Experimental Galactosemia Produces Diabetic-like Retinopathy

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SUMMARY

Six normal dogs were made galactosemic by feeding a 30% D-galactose diet, and were followed up to 5 yr. For comparison, 10 normal dogs and 10 alloxan-diabetic dogs were concurrently fed the diet less the galactose supplement. Retinopathy occurred in each of four dogs glactosemic 3 or more yr, and was absent at lesser durations of galactosemia, and from normal dogs not given the galactose supplement. The retinopathy was marked by saccular capillary aneurysms, hemorrhages, nonperfused or acellular vessels, tortuous hypertrophic capillaries, loss of capillary pericytes, and other lesions typical of diabetic patients and alloxan-diabetic dogs. In galactose-fed dogs, blood galactose varied between 0 (fasted) and 250 mg/dl (postprandial), and glycosylated hemoglobin levels became supranormal. In contrast to diabetic dogs, blood levels of glucose, free fatty acids, and branched-chain amino acids were not elevated in the galactosemic dogs, and their serum insulin seemed normal. The results suggest that the level of blood hexose is itself an important determinant of retinopathy.

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Hyperglycemia, the principal symptom of diabetes mellitus, has remained of uncertain significance *in the etiology of the vascular complications of* diabetes. Elevation of the blood level of an isomer of glucose, namely D-galactose, is known to be capable of reproducing many of the effects of diabetes on peripheral nerve, lens, and cornea.[1–3] However, no retinopathy or other clinically significant vascular disease previously has been reported in this condition.

The capillary aneurysms and other lesions that are characteristic of diabetic retinopathy in patients have been shown to develop also in experimentally diabetic dogs, provided the dogs have remained chronically hyperglycemic for lengthy periods of time, usually 3–5 yr.[4,5] In an effort to evaluate the possible role of excessive blood hexose in the development of retinopathy, effects of experimental galactosemia have been explored by us in dogs fed a galactose-rich diet.

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METHODS

Healthy dogs of mixed breeds and either sex were accepted for study when $1\frac{1}{2}$–$2\frac{1}{2}$ yr old if they weighed 8–14 kg and had been found to have a normal glucose tolerance test (intravenous 1.0 glucose/kg wt), normal blood and urine chemistry, and eyes that were normal by opthalmoscopy and fluorescein angiography. Animals were housed in metabolism cages, and were assigned at random to one of the following three groups.

(1) Experimental galactosemia was produced in six dogs by feeding a dry ration containing 30% D-galactose. This diet was prepared by mixing pulverized dog food (Purina Lab Canine Diet No. 5006, Ralston-Purina Company, St. Louis, Missouri) with D(+)-galactose (purified anhydrous crystalline, Sigma Chemicals, St. Louis, Missouri), and compressing the mixture into pellets. The quantity of food offered daily was limited to that which a given dog would eat consistently, with $\frac{2}{3}$ of the total being offered in the morning and $\frac{1}{3}$ in the evening.

(2) Ten normal dogs were kept as untreated controls housed similarly and fed the diet minus the galactose supplement. The quantity of food offered was limited to that which would allow slow weight gain but not obesity.

(3) Diabetes was induced in 10 dogs by intravenous injection of alloxan monohydrate (50–60 mg/kg) after a 24-h fast. Subsequently, the above diet without added galactose was offered daily ad libitum, and insulin (NPH, isophane insulin suspension, Eli Lilly and Company, Indianapolis, Indiana) was injected each morning at a dose insufficient to prevent chronic hyperglycemia and glucosuria, as described previously.[5] These animals, although made diabetic

TABLE 1
Retinopathy in galactosemic dogs

Dog no.	Duration galactose-fed (mo)	Cataract*	Retinopathy
D424	6	+	Absent
D357	21	+ +	Absent
D460	32 (living)	+ +	Microaneurysms and hemorrhages
D452	36 (living)	+ +	Microaneurysms
D397	52 (living)	+ +	Microaneurysms (by 34 mo), hemorrhages and vessel nonperfusion; histologically at 40 mo 16 aneurysms OS†
D379	60	+ + + +	Histologically at 40 mo 36 aneurysms OS, and at 60 mo hemorrhages and 117 aneurysms OD†

*Cataract graded 0 (none) to + + + + (fundus visibility limited to the major retinal vessels).
†See text for additional retinal pathology.

for comparison with the galactose-fed animals, comprise a portion of a larger experiment in progress involving additional groups of alloxan-diabetic and pancreatectomized dogs to be reported elsewhere.

