worst day, 15 May, along with curves for the three components of extinction (*7*). An average curve for June 1981 is also shown, from which the excess extinction due to the cloud alone is inferred to be 0.3 mag (~ 25 percent).

Figure 4 shows the time variations of the cloud extinction at five representative wavelengths. Mean extinction values have been subtracted based on the data of Hayes (*8*), so that Fig. 4 displays just the cloud extinction plus a small component of the seasonally variable local aerosol (~ 0.05 mag). The cloud appeared slightly blue with the excess extinction at 0.350 μm being ~ 0.1 mag (10 percent) greater than that at 0.709 μm. At the time of greatest extinction, 15 May, the cloud seemed more nearly gray but may have been changing during the ~ 4-hour period required for each set of measurements. By early June the initial conditions prevailed. These changes imply that the particle size distribution during mid-May was different from that before and after.

From stellar photometry records we also gain information on the color and amplitude of extinction change. The extreme values are given in Table 1. Although the cloud was fairly gray in the visible, the falloff in the red indicates an upper limit to the particle size of about 1 μm. Detailed modeling would be required to better define the particle size distribution.

During early May we repeatedly looked for, but did not see, a Bishop's ring, the diffraction halo of somewhat variable size that was seen all around the world after the Krakatoa eruption of 1883 (*9*). A Bishop's ring was seen from Houston, Texas, in May 1982 (*10*). Almost every evening, about 10 minutes after sunset, the striated structure of the cloud became visible (Fig. 5). What was evident is reminiscent of the Meinels' (*11*) description of the November 1974 ash cloud. Fine ripple patterns having apparent lifetimes of ~ 1 minute formed in certain bands. The earth's shadow and antitwilight arch (*12*) were abnormally weak or invisible both in the morning and in the evening. At 30 minutes past sunset, the purple light was often well developed and crepuscular rays, modulated by distant clouds below the horizon, were serving as a twilight finale even in October.

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References and Notes

1. *Eos* **63**, 499 (1982).
2. W. C. Livingston, C. Mahaffey, B. Ye, in *Instrumentation in Astronomy IV*, D. L. Crawford, Ed. (Society of Photo-Optical Instrumentation Engineers, Bellingham, Wash., 1982), vol. 331, p. 249.
3. J. Harvey, personal communication.
4. M. A. H. Smith, personal communication; *Eos* **63**, 513 (1982).
5. F. Meyskens, personal communication; D. S. Berger and F. Urbach, *Photochem. Photobiol.* **35**, 187 (1982).
6. H. Tug and N. White, personal communication.
7. ———, G. W. Lockwood, *Astron. Astrophys.* **61**, 679 (1977).
8. D. S. Hayes, *Kitt Peak Nat. Observ. Newsl. No. 21* (1982), p. 5.
9. G. J. Symons, Ed., *The Eruption of Krakatoa and Subsequent Phenomena: Report of the Krakatoa Committee of the Royal Society* (Trübner, London, 1888).
10. J. W. Crampton, personal communication.
11. A. B. Meinel and M. P. Meinel, *Science* **188**, 477 (1975).
12. M. Minnaert, *The Nature of Light and Colour in the Open Air* (Dover, New York, 1954).
13. J. Africano, personal communication.
14. R. Joyce, personal communication.
15. We thank G. Mechler, K. Pierce, and J. Goad for helpful discussions.

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Potential Role of Galactokinase in Neonatal Carbohydrate Assimilation

Abstract. *Glucose given to the newborn human may result in hyperglycemia, suggesting that its utilization is impaired at this developmental stage. Galactose is thought to be a more appropriate carbohydrate source for the newborn. The enzymes involved in hexose phosphorylation may, in part, be responsible for these observations. A key regulatory enzyme of hepatic glucose assimilation, glucokinase, is diminished in newborns compared to adults, whereas galactokinase activity is increased. When newborn dogs were fasted and then fed either glucose or galactose, their plasma insulin responses to glucose were similar, but the pups fed galactose demonstrated an attenuated systemic appearance rate of glucose. Hexose incorporation into hepatic glycogen and net glycogen synthesis was augmented in the galactose-fed dogs. In vitro, liver from neonatal dogs showed enhanced galactokinase activity relative to that for hexokinase or glucokinase. Neonatal hexose assimilation may be independent of insulin action and, instead, be related to the developmental presence of hexose phosphorylating enzymes.*

