Table 2. Effect of myoinositol in broths containing varying amounts of inorganic phosphate.

Additional phospate (%)	Additional inositol (%)	Maximum yield (μg/ml)	Stimulation (%)
	*Organic substrate**		
0	0.00	269	
0	.05	279	+ 4
0.1	.00	60	
.1	.05	117	+87
.2	.00	83	
.2	.05	118	+41
	Synthetic substrate†		
0.005	0.000	166	
.005	.050	120	−28
.015‡	.000	288	
.015	.050	350	+ 22
.030	.000	72	
.030	.050	111	+ 54
.050	.000	14	
.050	.050	17	+ 21

* Glucose-yeast extract medium. † Glucose-ammonium nitrate (*8*). ‡ Normal amount of phosphate in this substrate.

would be unusually high. This indicates that the formation of the inositol ring may be a limiting step at least in many cases in the formation of streptomycin.

Further support for the theory that myoinositol is a direct precursor for streptomycin is that myoinositol, to some extent, is capable of overcoming the depressing effect of high concentrations of phosphate. This is shown to be true for the organic substrate already described and for a simple medium of glucose and ammonium nitrate (*8*). The total activity in the high phosphate cultures did not reach that of the controls, but the percentage of stimulation was much higher in the presence of large amounts of phosphate (Table 2). The optimal proportion between inositol and phosphate was not determined. There is still the question of whether myoinositol is the natural precursor for the streptidine, although the indications are that it is the precursor. An interesting fact which some authors seem to have overlooked is that the myoinositol has a steric configuration other than that of the streptidine which has a scyllo configuration. This means that if the myoinositol before or after the attachment of the two guanido groups does not undergo any rearrangements, the glucosidic band connecting it with streptobiosamine must be established; at carbon atom 2 simultaneous inversion of the steric configuration at this atom takes place. Only one author (*9*) has tested scylloinositol which has the same steric configuration as streptidine. He found that scylloinositol did not increase the production of streptomycin; the compound was, however, capable of reversing the inhibitory effect shown by its oxidation product myoinosose.

HENRIK HEDING
Institute of Microbiology, Rutgers University, New Brunswick, New Jersey

References and Notes

1. S. K. Majumdar and H. J. Kutzner, *Appl. Microbiol.* **10**, 157 (1962).
2. D. J. D. Hockenhull, *Progr. Ind. Microbiol.* **2**, (**1960**), 131 (1960); personal communication.
3. G. D. Hunter, M. Herbert, D. J. D. Hockenhull, *Biochem. J.* **58**, 249 (1954).
4. J. B. Walker, *J. Biol. Chem.* **231**, 1 (1958).
5. S. C. Shen, C. P. Chen, M. L. Hsu, H. Wang, *Sheng Wu Hua Hsueh Yu Sheng Wu Wu Li Hsueh Pao* **2**, 253 (1962), also *Chem. Abstr.* **59**, 13131*b* (1963).
6. R. Nomi, *J. Bacteriol.* **86**, 1220 (1963).
7. G. D. Hunter and D. J. D. Hockenhull, *Biochem. J.* **59**, 268 (1955).
8. J. H. Ferguson, H. T. Huang, J. W. Davisson, *Appl. Microbiol.* **5**, 339 (1957).
9. G. D. Hunter, *Giorn. Microbiol.* **2**, 312 (1956).
10. Supported by National Science Foundation grant G-13950 and conducted under the supervision of Dr. Selman A. Waksman.

12 December 1963

Conversion of Leucoanthocyanins into the Corresponding Anthocyanidins

Abstract. *Purified preparations of leucoanthocyanins from several sources were heated in butanol–hydrochloric acid solutions from 50° to 90°C and the rate of production of cyanidin was measured. From the temperature dependence of this rate, the calculated energy of activation was of the order of magnitude of 20,000 calories. The rate-limiting step in this reaction was similar for two preparations tested, and of lower energy for a third, presumably of lower degree of polymerization.*

