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10. Rats raised for 10 to 12 weeks on either lab chow or fat-free diets were injected with the tritiated amino acid mixture (3 μc per gram of body weight). Rhodopsin was obtained by agarose column chromatography. Plots of specific activity of rhodopsin against time for control animals showed a complete turnover of rhodopsin in 9 to 10 days, with a peak of radioactivity at 4 to 5 days. In contrast, specific activity of rhodopsin in experimental animals was much lower than for controls and did not show any peak of radioactivity or apparent turnover.

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Polyol Accumulation in Galactosemic and Diabetic Rats: Control by an Aldose Reductase Inhibitor

Abstract. *An orally active inhibitor of aldose reductase, 1,3-dioxo-1H-benz[de]-isoquinoline-2(3H)acetic acid (AY-22,284), prevented cataractous changes in cultured lenses exposed to high concentrations of galactose. When given orally, AY-22,284 markedly decreased the accumulation of polyols in the lenses and sciatic nerves of galactosemic rats and rats with streptozotocin-induced diabetes. In addition, treatment of galactosemic rats with AY-22,284 effectively suppressed the formation of cataracts.*

Insulin therapy has greatly reduced death from acute complications of diabetes. However, the resulting longevity of diabetic patients has led to complications such as neuropathy, nephropathy, retinopathy, and cataracts. The sorbitol pathway appears to be involved in metabolism of the excess glucose in diabetic tissues; this may lead to development of some of these complications (*1*). In this report we describe the effects of the compound AY-22,284 in galactosemic and diabetic rats. The changes in treated animals are consistent with the capacity of AY-22,284 to inhibit aldose reductase, one of the enzymes of the sorbitol pathway.

The presence of the sorbitol pathway has been established in several tissues, in which it is localized in certain cell types—for example, the lens (*2*), Schwann cells of peripheral nerves (*3*), kidney papilla (*4*), and the islets of Langerhans in the pancreas (*5*). The sorbitol pathway consists of two enzymes, aldose reductase (E.C. 1.1.1.21) and sorbitol dehydrogenase (E.C. 1.1.1.14):

$$\text{D-Glucose} + \text{NADPH} + \text{H}^+ \xrightarrow[\text{reductase}]{\text{aldose}} \text{sorbitol} + \text{NADP}^+$$

$$\text{Sorbitol} + \text{NAD}^+ \xrightarrow[\text{dehydrogenase}]{\text{sorbitol}} \text{fructose} + \text{NADH} + \text{H}^+$$

(NADPH, reduced nicotinamide adenine dinucleotide phosphate; $NADP^+$, nicotinamide adenine dinucleotide phosphate; NAD^+, nicotinamide adenine dinucleotide; NADH, reduced nicotinamide adenine dinucleotide.)

Aldose reductase has low substrate specificity and reduces several aldoses and other compounds containing an aldehyde group (*6*). Since the affinity of aldose reductase for glucose and galactose is low (Michaelis constants approximately 70 and 20 m*M*) (*6*), maximal rates of aldose reductase–catalyzed formation of sorbitol or galactitol can be attained only with high intracellular concentrations of glucose, as in diabetes, or of galactose, as in galactosemia. The polyols thus formed accumulate because they are neither efficiently metabolized nor readily diffused through cell membranes. Accumulation of polyols causes hypertonicity followed by osmotic swelling. This sequence of events is the common mechanism of sugar cataract formation (*2, 7, 8*). Sugar cataracts like those in diabetic and galactosemic patients can be produced in experimental diabetic rats and in rats fed galactose or xylose (*9*), and can be simulated (*8*) and prevented (*10*) in lens cultures.

Recognition of the role of aldose reductase in the pathogenesis of sugar cataracts led us to consider the possible involvement of the sorbitol pathway in diabetic neuropathy. Sorbitol and fructose, present in the nerves of normal rats, are increased in amount in the nerves of rats with diabetes produced by alloxan (*11*) or streptozotocin (*12*); aldose reductase is localized in the Schwann cells (*3*), which are involved in myelin formation and maintenance; and damage of Schwann cells results in

Table 1. Effect of AY-22,284 on galactitol accumulation in galactosemic rats. For lens measurements, rats had access to a diet containing 10 percent galactose for 3 days; for measurements in sciatic nerve, a diet with 30 percent galactose was given for 7 days. The diet of treated animals contained 0.7 percent AY-22,284 (approximately 1.2 g/kg per day). Eight rats were in each group. Galactitol was determined in trichloroacetic acid extracts by a modification of a method for glycerol determination (*19*). Values have been corrected by subtracting the background galactitol contents found in normal rats. In separate experiments, similar values were obtained by gas-liquid chromatographic analyses for the lens (by J.H.K.) and sciatic nerve (by K.H.G.); S.E., standard error.

