Effect of Galactose-1-Phosphate on Glucose Oxidation by Normal and Galactosemic Leukocytes

Abstract. During incubation with galactose, galactosemic leukocytes accumulated more galactose-1-phosphate than did normal leukocytes. Concomitant determination of glucose oxidation, with C^{14} glucose, revealed no inhibition of the hexosemonophosphate pathway. These results are at variance with recent studies in rat lens tissue, which suggests that intracellular galactose-1-phosphate depressed glucose-6-phosphate dehydrogenase activity and the oxidative pathway.

Studies by Kalckar and his associates (*1*) have established that patients with congenital galactosemia are deficient in the enzyme galactose-1-phosphate uridyl transferase. This deficiency results in an inability to utilize dietary galactose, but endogenous synthesis of essential galactose-containing substances continues normally via uridine diphosphoglucose and uridine diphosphogalactose-4-epimerase. Since the clinical manifestations of galactosemia are related to dietary galactose, investigators have sought to explain the toxic features of the disease through the direct or indirect effects of galactose or α-D-galactose-1-phosphate (Gal-1-P), the two compounds that accumulate as a result of the metabolic block.

In 1956 Schwarz and his associates (*2*) found increased levels of Gal-1-P and diminished uptake of O_2 in galactosemic red blood cells. They also reported an accumulation of this ester in the cataractous lenses of rats fed a high-galactose diet, and they suggested that Gal-1-P interferes with glucose metabolism, this interference resulting in the toxic features of the disease (*3*). Lerman (*4*) has recently reported that in the galactose cataracts of rats there is a significant inhibition of glucose-6-phosphate (G-6-P) dehydrogenase and a resultant stunting of glucose metabolism via the hexosemonophosphate pathway. He has postulated that since this pathway is a principal source for glucose utilization in lens (*5*), inhibition by Gal-1-P may explain the cataracts in the experimental animal and galactosemic patient. Because human leukocytes have abundant G-6-P dehydrogenase (*6*) and an active hexosemonophosphate pathway (*7*), metabolize galactose and glucose readily in vitro (*8*), and are easily obtained from normal and galactosemic subjects, they provide an excellent experimental model to test for inhibition of the hexosemonophosphate pathway by Gal-1-P.

In the studies described below, leukocytes were obtained by fibrinogen sedimentation, as described by Skoog and Beck (*9*). Aliquots of the leukocyte suspension containing 2 to 3 × 10^7 cells were preincubated in modified Warburg flasks for 1 hour with glucose and galactose, as indicated in Table 1. Glucose-1-C^{14} or glucose-6-C^{14} (0.45 μc in 20 μl) was then added to alternate flasks, and the incubation was continued for an additional hour. The reaction was stopped with acid, and the $C^{14}O_2$ was trapped in 1 ml of hyamine base, added to the center well, and then transferred to a vial and counted in a TriCarb liquid scintillation counter (counting efficiency 50 percent). A ratio greater than unity for the amount of $C^{14}O_2$ derived from glucose-1-C^{14} as compared to that from glucose-6-C^{14} has been taken as evidence for the presence of the hexosemonophosphate shunt in the tissue under study (*10*). In each experiment an additional flask containing leukocytes and 50 mg of glucose and 100 mg of galactose per 100 ml was incubated for 2 hours, and the accumulated intracellular Gal-1-P was determined by the enzymatic method of Kirkman and Maxwell (*11*), after the centrifuged cells had been washed with normal saline and broken in a sonic oscillator.

Table 1 contains the results of these studies on four normal subjects and four patients with congenital galactosemia. The accumulation of Gal-1-P was significantly greater in the galactosemic cells, averaging 10.6 μg per 2 × 10^7 cells, as compared to 2.6 μg for an equal number of normal cells. The ratios of $C^{14}O_2$ from C^1- and C^6-labeled glucose did not change appreciably in either group, a finding that suggests that under the conditions of these experiments there was no inhibition of glucose oxidation via the hexosemonophosphate pathway. When galactose was present as a substrate there was slight lowering of counts from both C^1- and C^6-labeled glucose. From results of other studies involving C^{14}-labeled galactose or glucose incubated with unlabeled glucose or galactose, respectively, we have assumed that the decreased counts are due to dilution of the specific activity of the CO_2 pool and/or competition between the hexoses for membrane transport or phosphorylation with available adenosine triphosphate (*12*).

