the freezing point was measured as digestion proceeded (Fig. 3). Apparent cleavage of peptide bonds resulted in nearly complete inactivation of the glycoprotein, which was accompanied by the appearance of dialyzable protein containing carbohydrate. Digestion with subtilisin (strain BPN′) (Nagarse) also inactivated the glycoprotein. Thus the freezing-point-depressant activity is an intrinsic property of the glycoprotein molecule and is not due to the presence of small amounts of substances of low molecular weight which could not be separated from the glycoprotein by dialysis. If the freezing-point-depressant activity were due to the presence of retained ions, no loss in activity would be observed upon degradation of the glycoprotein molecule.

Chemical analysis by the method of Lowry *et al.* (*5*), in which bovine serum albumin was used as a standard, indicated that the glycoprotein is 50 percent protein (by weight). An estimation of the total carbohydrate content by the phenol-sulfuric acid method (*13*), in which dextrose was used as a standard, indicated that the glycoprotein is 34 percent carbohydrate (by weight). Amino acid analysis after hydrolysis for 24 hours in 6*N* HCl at 110°C indicated that alanine and threonine were the only amino acids present. Galactosamine accounted for approximately 20 percent of the hydrolyzed glycoprotein. The glycoprotein is soluble in most protein-precipitating agents, such as 10 percent TCA, zinc hydroxide, and 3 percent tungstic acid solution; however, it is insoluble in acetone and 95 percent ethanol.

The concentrations of nonprotein nitrogen, TCA-soluble protein, and carbohydrate in the serums of the Antarctic fishes are four to five times higher than those in most temperate marine fishes. The solubility in TCA and the chemical composition of the glycoprotein suggest that these high concentrations are largely due to the presence of the glycoprotein. In the deepwater populations of *T. bernacchii* and *T. hansoni* where the serum freezing points are 0.1°C higher than those of the shallow-water populations, the concentrations of nonprotein nitrogen, TCA-soluble protein, and carbohydrate are correspondingly lower. This most likely is an indication of lower amounts of the glycoprotein in the deepwater populations. A similar correlation between higher serum freezing points and lower serum concentrations of nonprotein nitrogen and TCA-soluble constituents was also found in another benthic Antarctic fish, *Notothenia larseni*, which has a serum freezing point of −1.51°C (*14*).

ARTHUR L. DEVRIES
Department of Food Science and Technology, University of California, Davis 95616
DONALD E. WOHLSCHLAG
University of Texas, Marine Science Institute, Port Aransas 78373

References and Notes

1. J. L. Littlepage, in *Biology of the Antarctic Seas*, M. O. Lee, Ed. (American Geophysical Union, Washington, D.C., 1965), vol. 2, p. 6.
2. P. K. Dayton, G. A. Robilliard, A. L. DeVries, *Science*, **163**, 273 (1969).
3. A. Keys, *J. Biol. Chem.* **119**, 389 (1937).
4. S. Natelson, *Microtechniques of Clinical Chemistry* (Thomas, Springfield, Ill., 1963).
5. O. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951).
6. J. H. Roe, *ibid.* **212**, 335 (1954).
7. R. C. C. Steel and J. H. Torrie, *Principles and Procedures of Statistics* (McGraw-Hill, New York, 1960), pp. 73–74.
8. M. S. Gordon, in *Handbook of Physiology*, D. B. Dill, Ed. (American Physiological Soc., Washington, D.C., 1964), vol. 4, pp. 697–713.
9. M. S. Gordon, B. H. Amdur, P. F. Scholander, *Biol. Bull.* **122**, 52 (1962).
10. R. E. Feeney, D. T. Osuga, H. Maeda, *Arch. Biochem. Biophys.* **119**, 124 (1967).
11. P. Andrews, *Biochem. J.* **95**, 595 (1965).
12. H. K. Schachman, in *Methods in Enzymology*, S. Colowick and N. O. Kaplan, Eds. (Academic Press, New York, 1957), vol. 4, pp. 32–103.
13. M. Dubois, K. A. Gilles, J. K. Hamilton, P. A. Rebers, F. Smith, *Anal. Chem.* **28**, 350 (1956).
14. A. L. DeVries, thesis, Stanford University (1968).
15. Supported in part by NSF grants GA–45 and GA–661, administered by Drs. D. E. Wohlschlag and A. C. Giese, respectively. Part of this work was carried out in the Department of Food Science and Technology, College of Agricultural and Environmental Sciences, University of California, Davis, by A.L.D. with support of NSF grant GA–991 administered by Dr. R. E. Feeney. We thank A. C. Giese and R. E. Feeney for reading the manuscript, and members of the U.S. Navy Task Force 43 and the U.S. Antarctic Research Program for their support. A.L.D. was supported by an NIH postdoctoral fellowship [1-F2-AM-38,350-01 (h)] while in the terminal phases of this study.