Group	Galactitol (micromoles per gram of tissue)	
	Lens (mean ± S.E.)	Sciatic nerve (mean ± S.E.)
Control	14.43 ± 0.77	3.29 ± 0.82
AY-22,284	7.19 ± 0.49*	0.82 ± 0.16†
Change	− 50 percent	− 75 percent

* $P < .01$. † $P \leqq .02$.

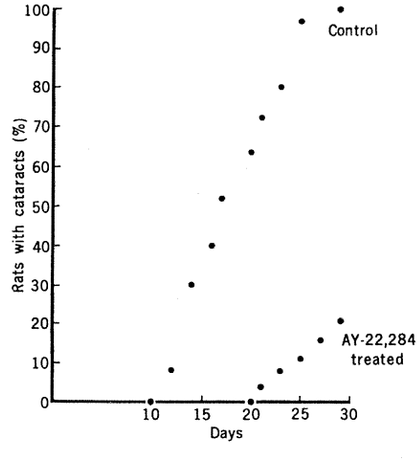
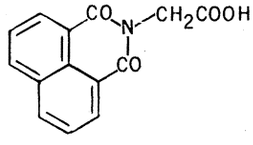


Fig. 1. Effect of AY-22,284 (0.7 percent of diet, or approximately 0.96 g/kg per day) on formation of macroscopically detectable cataracts in rats fed a diet containing 30 percent galactose. There were 23 or 24 rats per group.

segmental demyelination both in experimental and human diabetes. These results suggest a possible relation between sorbitol accumulation and acute damage of Schwann cells (*1*).

If aldose reductase is involved in the development of some diabetic complications, then inhibition of aldose reductase might provide a pharmacological approach to prevention of such complications. We used an aldose reductase inhibitor (*10*) to establish the crucial role of aldose reductase in initiating sugar cataract formation: Tetramethyleneglutaric acid (TMG) inhibited polyol formation in isolated rabbit lenses exposed to high galactose (*10*) or glucose (*13*) concentrations, thereby preventing lens swelling and other associated changes leading to cataract formation. In vivo, TMG had no effect in rats fed galactose.

We describe here the effects of an orally active inhibitor of aldose reductase, 1,3-dioxo-1H-benz[de]isoquinoline-2(3H)acetic acid (AY-22,284).



With DL-glyceraldehyde as substrate, aldose reductase from bovine lens is inhibited 45 percent by $1 \times 10^{-5}M$ AY-22,284 and 13 percent by $1 \times 10^{-6}M$ drug; with galactose as substrate, the enzyme is inhibited 79 and 35 percent by AY-22,284 concentrations of $1 \times 10^{-5}M$ and $1 \times 10^{-6}M$, respectively.

The effectiveness of AY-22,284 was tested in a lens culture system used in studies with TMG (*10*). Paired rabbit lenses were studied, one placed in control medium and the other in medium containing 30 mM galactose (*14*). A lens incubated overnight in galactose medium accumulated approximately 7 μmole of galactitol (*15*) and gained 20 mg of water. Addition of AY-22,284 decreased the amount of galactitol formed, thus reducing the degree of lens swelling. The decrease was concentration-dependent: with $1 \times 10^{-4}M$ drug, the reduction in galactitol accumulation and lens swelling was approximately 50 percent, and with $3 \times 10^{-4}M$ drug, the reduction was 70 to 75 percent. It appears that $1 \times 10^{-3}M$ AY-22,284 almost completely blocked aldose reductase activity, since little difference in galactitol and water contents was observed in the lens at this drug concentration as compared to the lens in control medium. These results suggest that, in this test system, AY-22,284 is at least ten times more effective than TMG.