Using normal and galactosemic human leukocytes in the present study, we were unable to find a specific inhibition of glucose oxidation via the hexosemonophosphate pathway under conditions where there was a considerably greater accumulation of Gal-1-P than was recorded in experiments in which lenses from galactose-fed rats were used. From our data obtained in leukocytes it cannot be concluded that glucose metabolism in other tissues is unaltered by the presence of Gal-1-P. The present observations indicate that the accumulation of this sugar phosphate in galactosemic tissue is not necessarily associated with inhibition of the oxidative pathway of glucose metabolism.

Table 1. Production of $C^{14}O_2$ and accumulation of galactose-1-phosphate in normal and galactosemic leukocytes. The 2-hour incubations were at 37°C in a Dubnoff shaker, in an atmosphere of 95 percent O_2 and 5 percent CO_2. Each counting is the average for triplicate flasks containing 5 ml of leukocyte suspension plus 1.5 ml of Krebs-Ringer bicarbonate buffer with added sugars as indicated. The C^{14}-labeled glucose was added after 1 hour.

Subject	Gal concn.* (mg/100 ml)	$C^{14}O_2$ [count/min (2 × 10^7 white cells)]		C^1/C^6	Gal-1-P accumulation	
		Substrate: Gluc.-1-C^{14}	Substrate: Gluc.-6-C^{14}		(μg/2 × 10^7 white cells)	(mg/100 g)†
		Galactosemic				
T.B.	0	1590	76	21		79
T.B.	100	1274	64	20	9.5	
M.O.D.	0	891	50	18		95
M.O.D.	100	984	42	23	11.3	
P.R.	0	1010	26	39		106
P.R.	100	815	22	36	12.7	
E.W.	0	2095	58	36		73
E.W.	100	1690	46	37	8.8	
		Normal				
J.C.	0	882	50	18		39
J.C.	100	666	43	16	4.7	
A.W.	0	1362	58	23		15
A.W.	100	1156	65	18	1.8	
S.S.	0	507	25	20		32
S.S.	100	494	19	26	3.9	
C.S.	0	1120	38	40		0
C.S.	100	1022	25	41	0	

* Glucose concentration, 50 mg/100 ml in each flask. † Based on net weight of 10^7 white blood cells=6 mg (*13*). Reported data on Gal-1-P in lens tissue are expressed in mg/100 g (*3*).

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Molybdenum as a Factor Limiting Primary Productivity in Castle Lake, California

Abstract. A trace-element deficiency is evident from carbon-14 bioassays of Castle Lake's natural phytoplankton populations. Increase in photosynthetic rates with the addition of molybdic acid or sodium molybdate has been demonstrated throughout the year. It is likely that other trace elements may also be found to be limiting factors in lakes having a limited watershed.

A study of primary productivity and limiting factors in a number of northern California lakes was started in March 1959. Castle Lake was among the lakes selected for this study. It is in a cirque basin at an elevation of 5200 feet in the Klamath Mountains, 11 miles southwest of Mt. Shasta, California. Its surface area is 19.4 hectares, and the lake has a maximum depth of 36.5 m. Studies on trout production in the lake have been in progress since 1938 (*1*).

A rapid carbon-14 bioassay technique developed for limiting-factor investigations in Alaskan lakes in 1957 (*2*) revealed changes in photosynthetic rate under various nutrient conditions. The lake's natural, unconcentrated phytoplankton population was studied under both field and laboratory conditions. Cultures in the field were contained in sterile, screw-cap, 500-ml Pyrex erlenmeyer flasks fastened to a floating crib anchored in the lake near shore. This arrangement provided lake-surface conditions of temperature and light and maintained the plankton population in suspension through wave motion of the crib. In the laboratory, cultures were held at a constant (10°C) temperature under a 40-watt fluorescent light.