Newborn mammals given carbohydrates show an attenuated rate of systemic glucose utilization (*1*, *2*). Newborn rats and humans show a diabetic-like glucose tolerance curve in response to exogenous glucose (*3*, *4*). Newborn humans of low birth weight develop excessively high concentrations of blood glucose when glucose is administered intravenously at conventional rates (*5*). The higher mortality rate of such infants may, in part, be related to their glucose intolerance (*6*). As the normal infant matures, glucose tolerance increases toward adult values (*3*, *4*).

The utilization of galactose by newborn humans has been described as optimal (*7*). Hepatic tissue of neonatal rats utilize galactose more readily than hepatic tissue from adult rats; galactose is oxidized and disappears more rapidly from the less mature tissue. In the newborn rat and dog, galactose is incorporated into hepatic glycogen more rapidly than glucose, whereas glucose is incorporated more rapidly in the adult rat liver (*8*, *9*). Newborn dogs given galactose show lower rates of systemic glucose appearance than newborn dogs given equivalent amounts of glucose (*9*). Because the liver is the major site of disposition of orally administered carbohydrate, we suggested that hepatic carbohydrate uptake is augmented in the newborn when galactose, rather than glucose, is administered. This might be explained on the basis of the availability of the enzymes that phosphorylate these monosaccharides: galactokinase, hexokinase, and glucokinase. We have studied these enzymes in fed and fasted newborn beagle pups during the first day after birth. The results confirm our earlier studies (*9*) and suggest that high levels of galactokinase activity may account for the better utilization of galactose than glucose in the newborn human.

Umbilical artery and venous catheters were placed in newborn pups delivered by Cesarean section at term (*9*). Fasting glucose turnover was measured by the primed-continuous infusion of [6-^{3}H]glucose and analysis of the data with Steele's equations (*9*). The pups were then randomly fed physiological quantities (0.625 g/kg) of an isotonic solution of either [U-^{14}C]glucose or [U-^{14}C]galactose, and blood glucose and galactose concentrations, as well as glucose kinetics, were determined as described (*9*). Four hours after enteric feeding, hepatic tissue was quickly sampled and cooled rapidly to the temperature of liquid nitrogen; hepatic glycogen, galactokinase, hexokinase, and glucokinase activity were then analyzed (*9–11*).

Concentrations of glucose in the blood achieved peak values 1 hour after equivalent amounts of either glucose or galactose were fed, but the peak value was highest after glucose (Table 1). The glucose concentrations declined to prefeeding values 4 hours after feeding. Galactose was not present in the fasting pups' blood nor in that of the pups fed glucose, but when galactose was fed, the blood galactose concentrations peaked earlier than glucose (30 minutes after feeding) and returned to undetectable levels by 4 hours. The plasma insulin response to galactose was similar among glucose or galactose fed pups and therefore probably had little effect on substrate appearance or tissue utilization. Fasting rates of glucose turnover were the same in both groups. The glycogen content of the liver in age-matched control pups from our laboratory is 354 ± 38 μmole per gram of wet tissue. In the present experiments, the hepatic glycogen content of the pups fed glucose or galactose increased, but the increase was greatest in the pups fed galactose (Table 1). In addition, the incorporation into glycogen of ^{14}C from enteric substrates and of ^{3}H from intravenously infused glucose was enhanced in the galactose-fed pups. The specific activity of the infusion-derived [^{3}H]glucose in glycogen was 76.2 ± 26.2 dpm/μmole after the galactose feeding compared to 26.9 ± 4.7 dpm/μmole after glucose ($P < .01$) (Student's t-test). The specific activity of the enteral-derived [^{14}C]glucose in glycogen was 122.6 ± 38.4 dpm/μmole after galactose, whereas it was only 20.7 ± 4.2 dpm/μmole after glucose ($P < .01$). Although hexose oxidation was not quantified, we found that galactose had an augmenting effect on the utilization of hepatic monosaccharide for glycogen synthesis. These data suggest that when galactose is fed to newborn dogs systemic glucose appearance is attenuated whereas hepatic hexose uptake is augmented.

