Aldose Reductase Inhibition and Retinopathy

I would like to comment on the article by Drs. Ronald L. Engerman and Timothy S. Kern entitled "Aldose Reductase Inhibition Fails to Prevent Retinopathy in Diabetic and Galactosemic Dogs" published in *Diabetes* (1).

First, it appears that much of the galactosemic dog data has been published previously. The data presented in Table 1 (1) on the 9 placebo dogs of the galactosemic groups are identical to the data presented in Table 1 of an article published previously (2) (i.e., $n = 9$, $HbA_1 = 6.4 \pm 0.4\%$, glycated plasma protein = 66 ± 6 nmol/g; duration = 60 months appears in both). Curiously, the remainder of the data on the same line are numerically identical to the data in Table 2 of the previous publication (2) even though the *n* value was reported to be 9, 5, and 6 rather than 9 for erythrocyte hexitol (1,475 ± 517 nmol/g Hb), muscle hexitol (222 ± 148 nmol/g protein), and retinal galactitol (23.3 ± 7.2 nmol/mg DNA), respectively. Data presented for the sorbinil-treated galactosemic group in Table 1 (1) also are numerically identical to most of the data published in the earlier article (2) in both Table 1 (i.e., $n = 10$; $HbA_1 = 6.7 \pm 0.4\%$; duration = 60 months) and Table 2 (i.e., erythrocyte hexitol = 134 ± 34 nmol/g Hb; muscle hexitol = 25 ± 9 nmol/g protein). Although the data are numerically the same, the *n* values were listed as 10 and 5 in one publication (2) and 10 in the other (1). Also, Table 2 of the article (1) has entries on data lines 3 and 5 of both columns 3 and 5 that are the same as entries in Table 22, data lines 6 and 7 of columns 1 and 3 of yet another publication (3). Note that the authors cite, in METHODS, a 1984 paper (their reference 8) rather than citing an article with much identical data (2) that appears to have involved the same dogs and gives more precise and relevant methods.

Second, the authors state that their dogs varied in age from 1.5 to 2.5 years, but fail to mention that they came from different strains (personal communication) and how these strains might be expected to differ in their responses to treatment at different ages. A serious matter is the failure to address appropriately the fact that all the diabetic and galactose-fed dogs had cataracts whether they were given an aldose reductase inhibitor or placebo. Brief comments (e.g., ". . . technical problems such as variations in photo quality during cataract development") (1) obscure the important issue that cataract formation indicates an inadequate and/or inconsistent drug dose. Various aldose reductase inhibitors have been shown to prevent both lenticular polyol accumulation and the diabetes (sugar) cataract in all other animal models (4). Because the experimental design did not include adjustment of the drug dose so cataracts would be prevented, it is not surprising that retinopathy was not prevented. Although an independent study (5) on galactose-fed dogs also failed to prevent cataracts with aldose reductase inhibitor treatment, the investigators documented the occurrence of cataracts and carefully followed their early progression (6). They found that, although the cataracts were not prevented by any of the doses used, both cataracts and retinopathy were delayed in a dose/drug potency-related manner.

The retinal polyol measurements are helpful, but they do not provide information on the capillary pericytes where elevated polyol levels might be expected to have the greatest effect. Pericyte degeneration is one of the earliest capillary lesions and has been considered by many to trigger the subsequent cascade of angiopathies leading to diabetic retinopathy, mainly a microvascular disease. A retention of high concentrations of polyol in the pericytes until their demise would have been missed because the measurements were done on the total retinal volume (of which the pericytes represent only a minute percentage) and after 60 months (when a large proportion of the pericytes were degenerated in the galactose-fed dogs).

Third, it is extraordinary that a report that depends entirely on critical morphological studies does not have a single micrograph for documentation. The article does not demonstrate a capability of *1*) adequate funduscopy in dogs, *2*) quality electron microscopy for the determination of basement membrane thickening, or *3*) the ability to make the artifact-free preparations that are needed for accurate recognition of microaneurysms and pericyte ghosts.