In June 1959 cultures in both the lake and laboratory showed very significant increase in carbon fixation with the addition of potassium, sulfate, or a trace-element mixture. Subsequent cultures in which trace elements were added separately demonstrated that molybdenum was the stimulating micronutrient. Addition of 0.100 part of molybdenum (as Na_2MoO_4) per million increased the rate of photosynthesis of the lake phytoplankton over a 4-day period in June (Fig. 1). This was accompanied by a high rate of primary productivity in the lake. The addition to cultures of 0.100 part of molybdenum (as molybdic acid) per million had the same effect as the addition of Na_2MoO_4.

Other experiments started throughout the summer, fall, and winter of 1959 gave nearly identical results. Responses early in the season were greater; responses diminished somewhat with the seasonal decline in primary productivity and perhaps with rainfall in August and September. By October, 0.050 part of molybdenum per million was more effective than higher concentrations. In cultures maintained in the lake under 1 m of ice on 10 January 1960, the addition of 0.025 part of molybdenum per million gave a significant response during a 4-hour period. Although an increase in the rate of photosynthesis was consistently evident with the addition of from 0.001 to 0.050 part of molybdenum per million, the optimum was about 0.025. In the early summer of 1960, the addition of 0.005 part per million gave a greater response than higher concentrations.

The essential role of molybdenum in the growth of higher plants has been recognized since 1939 (*3*). It was demonstrated as a requirement for *Chlorella* in 1953 (*4*) and for *Scenedesmus* in 1955 (*5*). The low levels of molybdenum evident in a number of inland waters and the involvement of molybdenum in nitrogen reduction and fixation have suggested its possible importance in lakes (*6*). Although this is the first time molybdenum has been reported to be a limiting factor in lakes, it is probable that it and other micronutrients will be found in limiting concentrations in lakes with restricted watersheds.

The importance of molybdenum in the formation of nitrate reductase and in nitrogen fixation is well established (*7*). In attempting to identify more specifically the factors involved in the molybdenum deficiency in winter, Mo. NH_4^+ and ND_3^- were added singly and in combination. An almost totally nannoplankton population is characteristic of Castle Lake under ice conditions. Lack of response in *in situ* winter cultures with NH_4^+ or NO_3^-, and response to the addition of Mo and NH_4^+ would seem not to favor a reductase requirement. Nitrogen fixation by these microplankton remains a possibility as does a deficiency in more than a single enzyme system.

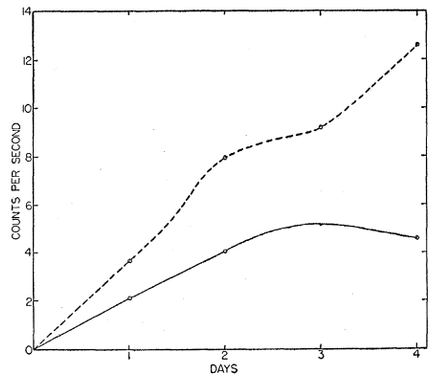

Fig. 1. Stimulation in the rate of carbon fixation (as measured by C^{14} uptake) with the addition of molybdenum to a culture of Castle Lake's natural phytoplankton population maintained at lake-surface temperature and light. Solid line, the control; broken line, the culture with 0.10 part of molybdenum per million added. This experiment was started on 29 June 1959.

The alder trees (*Alnus tenuifolia* Nutt) that are abundant along the east shore of Castle Lake make an appreciable nitrogen contribution to the lake, principally in the form of NO_3^- (*8*). Reduction of this nitrate by phytoplankton presumably would require molybdenum, which is in the lake in suboptimal concentrations. Analysis of the alder leaves showed molybdenum to be present in trace quantities (< 0.1 part per million). The nitrogen-fixing alder trees and other plants may be competing with the lake for the available Mo, K^+, and SO_4^{--} which would otherwise be added by the springs draining the alder-covered shore line. Culture experiments carried out during June and July of 1960 showed a lower response to the addition of molybdenum than those of the previous year. Precipitation between March and August of 1959 was only 35 percent of the mean annual precipitation for those months, and thus it seems likely that replenishment of molybdenum by inflow is a critical factor in reducing the level of deficiency (*9*).

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