Eyes were examined ophthalmoscopically and, in some cases (see RESULTS), also histologically, as previously described.[5] Fluorescein angiograms, together with stereo color photos of 16 standardized fields of each fundus, were obtained before the start of the experiment and at intervals of 4–6 mo thereafter. The condition of the retina was determined ophthalmoscopically by comparison of the successive angiograms and color photos in vivo, and histologically by study of trypsin digests of retina.

Venous blood samples were obtained at 8–9 a.m. after an overnight fast, and were assayed by methods reported previously,[5,6] and as follows. Serum insulin was measured by radioimmunoassay (RSL Kit, Isolab, Inc., Akron, Ohio) in animals when fasted and again 2 h after each had consumed 14 g diet/kg body wt. Glycosylated hemoglobin was measured using minicolumns (Isolab, Inc.) and freshly drawn erythrocytes washed 4 h. Blood glucose and plasma galactose were measured by gas chromatography or, respectively, glucose- or galactose-oxidase methods. To assess the diurnal variation of blood glucose and galactose, blood was obtained at intervals of 2–6 h throughout the day on about three occasions each year.

RESULTS

Three of the galactose-fed animals have been killed for study, and three are at present alive after 32–52 mo on the galactose-rich diet (Table 1). Cataracts have invariably appeared, but it has been possible to continue ophthalmoscopy for the durations shown in Table 1 in all except one of the dogs (D379), an animal in which cataracts obscured the fundus after the first year of study.

Capillary aneurysms and other retinal lesions like those of diabetes have appeared in the four animals galactose-fed for 32 mo or more (Figure 1), and have been absent from animals studied lesser durations, as well as from normal animals not fed the galactose supplement. Microaneurysms were first detected at 32–36 mo duration by fluorescein angiography. The presence of retinopathy was confirmed histologically upon surgical enucleation of an eye from two dogs (D379 and D397) at 40 or 48 mo duration, and subsequent necropsy of one dog (D379) at 60 mo duration. The retinas obtained at 40, 48, and 60 mo contained supranormal num-