Since hepatic hexose phosphorylation may be important in controlling the disposition of galactose and glucose, developmental alterations of hexokinase, glucokinase, and galactokinase may account for our observed results. Hexokinase is present in newborn liver and varies little in activity with maturation (*3*). Because of its relatively low Michaelis constant (K_M), hexokinase cannot augment the hepatic phosphorylation of glucose during periods of alimentation if concentrations of glucose exceed this enzyme's limiting K_M (*12*). Furthermore, hexokinase is subject to feedback inhibition by glucose 6-phosphate.

In adults, glucokinase is hypothesized to be the key enzyme of hepatic glucose phosphorylation during periods of alimentation. This is because of its very high K_M which enables it to function in the range of blood glucose concentrations during alimentation (*12*). It is also not subject to end-product inhibition. Glucokinase activity is low or absent in most newborn species (*3*). In the developing rat, the incorporation into glycogen of radioactivity from administered glucose does not occur until glucokinase activity becomes present (*13*). The increase in glucokinase activity parallels the developmental transition from neonatal to adult ability to utilize exogenous glucose; glucose tolerance among rats correlates well with the appearance of glucokinase (*3*). In adult humans and rats, glucokinase activity is thought to control the rate of hepatic glucose utilization, because the activity of this enzyme in fasted or diabetic patients diminishes as intolerance to glucose develops (*3, 14*).

Galactokinase activity in the liver of newborn rats and humans is relatively high (*10, 15*). This enzyme is less susceptible to end-product inhibition, and could be responsible for the enhanced galactose clearance during the newborn period in addition to the augmented incorporation of galactose into hepatic glycogen. In neonatal dogs (fasted or fed), galactokinase activity (18.86 ± 0.66 nmole per minute per milligram of protein, $P < .001$) was the highest of all three hexose phosphorylating enzymes (hexokinase 7.42 ± 0.88 nmole/min-mg protein; glucokinase 8.09 ± 0.78 nmole/min-mg protein). These data were derived from four age-matched, fasted pups plus eight pups from the present study that were fed either glucose or galactose. There were no differences between hepatic enzyme activities among fasted or fed groups; in addition, enzyme activity was not affected during the first 24 hours of newborn fasting (*16*). Although the K_M's were not determined, murine hepatic galactokinase in newborn rats has been shown to have a higher maximum velocity (V_{max}) and a higher K_M for galactose than in adults (*17*). The total activity of murine galactokinase exceeds adult values by 400 percent. Since both the newborn rat and dog demonstrate augmented galactose incorporation into glycogen, this may be due to the high activity of galactokinase. Whether newborn canine galactokinase has a more favorable K_M and V_{max} than the adult canine liver enzyme warrants further investigation.

Augmented glycogen synthesis, higher rates of hexose incorporation into hepatic glycogen, and lower rates of systemic glucose appearance after galactose feeding all suggest that galactose is utilized better than glucose by the liver of the newborn dog. The lower activity of glucokinase in this developmental period may explain, in part, the observed glucose intolerance of the newborn, whereas the enhanced utilization of galactose may be related to the availability of galactokinase.

Table 1. Concentrations of circulating hexose and insulin, rate of systemic glucose appearance, and hepatic glycogen content in newborn pups. The data are expressed as mean values ± the standard error of the mean. N.D., not determined.

Carbohydrate fed by stomach tube	Concentration in blood (m*M*)		Plasma insulin (μU)	Systemic rate of appearance of glucose (μmole/kg-min)	Hepatic glycogen (μmole/g)
	Glucose	Galactose			
			Fasting conditions		
Glucose ($N = 5$)	6.03 ± 0.91	Undetectable	2.34 ± 0.40	31.3 ± 8.1	N.D.
Galactose ($N = 6$)	5.47 ± 0.79	Undetectable	2.75 ± 0.54	29.4 ± 57	N.D.
			Peak values at 4 hours		
Glucose	11.22 ± 0.63	Undetectable	13.60 ± 1.24	168.9 ± 9.5	412.0 ± 41
Galactose	8.76 ± 0.93*	1.22 ± 0.9	11.71 ± 0.98	72.3 ± 3.1†	587.0 ± 52‡

*$P < .05$. †$P < .001$. ‡$P < .01$.