Colorless precursors of anthocyanidins occur either as the pesudobase (*1*) or leucoanthocyanin (*2*), also termed anthocyanogens, proanthocyanidins, or flavolans (*3*). Since Rosenheim (*2*) first demonstrated the presence of a colorless precursor of oenidin (cyanidin) in grape leaves, which was converted into the colored oenidin during boiling with 20 percent hydrochloric acid, the existence and widespread distribution of leucoanthocyanins in plant tissue has been demonstrated qualitatively (*4–9*). While the Robinsons (*4*) used solubility and color reactions for qualitative identification, Bate-Smith (*6*) first introduced paper chromatography for the identification of anthocyanidins formed from leucoanthocyanidins, and this technique was extended by Roux (*8, 9*). Cyanidin is the chief anthocyanidin pigment obtained from plant leucoanthocyanins, although delphinidin and others have been reported in some cases. The conversion was obtained by heating aqueous extracts with hydrochloric or sulfuric acid (the Robinsons (*4*) used boiling 10 percent HCl, Bate-Smith (*6, 7*) used hot 2*N* HCl, heating the reaction in a boiling water bath). Quantitative methods for their determination were introduced by Pigman *et al.* (*10*) and modified by others (*11–13*). More empirical modifications were reported by Luh *et al.* and by Nakayama and Chichester (*14*). Pigman *et al.* (*10*) introduced the use of *n*-propanol solutions of hydrochloric acid instead of aqueous or methanol solutions and reported some observations on the kinetics of conversion of spruce leucoanthocyanins to cyanidin. Swain and Hillis (*11*) introduced the use of *n*-butanol in place of the more volatile *n*-propanol, and reported that the conversion was not quantitative even over the range of 50 to 400 μg of leucoanthocyanin. While it is known that the conversion of anthocyanogen into the corresponding anthocyanidin, on heating with alcohol containing HCl, is not complete, yields of 10 percent or less being obtained, the kinetics of the reaction and its mechanism have not been investigated. Pigman *et al.* (*10*) reported that the conversion in *n*-propanol containing HCl was reduced in rate and extent by the presence of water. They obtained a higher yield of anthocyanin in 0.03*N* HCl in the absence of water than in 1.8*N* HCl in the presence of 20 percent of water, by volume. This result was confirmed and extended by Roux and Bill (*15*) who reported yields of about 40 percent with anhydrous 0.03 to 0.02*N* HCl in *n*-propanol in comparison with 3 percent yields in aqueous 3*N* HCl and 20 percent yield with 3*N* HCl in *n*-propanol under the conditions of Pigman *et al.* (*10*). They proposed that the conversion of flavan-3, 4-diols into anthocyanidin occurs by dehydration at the diol group followed by oxidation or disproportionation. Molecular size limited the extent of conversion (*13, 16*). The yield of fise-

Table 1. The percentage conversion of three leucoanthocyanin preparations into cyanidin on heating with a solution of *n*-butanol in HCl.

Temperature (°C)	Number of minutes					
	5	10	15	20	30	60
	Grape phlobatannin					
50	0.10	0.56	0.42	0.56	0.63	1.15
60	0.90	0.90		1.90		2.84
70	0.94	1.70	2.07	2.59	3.59	5.27
80	2.09	3.45	4.48	4.96	5.93	7.32
90	4.23	6.05	7.09	7.80	8.70	9.92
	Cacao					
70	2.00	2.92	3.87		6.04	8.24
90	4.79	5.85*				
	Melacacidin					
50	0.25	0.46	0.61	0.82	1.14	1.32
80	0.39	1.08	1.90	2.33	3.24	5.84
90	1.83	2.89	3.88	4.50	5.63	7.04

* After 8 minutes.

Table 2. First order reaction rate constants (K) of conversion of three leucoanthocyanin preparations into cyanidin after heating with a solution of *n*-butanol in HCl.

$K \times 10^4$					E
50°C	60°C	70°C	80°C	90°C	Cal
		Grape leucoanthocyanin			
5.74	6.30	16.0	29.5	84.0	21,000
		Cocao leucoanthocyanin			
		17.1		35.1	23,000
		Melacacidin leucoanthocyanin			
			9.83	21.4	12,000

tinidin was reported (*16*) to be about 24 percent from the monomeric leucofisetinidin and to decrease to about 7 percent for the trimeric tannin and to 5 percent for pentameric or decameric tannins. Pigman *et al.* (*10*), on the basis of qualitative observations, indicated that the reaction occurred in stages but did not specify them.

To obtain additional information on the mechanism of the conversion, the rate of conversion of several leucoanthocyanin preparations in a solution of *n*-butanol in HCl at several temperatures was determined. The reaction was followed by heating 5 ml of ethanol solution of leucoanthocyanin containing 5 to 50 mg of leucoanthocyanin preparation (*17*) with 50 ml of *n*-butanol containing 5 percent by volume of concentrated hydrochloric acid at various temperatures. Portions were removed and cooled in an ice bath and their absorbance was determined at 550 mμ in a 1-cm quartz cell in a Beckmann Model DU spectrophotometer. The concentration of cyanidin produced was obtained from the absorbance of a pure preparation of cyanidin in *n*-butanol-HCl in the range of 0 to 10 μg/ml. The concentration of leucoanthocyanin preparation usually was 0.091 mg/ml and the hydrochloric acid was approximately 0.66N.