Table 2. Effect of AY-22,284 on glucose, sorbitol, and fructose accumulation in the sciatic nerve of diabetic rats. Rats were rendered diabetic by single intravenous injection of streptozotocin (85 mg/kg). For the next 3 weeks, they had access to a control diet or a diet containing 0.6 percent AY-22,284 (approximately 1.1 g/kg per day). There were 13 or 14 animals per group. Glucose was determined enzymatically (*20*), sorbitol was measured by a modification of a method for glycerol determination (*19*), and fructose was determined by the method of Zender and Falbriard (*21*). The data have been corrected by subtracting values found in normal rats. Similar results were obtained in diabetic rats for 4 weeks that received subcutaneous injections of AY-22,284 (500 mg/kg per day) for the last 3 days (by K.H.G.).

Group	Accumulation (micromoles per gram of tissue)		
	Glucose (mean ± S.E.)	Sorbitol (mean ± S.E.)	Fructose (mean ± S.E.)
Control	11.8 ± 1.0	2.03 ± 0.11	4.55 ± 0.33
AY-22,284	12.0 ± 1.5	1.15 ± 0.16*	2.28 ± 0.22*
Change		− 43 percent	− 50 percent

* $P < .001$.

The effect of AY-22,284 in vivo was investigated in rats fed galactose and in rats with streptozotocin-induced diabetes. In rats fed galactose, galactitol accumulation in the lens and sciatic nerve was significantly reduced when 0.7 percent AY-22,284 was added to the diet (Table 1). Prior treatment of rats with AY-22,284 had no significant effect on the absorption of galactose, as determined by galactose concentrations in blood (*16*). Groups of six rats had access to a diet containing 0.7 percent AY-22,284 for 5 days before receiving a single oral dose of galactose (14 g per kilogram of body weight). Serum galactose concentrations (in milligrams per 100 ml; mean ± standard error) in controls at 1 and 2 hours after galactose administration were 402 ± 30 and 262 ± 26, respectively; in rats treated with AY-22,284, the corresponding values were 438 ± 20 after 1 hour and 293 ± 32 after 2 hours.

The interference of AY-22,284 with aldose reductase in vivo was demonstrated by the markedly decreased incidence of cataract formation in rats fed a diet containing 30 percent galactose. Although cataracts were detected after 29 days in all control animals, only 20 percent of rats treated with AY-22,284 had cataracts (Fig. 1). A similar delay was observed in rats pair-fed a diet containing 40 percent galactose (*17*). AY-22,284 also delayed the appearance of the motor nerve conduction defect in these rats (*18*).

In rats with streptozotocin-induced diabetes, addition of AY-22,284 to the diet for 3 weeks markedly reduced sorbitol and fructose levels in the sciatic nerve without affecting glucose levels in the nerve (Table 2) or in serum. Serum glucose concentrations (in milligrams per 100 ml; mean ± S.E.) on day 1 were 369 ± 11 for controls and 365 ± 14 for treated animals; on day 21, these values were 358 ± 11 for controls and 342 ± 14 for treated animals.

The results with AY-22,284 in galactosemic and diabetic rats are consistent with the capacity of this drug to inhibit aldose reductase. Good absorption and rapid excretion following oral administration, coupled with lack of apparent toxicity, suggest that AY-22,284 may be useful in further studies of the sorbitol pathway and its implication in the development of diabetic and galactosemic complications.

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Luteinizing Hormone–Releasing Factor Potentiates Lordosis Behavior in Hypophysectomized Ovariectomized Female Rats

Abstract. *Subcutaneous injection of luteinizing hormone–releasing factor (LRF) in estrogen-primed hypophysectomized, ovariectomized female rats facilitates the appearance of the lordosis response. The LRF effect on lordosis was seen 90, 180, and 360 minutes after injection. This effect could help to synchronize the female's mating behavior with the ovulatory discharge of luteinizing hormone.*

Luteinizing hormone–releasing factor (LRF) recently has been isolated from the hypothalamus, chemically identified, and synthesized (*1*). Injected systemically, it can cause luteinizing hormone discharge from the female rat pituitary within minutes (*2*). During the estrous cycle of the female rat the ovulatory discharge of luteinizing hormone from the pituitary normally is synchronized with behavioral receptivity, indicated by lordosis responses to mounts by the male (*3*). Therefore, we conducted the following experiments to see if LRF could facilitate lordosis in the female rat.