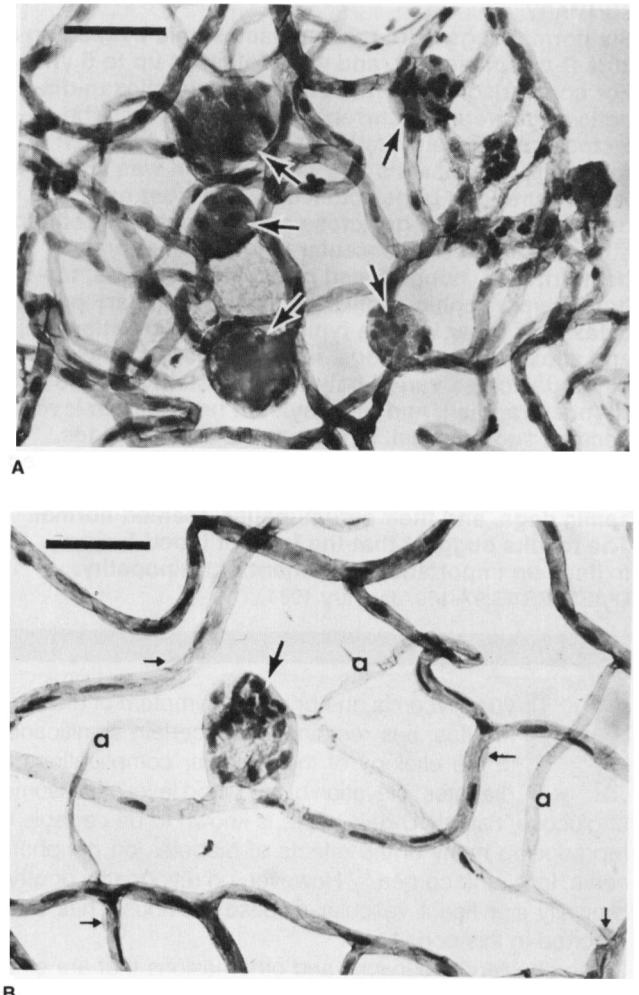

FIGURE 1. Retinopathy in experimental galactosemia. Capillary aneurysms (large arrows), acellular capillaries (a), and ghosts of intramural pericytes (small arrows) in the trypsin-digested retinal vasculature from two animals galactose-fed 40 and 48 mo. (Bar, 50 μm; hematoxylin and periodic acid–Schiff stain.)

TABLE 2
Blood chemistry of galactosemic and diabetic dogs (mean ± SD)

	Normal (N = 10)	Alloxan-diabetic (N = 10)	Galactose-fed (N = 5)
Duration of study (mo)	15–60	20–60	21–60
Galactose, daily maximum (mg/dl plasma)	0	0	193 ± 32*
Glucose, diurnal range (mg/dl blood)	60–98	261–450	49–87
Glycosylated Hb (%)	6.3 ± 0.5	11.1 ± 1.1*	7.3 ± 0.5*
Body weight (% of baseline)	111 ± 8	103 ± 11	114 ± 8
Insulin (μU/ml serum)	(N = 5)	(N = 3)	
Fasted	15 ± 7	5 ± 3	13 ± 4
Fed	44 ± 18	6 ± 3*	46 ± 36
NEFA (μeq/L serum)	639 ± 129	1454 ± 225*	659 ± 142
Triglycerides (mg/dl serum)	32 ± 12	39 ± 13	33 ± 4
Cholesterol (mg/dl serum)	189 ± 60	253 ± 44*	221 ± 33
Branched-chain amino acids	302 ± 64	672 ± 81*	264 ± 35
(nmol/ml plasma)†	(N = 4)	(N = 4)	(N = 4)

*Significantly different from normal, $P < 0.05$.
†Sum of valine, leucine, and isoleucine.

bers of acellular capillaries and pericyte ghosts, an occasional varicose (tortuous, hypertrophic) capillary, and, respectively, 16, 36, and 117 saccular capillary aneurysms per retina.

Dot and blot retinal hemorrhages have appeared in three of the four animals galactose-fed 32 mo or more, and one animal has developed a cotton wool spot that during fluorescein angiography corresponded with an area of vessel nonperfusion. Normal dogs given the diet without the galactose supplement for comparable lengths of time have retained normal-looking fundi. Severe cataracts developed in all diabetic dogs within the first year of study, thereby obscuring the fundus. Three dogs were killed after 60 mo of alloxan diabetes, and the trypsin digests of their retinas confirmed the occurrence of the capillary aneurysms and other lesions previously described by us in diabetic dogs,[4,5] and now described in galactosemic dogs.

In animals offered the galactose-rich diet, blood galactose concentrations were found to vary from hour to hour throughout the day, from values near 0 after an overnight fast, up to values of about 150–250 mg/dl later in the day, after the galactose-rich diet had been eaten. Glycosuria occurred daily in these animals and consisted usually of 3–5% reducing sugar containing up to 0.25% glucose. The animals nevertheless retained a healthy appearance and tended to continue to gain weight.

Blood glucose and serum insulin concentrations showed no abnormality in the galactosemic animals, in contrast to the alloxan-diabetic animals studied concurrently (Table 2). In the diabetic animals, serum insulin tended to be subnormal, and failed to show a postprandial rise. In contrast, the fasting and postprandial values for serum insulin in galactosemic animals were comparable to those of normal animals. In addition, the blood levels of nonesterified fatty acids, cholesterol, and branched-chain amino acids remained normal in the galactosemic animals, in contrast to the supranormal levels observed in diabetic animals.