The conversion into cyanidin varied with the heating conditions from 0.10 percent to almost 10 percent (Table 1). Assuming that the equivalent weight of the leucoanthocyanin equaled the molecular weight of cyanidin, we found that when the logarithm of the concentration of unconverted leucoanthocyanin was plotted against time, the graph was linear for the first 10 minutes at higher temperatures (above 60°C) and for the first 60 minutes at lower temperatures (60°C and below). The first-order specific reaction rate constants calculated from these graphs are shown in Table 2. The Arrhenius constant corresponding to these rate constants, calculated from the slope of the curve of log K versus $1/T$ varies from 23,000 calories for cacao leucoanthocyanin to 12,000 for melacacidin. A preparation of leucocyanidin obtained by reducing taxifolin with borohydride in ethanol according to the procedure of Brown (*18*) yielded a compound which formed cyanidin in the cold on addition to acidified butanol. These observations indicate that the energy required for the rate-limiting step is smaller the lower the degree of polymerization of the compound tested.

M. A. JOSLYN
J. L. GOLDSTEIN
Department of Nutritional Sciences, University of California, Berkeley

References and Notes

1. R. Willstätter and A. E. Everest, *Ann.* **401**, 205 (1913).
2. O. Rosenheim *Biochem. J.* **14**, 178 (1920).
3. K. Freudenberg and K. Weinges, *Ann.* **613**, 61 (1958); *Tetrahedron* **8**, 336 (1960); K. Winges, *Chem. Ber.* **94**, 3032 (1961).
4. G. M. Robinson and R. Robinson, *Biochem. J.* **27**, 206 (1933).
5. G. M. Robinson, *J. Chem. Soc.* **1937**, 1157.
6. E. C. Bate-Smith, *Biochem. J.* **58**, 122 (1954).
7. ——— and N. M. Lerner, *ibid.*, p. 126.
8. D. G. Roux, *Nature* **179**, 305 (1957).
9. ———, *ibid.* **180**, 973 (1957).
10. W. Pigman, E. Anderson, R. Fischer, M. A. Buchanan, B. L. Browning, *Tappi* **36**, 4 (1953.)
11. T. Swain and W. E. Hillis, *J. Sci. Food Agr.* **10**, 63 (1959).
12. G. Harris and R. W. Ricketts, *J. Inst. Brewing* **65**, 331 (1959); W. D. McFarlane, *ibid.* **67**, 502 (1961).
13. D. W. Manson, *Tappi* **43**, 59 (1960).
14. B. S. Luh, S. J. Leonard, D. S. Patel, *Food Technol.* **14**, 53 (1960); T. O. M. Nakayama and C. O. Chichester, *Nature* **199**, 72 (1963).
15. D. G. Roux and M. C. Bill, *Nature* **183**, 42 (1959).
16. D. G. Roux and E. Paulus, *Biochem. J.* **82**, 320 (1962).
17. The grape leucoanthocyanin preparation was a grape phlobatannin preparation from White Colombard grape pomace obtained from Dr. F. DeEds of the Western Regional Research Laboratory, U.S. Department of Agriculture, Albany, Calif.; the cacao leucoanthocyanin preparation was obtained from Dr. T. Swain from a preparation obtained by the procedure of W. G. C. Fosyth, *Biochem. J.* **51**, 511, 516 (1952); the melacacidin was supplied by Dr. W. Bottomley.
18. H. C. Brown, *Hydroboration* (Benjamin, New York, 1962). The reduction of taxifolin by sodium borohydride to leucocyanides was reported first by T. Swain, *Chem. and Ind.* **1954**, 1144 (1954), but the experimental details were not given. These were given later by A. K. Ganguly and T. R. Seshadri, *Tetrahedron* **6**, 21 (1959).
19. This work was supported in part by U.S. Public Health grant No. EF 00080.

18 November 1963

Phospholipid-Sugar Complexes in Relation to Cell Membrane Monosaccharide Transport

Abstract. *Phospholipids extracted from "ghosts" of human erythrocytes or from other sources carry substantial quantities of glucose or other monosaccharides from the dry state into highly nonpolar solvents. Various characteristics of this weak association phenomenon show suggestive parallels with known properties of the mediated sugar-transfer system in the membrane of the intact red cell.*

Much evidence has accumulated in support of the thesis that the penetration of simple sugars into various types of cells in the vertebrate body involves a transient physicochemical association of the translocated sugar molecules with some special component of the barrier (presumably the plasma membrane) at the cell surface. We have therefore tried to extract such components from the stromata of human red blood cells, in the hope of duplicating in an inanimate system some of the sugar-transport properties which have been defined for these cells. The molecules bearing the apparent reactive sites have been pictured as acting either as carriers traversing the membrane in combination with the sugar (*1*, *2*), as relayers transferring the sugar along a path of relatively fixed adjacent sites (*3*), or as modifiers rendering the sugar capable of penetrating the membrane on its own, as by inducing the formation of less hydrophilic dimers (*4*). In all of these concepts a specialized monosaccharide-accepting site of considerable sterospecificity, sensitive to a variety of defined pharmacological agents, is presumed.

Since the critical structural component of the membrane is generally considered to be the lipid layer, and since in recent years attention has been particularly directed to phospholipids as possible carrier-like participants in cation transport through cell membranes (*5*), we applied lipid solvents to the