Twenty Sprague-Dawley female rats, weighing 230 to 250 g, were obtained hypophysectomized and ovariectomized from Hormone Assay Laboratories. Upon receipt in our laboratory they received a subcutaneous injection of 10 μg of estradiol benzoate, which tends to facilitate later behavioral responses to estrogen, and then remained undisturbed for 2 weeks. They were maintained on a reversed light cycle (lights off from 10 a.m. to 10 p.m.), were fed normal lab chow and water, and also had available for drinking a bottle of physiological saline with terramycin and 2 percent sucrose.

Each testing protocol was begun by subcutaneous injection of 2 μg of estradiol benzoate in sesame oil. Sixty-five hours later, at the beginning of the dark phase of the daily light cycle, each rat was given a subcutaneous injection of either the physiological saline vehicle control or commercially available synthetic luteinizing hormone–releasing factor (LRF, Beckman Instruments, Spinco Division). The LRF was given in either of two doses, 0.4 μg or 4 μg, dissolved in 0.3 ml of physiological saline. In different weeks of testing, each rat received the control injection and both LRF doses, counterbalanced for order across the 20 rats. For additional confirmation, tests of some rats were repeated at the same dose, and no marked order effects were observed. After a vehicle control or LRF injection, rats were tested for lordosis with a vigorous stud male 10, 30, 90, 180, and 360 minutes after the injection. Lordosis was scored when the back was arched and the head raised during a mount by the male. Differences in the lordosis quotient [(number of lordoses by female/number of mounts by male) × 100] were evaluated statistically by the sign test (*4*), while differences in the percent of tests positive for lordosis were evaluated by the nonparametric McNemar test (*4*). Upon autopsy, complete ovariectomy was confirmed. Completeness of hypophysectomy was confirmed by observing the lack of body weight increase over the weeks (due to lack of pituitary growth hormone), and at the end of the experiment by microscopic examination of the sella turcica and of histological sections through the median eminence.

Under the conditions of this experiment, estrogen alone, followed only by the vehicle control injection, did not lead to high levels of lordosis behavior (Table 1). The lower dose of LRF gave small increases in lordosis, of borderline statistical significance. However, the higher dose of LRF caused significant increases in the occurrence of lordosis (Table 1). These were reflected in both the number of tests positive for lordosis and in the lordosis quotient, and appeared in the tests 90, 180, and 360 minutes after LRF injection. Additional tests, using manual stimulation of lordosis by the experimenter (scratching the female's flanks and exerting pressure on the base of the tail and on the perineal regions), also showed that the higher dose of LRF facilitates lordosis (*5*).

Thus, LRF in sufficient doses can facilitate lordosis in the estrogen-primed female rat. After submission of this report, Moss and McCann (*6*) reported independently that LRF can trigger lordosis in ovariectomized rats primed with estrone and that thyrotropin-releasing factor is ineffective. Since in our experiments the subjects were also hypophysectomized, the effect of LRF could not have been due to

Table 1. Facilitation of lordosis behavior by luteinizing hormone-releasing factor (LRF) in estrogen-primed hypophysectomized, ovariectomized female rats. Some rats were tested more than once at the same dose, so the number of tests is greater than the number of rats. The mean score was taken for each animal at each time point and dose; S.E., standard error.

Treatment	Rats (No.)	Tests positive for lordosis (%)					Lordosis quotient* (mean ± S.E.)				
		Minutes postinjection					Minutes postinjection				
		10	30	90	180	360	10	30	90	180	360
Saline control	20	5	0	8	7	0	6 ± 4	0	5 ± 3	4 ± 3	0
LRF											
0.4 μg	20	0	0	13	20	15	0	0	10 ± 5	18 ± 7	11 ± 6
4.0 μg	20	0	7	46†	57†	50†	0	4 ± 3	32 ± 6†	42 ± 7†	34 ± 7†

* (Number of lordoses by female/number of mounts by male) × 100; calculated from results for all tests, including those where the quotients equal zero. † Significantly different from saline controls ($P < .01$).