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References and Notes

1. S. Varma *et al.*, *Metabolism* **22**, 1367 (1973).
2. W. Sherwood, D. Hill, G. Chance, *Pediatr. Res* **11**, 874 (1977).
3. D. Walker and G. Holland, *Biochem. J.* **97**, 845 (1965).
4. H. Sutherland and J. Stowers, in *Pregnancy and*

the Newborn (Churchill Livingstone, New York, 1976), pp. 106–126.
5. H. S. Dweck, Y. W. Brans, J. E. Sumners, G. Cassady, *Biol. Neonat.* **30**, 261 (1976).
6. M. Zaref, R. Pildes, D. Vidyassager, *Diabetes* **25**, 428 (1976).
7. S. Segal, H. Roth, D. Bertoli, *Science* **142**, 1311 (1963).
8. F. Ballard and I. Oliver, *Biochem. J.* **90**, 261 (1963).
9. R. Kliegman, E. Miettinen, S. Kalhan, P. Adam, *Metabolism* **30**, 1109 (1981).
10. Y. Shin-Buehring, T. Beier, A. Tan, M. Osang, J. Schaub, *Pediatr. Res.* **11**, 1003 (1977).
11. A. Faulkner and C. Jones, *Arch. Biochem. Biophys.* **175**, 477 (1976).
12. E. Newsholme and C. Start, *Regulation in Metabolism* (Wiley, New York, 1976), pp. 262–267.
13. F. Ballard and I. Oliver, *Biochim. Biophys. Acta* **71**, 578 (1963).
14. C. Sharma, R. Manjeshwar, S. Weinhouse, *J. Biol. Chem.* **238**, 3840 (1963).
15. D. Walker and H. Khan, *Biochem. J.* **108**, 169 (1968).
16. R. M. Kleigman, E. L. Miettinen, S. Morton, unpublished data.
17. P. Cuatrecasas and S. Segal, *J. Biol. Chem.* **240**, 2382 (1965).
18. We thank G. Campbell and A. Sutton for technical assistance, S. D. Sachs for care of the beagles, and S. Hartman for preparation of the text. Supported by NICHHD grant HD05740 and Diabetes Association grant 40RIA-79. R.M.K. is a recipient of a Research Investigator Award, Diabetes Association of Greater Cleveland.
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Virus-Induced Autoimmunity: Monoclonal Antibodies That React with Endocrine Tissues

Abstract. *Mice infected with reovirus type 1 develop an autoimmune polyendocrine disease. Spleen cells from these mice were fused with myeloma cells and the culture fluids were screened by indirect immunofluorescence for autoantibodies reactive with normal mouse tissues. A large panel of cloned, stable antibody-producing hybridomas has been obtained. Fourteen of the hybridomas make autoantibodies that react with cells in the islets of Langerhans, 24 with cells in the anterior pituitary, 11 with cells in gastric mucosa, and 5 with nuclei. Except for the antibodies to nuclei, the monoclonal autoantibodies are organ-specific. Some, however, show broad cross-species reactivity, recognizing similar antigenic determinants in mouse, rat, pig, and human organs, whereas others recognize determinants only in rodent tissues. Several of the antigens recognized by these monoclonal autoantibodies have been identified as hormones (for example, glucagon, growth hormone, and insulin).*

There are a large number of important human diseases of undetermined etiology that have an autoimmune component (*1*). In some of these diseases, such as systemic lupus erythematosus, the autoimmune component is very broad, involving many different organs and tissues (*2*). In insulin-dependent diabetes mellitus and polyendocrinopathy, the autoimmune component involves primarily, but not exclusively, endocrine organs (for example, pancreas, anterior pituitary, thyroid, and gastric mucosa) (*3*). In still other diseases, such as myasthenia gravis, the autoimmune response is further restricted, being directed predominantly against the acetylcholine receptor (*4*).