23 August 1968; revised 4 November 1968

Do Trehalose and Trehalase Function in Renal Glucose Transport?

Abstract. *Maximum catalytic capacity for renal trehalase varied from 20 micromoles (of glucose produced per minute per gram of fresh tissue) for the armadillo down to zero for the rat, the cat, most turtles, fishes, and all birds and snakes investigated. Labeled trehalose could not be demonstrated in the kidney of the rabbit and the rat after injection with labeled glucose. These data do not support the hypothesis that trehalase and trehalose function in the transport of glucose through the kidney.*

After discovering trehalase in the blood serum of several mammals and turtles (*1*), we determined the trehalase activity in the kidneys of a representative number of mammals, birds, reptiles, amphibians, and fishes. Tissues were removed as quickly as possible and used fresh or after storage at −20°C. Absence of trehalase was not due to lability during storage. In rats, no activity was found, even when the assay was started within minutes after death. Human kidneys were removed 4 to 10 hours after death. Tissues were homogenized, diluted with water to a concentration of 100 mg/ml, and centrifuged at 1500 rev/min for 10 minutes. The supernatants contained 0.6 to 1.0 percent protein (60 to 100 mg per gram, fresh weight) and were assayed as described for serum (*1*); all reactions followed zero-order kinetics. By incubating homogenates with low activities up to 48 hours, we determined the sensitivity to be in excess of 0.005 μmole/min.

The rabbit kidney cortex contains all the enzymes for the synthesis of trehalose, and trehalase is localized within the cortical tubules of the human, the mouse, and the rabbit (*2*). Sacktor proposed that, in mammals, the reaction

2 Glucose → trehalose → 2 glucose

functions in a mechanism for tubular reabsorption of glucose; rapid synthesis of trehalose would provide a steep glucose concentration gradient between the glomerular filtrate and the tubular cell (*2*). A similar hypothesis has been used by Treherne to rationalize the rapid synthesis of blood trehalose from absorbed monosaccharides in insects (*3*).

The fasting normal human with a blood glucose concentration of 0.09 percent reabsorbs about 150 mg/min, which is 0.5 mg per gram of kidney or 0.8 mg per gram of cortex. The kidney would then have to produce from trehalose 2.8 μmole of glucose per minute per gram (4 μmole per gram of cortex); when the maximum tubular reabsorptive capacity is used, after a large sugar

Table 1. Trehalase activity in tissues of human males. Results are given as the maximum catalytic capacity in micromoles of glucose, produced per minute per gram of fresh tissue, at *p*H 6.2, and at 38°C.

Patient		Liver	Kidney
Age (yr)	Cause of death		
		Cortex	
62	Pulmonary carcinoma	0.04	0.7
48	Accident	0.08	1.2
68	Myocardial infarction	0.20	2.3
60	Myocardial infarction	0.37	2.2
		Whole	
43	Myocardial infarction	0.07	1.4
66	Thrombosis, with chronic renal disease and occlusion of renal arteries	0.015	0.22

load, or in diabetes, at least 5.5 μmole of trehalase activity would be required to guard against net accumulation of trehalose in the tubular cell or leakage of trehalose into the blood. Similar trehalase activities should be present in the kidneys of all animals for which such a mechanism of glucose reabsorption is postulated.

Sacktor's values (*2*) in human cortex (4.2, 2.4, and 0.75 μmole) and the values in Table 1 fall somewhat short of this requirement, but they may be sufficient to support the hypothesis because, of necessity, these kidneys were removed several hours after the patients died, which may have diminished enzyme activity. Interestingly, the kidney in which the renal artery was occluded had a very low activity.

Trehalase activity in liver was much lower than in kidney (Table 1), but higher than serum acitvity (0.00 to 0.01 μmole) in man (*1*).

A serious objection to the hypothesis is the absence of trehalase from the kidney of the cotton rat, the Wistar rat, the house cat, all birds and snakes, and most turtles and fishes (Table 2). The absence or near-absence of trehalase in the rat, the cat, and, in addition, in two goat species was also observed by Sacktor (*2*).

Kidneys of species which are capable of rapid trehalose synthesis, but which lack trehalase, might still be able to function according to the hypothesis, if trehalase were present in serum or in liver. Indeed, several mammals have an active trehalase in liver and serum (*1*), which could hydrolyze trehalose spilled into the serum by the kidney. However, the species that lacked trehalase in the kidney showed no activity in serum or in liver.