DISCUSSION

Retinopathy had not previously been reported to occur with galactosemia, but rarely has the duration of galactosemia in experimental animals exceeded more than a few months, and no large experimental animals such as dogs seem to have been examined. In the present study, experimental galactosemia is found to result in the development of retinal lesions morphologically identical with those typical of diabetes in patients, and of spontaneous or experimentally induced diabetes in dogs.[7] The retinopathy is marked by the development of saccular capillary aneurysms, retinal hemorrhage, loss of capillary pericytes, capillary nonperfusion and atrophy, and varicose capillaries. Measurements of retinal capillary basement membrane thickness[8] are currently in progress, and data from the one animal killed after 60 mo of galactosemia indicate that the mean thickness in that dog (2533 Å) exceeds by more than two standard deviations the mean for eight normal dogs of comparable age in this laboratory (1827 Å ± 282 SD).

The morphologic similarity between diabetic retinopathy and the retinopathy of experimental galactosemia suggests that the mechanisms responsible for the microvascular abnormalities in diabetes and galactosemia may share in common a single final pathway. In both diabetes and galactosemia the elevation of a blood hexose is found to be followed by the development of distinctive retinal lesions. It is noteworthy that the diabetic-like retinopathy in our galactose-fed animals developed in the apparent absence of a number of metabolic disorders commonly associated with diabetes mellitus, namely in the absence of the abnormal blood levels of insulin, nonesterified fatty acids, cholesterol, and branched-chain amino acids. The available evidence suggests that excessive blood hexose may itself be an important determinant of microvascular disease.

It is not clear at present whether or not chronic galactosemia in humans may be associated with retinopathy. Since galactosemic patients can be treated readily with a galactose-poor diet, and an alternative pathway for the metabolism of galactose may develop during childhood,[9] it seems possible that galactosemia in patients might be insufficiently severe and/or protracted to elicit retinal changes such as those observed in the present dogs. A few galactose-intolerant patients have been reported who have survived many years without dietary restriction,[10,11] but no information is available concerning the ophthalmoscopic or histologic condition of their retinas.

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REFERENCES

[1] Datiles, M., Kador, P. F., Fukui, H., Hu, T. S., and Kinoshita, J.: Corneal reepithelialization in galactosemic rats. Invest. Ophthalmol. Vis. Sci. 1983; 24:563–69.

[2] Kinoshita, J.: Mechanisms initiating cataract formation. Invest. Ophthalmol. 1974; 13:713–24.

[3] Sharma, A. K., Thomas, P. K., and Baker, R. W.: Peripheral nerve abnormalities related to galactose administration in rats. J. Neurol. Neurosurg. Psychiatry 1976; 39:794–802.

[4] Engerman, R. L., Davis, M. D., and Bloodworth, J. M. B.: Retinopathy in experimental diabetes. *In* Diabetes. Rodriguez, R., and Vallance-Owen, J., Eds. Amsterdam, Excerpta Medica, 1971:261–67.

[5] Engerman, R., Bloodworth, J. M. B., and Nelson, S.: Relationship of microvascular disease in diabetes to metabolic control. Diabetes 1977; 26:760–69.

[6] Kern, T. S., and Engerman, R. L.: Amino acid concentrations in plasma and urine of experimentally diabetic dogs. Res. Exp. Med. (Berl.) 1983; 182:185–92.

[7] Engerman, R. L., and Kern, T. S.: Experimental galactosemia produces diabetic-like retinopathy. Diabetes 1982; 31 (Suppl. 2):26A.

[8] Siperstein, M., Unger, R., and Madison, L.: Studies of muscle capillary basement membrane in normal subjects, diabetic and prediabetic patients. J. Clin. Invest. 1968; 47:1973–99.

[9] Cohn, R. M., and Segal, S.: Galactose metabolism and its regulation. Metabolism 1973; 22:627–42.

[10] Gitzelmann, R.: Hereditary galactokinase deficiency, a newly recognized cause of juvenile cataracts. Pediatr. Res. 1967; 1:14–23.

[11] Vogt, M., Gitzelmann, R., and Allemann, J.: Dekompensierte Leberzirrhose infolge Galaktosamie bei einem 52 jahrigen Mann. Schweiz. Med. Wochenschr. 1980; 110:1781–83.