Little is known about the factors that trigger the production of autoantibodies in these various diseases, but viruses have been suspected as one of the possible causes. Recently, we showed that SJL/J mice, infected with reovirus type 1, develop an autoimmune polyendocrine disease characterized by mild diabetes mellitus and retarded growth (*5*). Autoantibodies directed against normal pancreas, pituitary, and gastric mucosa were found in serum samples from the infected mice. The polyendocrinopathy has an immunological component, because immunosuppression prevents the development of autoantibodies, diabetes, and growth retardation (*6*). However, the role that these antibodies actually play in the pathogenesis of this disease has been difficult to evaluate because of the problem of obtaining large quantities of purified autoantibodies. To accomplish this end, we used hybridoma technology. Spleen cells from reovirus-infected mice were fused with myeloma cells, and the culture fluids were screened for autoantibodies. By this method we have obtained over 40 stable hybrids that produce monoclonal autoantibodies that react with a variety of normal mouse tissues.

Suckling SJL/J mice were inoculated with reovirus type 1 as previously described (*5*). Serum samples from animals with retarded growth were examined by indirect immunofluorescence (FA) for antibodies reactive with paraffin sections of normal mouse pancreas, pituitary, and stomach that had been fixed in Bouin's fluid. Mice with detectable autoantibodies were killed between 9 and 26 days after infection. Spleen cells were fused with P3-653Ag8 myeloma cells at a 10:1 ratio with the use of 50 percent polyethylene glycol 1000 (*7*). The fused cells were plated (2.5×10^5 cells per well) in 96-well microtiter plates containing unstimulated peritoneal exudate cells (5×10^3 cells per well) as a feeder layer. After selection of cells in HAT medium (hypoxanthine, aminopterin, thymidine), supernatant fluids from confluent cultures were examined by FA for antibodies to mouse adrenal glands, brain, muscle, pancreas, pituitary, salivary glands, skin, stomach, thymus, and thyroid. Hybrids producing autoantibodies were immediately cloned by limiting dilution (*8*) and were microscopically inspected for the presence of a single colony. Only cloned hybrids that remained productive upon passage are described in this report. Spleen cells obtained from mice less than 20 days after infection yielded

Table 1. Monoclonal autoantibodies against the anterior pituitary react with growth hormone. Abbreviation: N.D., not determined.

Hybridoma	Anterior pituitary (FA)*					Growth hormone (ELISA)†	
	Mouse		Rat	Pig	Human	Rat	Human
	Before	After‡					
8–4	400	8	20	2	<2	640	<10
6B–12	200	<2	20	<2	<2	80	<10
6B–7	80	<2	8	<2	<2	40	<10
2–9	200	16	20	<2	<2	320	20
6A–3	800	<2	50	16	<2	160	<10
5B–5	800	<2	800	400	800	2,560	20,480
5B–8	400	N.D.	400	20	20	<10	<10

*The antibodies, precipitated from supernatant fluids by 50 percent saturated ammonium sulfate, were tested for reactivity with anterior pituitary and purified growth hormone. All of these antibodies were immunoglobulin M. Paraffin sections from normal mouse, rat, pig, and human pituitaries (fixed in Bouin's fluid) were incubated with serial dilutions of autoantibody and then with antibody to mouse immunoglobulin conjugated with fluorescein isothiocyanate. The fluorescent antibody (FA) titer represents the reciprocal of the highest dilution giving positive fluorescence. †For enzyme-linked immunosorbent assay (ELISA), Immunlon-2 plates (Dynatech Laboratories) were coated with 5 μg of purified human or rat growth hormone (courtesy of National Pituitary Agency, Baltimore), incubated with serial dilutions of monoclonal antibodies, and then reacted with peroxidase-labeled antibody to mouse immunoglobulin as described (*5*). Absorbance was read with a Titertek microplate reader and titers were expressed as the reciprocal of the highest dilution giving a value twice the control readings. ‡The FA titer after incubation for 48 hours with 0.5 mg of purified rat growth hormone per milliliter.