If rapid conversion of glucose to trehalose provides the steep concentration gradient which allows passive absorption of glucose, the ratio of trehalose to glucose in the tubular cell should be high. However, the kidneys of rabbits and rats did not contain measurable quantities of trehalose.

The role of trehalose as an intermediate in glucose transport was further investigated by isotope studies. New Zealand White rabbits (which show high renal trehalase activity) and Wistar rats (which have no renal trehalase activity) were anesthetized and injected in the heart with a 5 percent solution of uniformly labeled glucose-C^{14} (25 mg per kilogram of body weight); 10 and 20 minutes later, the kidneys were removed and immediately boiled to stop further enzyme action. The aqueous extracts were concentrated, deproteinized, de-ionized, and chromatographed on paper in a butanol, ethanol, water system (52:30:18). The area with the approximate R_F value of trehalose was eluted and contained 10 to 30 percent as much radioactivity as the glucose fraction. The suspected trehalose eluate was then mixed with carrier trehalose and boiled with HCl or incubated with fresh rabbit kidney extract until it was completely hydrolyzed to glucose. Upon rechromatography, this glucose fraction contained less than 1 percent of the radioactivity, the remaining 99 percent still being in the trehalose area of the paper. These experiments do not support the hypothesis (*2*) that trehalose is an intermediate in glucose transport through the kidney in species with or without renal trehalase activity.

Table 2. Trehalase activity in whole kidneys of mammals (other than man) and of some other vertebrates. Results are in micromoles of glucose produced per gram of fresh tissue at *p*H 6.2, and at 38°C.

Species	Samples (No.)	Whole kidney
Other mammals		
Armadillo (*Dasypus novemcinctus*)	2	20
Gray fox (*Urocyon cinereoargenteus*)	1	14
Cottontail rabbit (*Sylvilagus floridanus*)	1	12
Cotton mouse (*Peromyscus gossypinus*)	5	7–20
White laboratory mouse	5	4–8
Raccoon (*Procyon lotor*)	2	4–9
Eastern wood rat (*Neotoma floridana*)	2	0.1–0.5
Cotton rat (*Sigmodon hispidus*)	1	0.00
Wistar rat	4	0.00
House cat	2	0.00
*Other vertebrates**		
Birds (20 species)		0.00
Snakes (12 species)		0.00
Turtles (7 species)		0.0–1
Frogs and toads (3 species)		0.5–2.5
Fishes (6 species)		0.0–0.5

* The complete list is available from the author.

EMILE VAN HANDEL
Florida State Board of Health, Entomological Research Center, Vero Beach 32960

References and Notes

1. E. Van Handel, *Comp. Biochem. Physiol.* **26**, 561 (1968).
2. B. Sacktor, *Proc. Nat. Acad. Sci. U.S.* **60**, 1007 (1968); W. Grossman and B. Sacktor, *Science* **161**, 571 (1968).
3. J. E. Treherne, *J. Exp. Biol.* **35**, 297 (1958).
4. Supported by NIH grant AI-06587. The human tissues were provided by Dr. L. E. McHenry, and the animal tissues by Drs. H. Kale and J. Edman. The analyses were performed by J. E. Guira.

30 September 1968; revised 4 December 1968

Antibiotic-Induced Surface Changes in Microorganisms Demonstrated by Scanning Electron Microscopy

Although available for several years, the scanning electron microscope has had little use by microbiologists. Bartlett (*1*) has examined surface structures of foraminifera, Williams and Davies (*2*) have studied actinomycetes, and Gray (*3*) has examined soil microorganisms.

Stereoscan electron microscopy permits the rapid examination of the surfaces of large numbers of organisms in three dimensions. Preparation of the material is relatively simple. The chief drawback to the machines now available is that the resolution (about 200 Å) is insufficient to resolve some of the surface structures, such as the flagella and fimbriae of some Gram-negative organisms. This considerably limits the value of the high magnifications obtainable, which are in excess of 100,000. Still, information about surface changes caused by antibiotics not obtainable with light microscopy or only laboriously reconstructed from electron microscopic studies can be obtained.

The Oxford strain of *Staphylococcus aureus* and a hemolytic streptococcus (Lancefield group A) obtained from clinical material, cultured in ordinary nutrient or glucose broth with or without ampicillin (100 μg/ml) for 1.5 to 2.5 hours, were fixed overnight in 1 percent glutaraldehyde in 5 percent sucrose solution. The suspensions were