical. It is inferred from these findings that the combination of glucose with the postulated intracellular carrier occurs at the cell barrier rather than within the cell. That glucose may enter the cells from the peritubular fluid side is suggested by the finding, in most experiments of the type illustrated in Fig. 1, of glucose concentration ratios somewhat less than the creatinine concentration ratios in the samples at or preceding the maxima. No marked change in this relationship was noted after phlorhizin.

These findings form the basis for the following tentative conclusions. (i) Glucose is transported by the renal tubule cells without breakdown and resynthesis of the six-carbon chain. (ii) The transport system is of the membrane carrier rather than of the cytoplasm carrier variety. (iii) The postulated glucose-carrier complex is not a macromolecule.

FRANCIS P. CHINARD,
W. ROWLAND TAYLOR,
MARY F. NOLAN, THEODORE ENNS
Departments of Physiological Chemistry and Medicine, Johns Hopkins School of Medicine, and Medical Division, Baltimore City Hospitals, Baltimore, Maryland

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22 January 1957

Protection of Sulfhydryl Groups against Ionizing Radiation

Sulfhydryl compounds have long been known to be among the most radiosensitive of all organic compounds. A number of them, such as cysteine, glutathione, cysteamine (β-mercapto ethylamine) and 2,3-dimercapto 1-propanol (BAL) have been used as protective agents against the action of ionizing radiation. Their protective action was assumed to be owing to their ability to act as "free radical acceptors"—that is, to compete with other components of the system for the free OH, H, or HO_2 radicals formed as primary products of radiolysis of water.

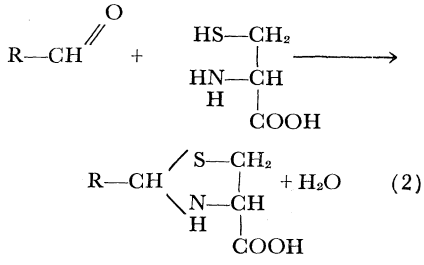
Recently, however, Eldjarn *et al.* (*1*), using "labeled" sulfur components, demonstrated that the protective action of cysteamine and cystamine ($HS—CH_2—CH_2NH_2$ and $NH_2 \cdot CH_2—CH_2—S—S—CH_2—CH_2 \cdot NH_2$) was due not so much to their ability to scavenge free radicals, but rather to a specific chemical reaction of cystamine with free sulfhydryl groups of the protein molecules. By this reaction, cystamine forms a disulfide linkage according to the scheme

$$\text{Protein—S—H} + \text{R—S*—S*—R} \rightarrow \text{protein—S—S*—R} + \text{H—S*—R} \quad (1)$$

where R—S*—S*—R represents the (labeled) cystamine and H—S*—R represents the resulting cysteamine. The resulting disulfide linkage appears to be much more resistant to the action of ionizing radiation than the "unprotected" sulfhydryl group, thus accounting for the effectiveness of these compounds as protective agents. The blocking of the sulfhydryl groups is temporary, and they are eventually restored by normal biological processes.

In the course of investigations of the development of odors on irradiation of food, we became interested in the formation of hydrogen sulfide and mercaptans as contributors to the odor problem, and consequently in methods of prevention of their formation. Because of the extreme radiosensitivity of the sulfur compounds involved, particularly cysteine and glutathione, effective reduction of the hydrogen sulfide formation by addition of free radical acceptors such as ascorbic acid did not appear to be promising.

The work of Eldjarn pointed out another approach—namely, masking these sulfhydryl groups by specific chemical reactions, rather than protecting them by competing reactions. Considerable work had been done in this direction. Schubert (*2*) and Vuataz (*3*) found that a number of carbonyl compounds were capable of reacting with sulfhydryl compounds even in dilute aqueous solutions, forming mercaptals, and reported that some of these no longer showed the characteristic sulfhydryl reactions, such as the purple nitroprusside color. The situation seemed to be particularly favorable in the presence of α-amino groups (such as in cysteine), since stable thiazole rings could be formed:



(2)

A number of aldehydes, dialdehydes, keto aldehydes, and keto acids were tried as additives, using approximately 100 percent excess. A nitroprusside test was made first; the mixture was then subjected to the action of atomic hydrogen and γ-rays. The techniques used for the production of hydrogen atoms as well as for treatment with γ-rays from a Co^{60} source have been described elsewhere (*4*). The best protection of cysteine against the action of atomic hydrogen was provided by glyoxal, formaldehyde, pyruvic acid, pyruvic aldehyde diacetyl, and glyceraldehyde, which reduced the amount of H_2S formed to 10 to 15 percent of that formed in unprotected solutions. By contrast, ascorbic acid used in tenfold excess reduced the amount of H_2S only to about one-half. Glutathione was protected best by glyoxal, pyruvic acid, and formaldehyde.

The protection against γ-rays, used at a level of 5×10^6 rep, is even more favorable. No measurable amount of H_2S was formed from cysteine solutions at *p*H 7 when either glyoxal or pyruvic acid was used as an additive in 100 percent excess. This compares favorably with ascorbic acid, which afforded only a 30 percent reduction of H_2S formation. With glutathione at *p*H 7, glyoxal (in 100 percent excess) prevented the formation of H_2S almost completely, pyruvic acid provided a 65 percent reduction; ascorbic acid used for comparison resulted in a 50 percent reduction of H_2S formation. A number of aldoses were tried but found to be ineffective, with the exception of glyceraldehyde. This is consistent with the assumption that sugars containing four or more carbons form cyclic hemiacetals and thus do not possess a reactive aldehyde group.