Fourth, most alarming is the apparent subjectivity and misrepresentation exhibited regarding the many publications on the galactose-fed rat model. On page 824, 2nd paragraph, the authors mention only 3 galactose-fed rats and ". . . 3 additional rats fed an aldose reductase inhibitor with the galactose diet" (1), thus giving the impression that success with aldose reductase inhibitors was limited to 3 rats. The readers of *Diabetes* deserve to know about the studies published in several respected journals that have been ignored, many of which clearly demonstrate prevention of various stages of retinopathy by various aldose reductase inhibitors in the galactose-fed rat (7–10). Including rats in the studies cited by the authors, a total of 36 rats from 3 different strains were studied in two independent laboratories in which two different aldose reductase inhibitors at 3 different doses were tested. All demonstrated prevention of capillary basement membrane thickening, which ". . . is widely accepted as the ultrastructural hallmark of diabetic microangiopathy. . . " (11). In addition, the prevention of the more advanced angiopathies such as microaneurysms,

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shunts, and hypercellular channels in rats fed a 50% galactose diet plus any 1 of 4 different aldose reductase inhibitors has been reported in an abstract (12).

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REFERENCES

1. Engerman RL, Kern TS: Aldose reductase inhibition fails to prevent retinopathy in diabetic and galactosemic dogs. *Diabetes* 42:820–25, 1993
2. Kern TS, Engerman RL: Retinal polyol and *myo*-inositol in galactosemic dogs given an aldose-reductase inhibitor. *Invest Ophthalmol Visual Sci* 32:3175–77, 1991
3. Kern TS: *Dog Model for Diabetic Retinopathy and Nephropathy (Workshop on Aldose Reductase Inhibitors)*. Washington, DC, National Institutes of Health, 1991, p. 65–73 (NIH publ. no. 91–3114)
4. Robison WG Jr, Laver NM: Ocular lesions in animal models of human diabetes. In *Lessons From Animal Diabetes IV Edition*. Shafrir E, Ed. London, Smith-Gordon, 1993, p. 146–63
5. Kador PF, Akagi Y, Takahashi Y, Ikebe H, Wyman M, Kinoshita JH: Prevention of retinal vessel changes associated with diabetic retinopathy in galactose-fed dogs by aldose reductase inhibitors. *Arch Ophthalmol* 108:1301–309, 1990
6. Sato S, Takahashi Y, Wyman M, Kador PF: Progression of sugar cataract in the dog. *Invest Ophthalmol Visual Sci* 32:1925–31, 1991
7. Robison WG Jr, Kador PF, Kinoshita JH: Retinal capillaries: basement membrane thickening by galactosemia prevented with aldose reductase inhibitor. *Science* 221:1177–79, 1983
8. Frank RN, Keirn RJ, Kennedy A, Frank KW: Galactose-induced retinal capillary basement membrane thickening: prevention of sorbinil. *Invest Ophthalmol Visual Sci* 24:1519–24, 1983
9. Robison WG Jr, Kador PF, Akagi Y, Kinoshita JH, Gonzalez R, Dvornik D: Prevention of basement membrane thickening in retinal capillaries by a novel inhibitor of aldose reductase, tolrestat. *Diabetes* 35:295–99, 1986
10. Robison WG Jr, Nagata M, Kinoshita JH: Aldose reductase and retinal capillary basement membrane thickening. *Exp Eye Res* 46:343–48, 1988
11. Williamson JR, Kilo C: Current status of capillary basement-membrane disease in diabetes mellitus. *Diabetes* 26:65–75, 1977
12. Laver N, Robison WG Jr, Kinoshita JH: The galactose-fed rat model for diabetic retinopathy (Abstract). *Invest Ophthalmol Visual Sci* 31:127, 1990

Response to Robison

Dr. Robison, in his opening paragraph, is mistaken in claiming that the *n* value for some measurements in our retinopathy paper (1) do not agree with those reported in our earlier paper (2). Apparently he overlooked the values for *n* shown for various measurements in our METHODS section (1; p. 821, right column, paragraph 1).

To properly characterize the diabetic and galactosemic groups in our retinopathy paper, in one of the tables we included data that would allow the severity of glycemia and Sorbinil's effects on polyol levels to be compared in the animals, even though some of the data on tissue chemistry of galactosemic dogs also appears in another publication. The tissue chemistry data in question are essential for interpretation of the drug's effect on retinopathy. In fact, the absence or scarcity of such essential data from the studies of rats and dogs cited by Dr. Robison has made it virtually impossible to reconcile their conclusions with ours. Contrary to the closing remark of the paragraph, our METHODS section does in fact cite the paper in question (2) (reference 19 in our paper). In retrospect, the reference should have been added to Table 1 also.

The suggestion by Dr. Robison that the Sorbinil dose should have been chosen based on cataractogenesis is overly simplistic and less satisfactory than the procedure we followed. Cataractogenesis is much slower in dogs than in the rats familiar to him and offers no more than belated evidence of lens dysmetabolism during previous months or weeks. Sorbinil dosages in our experiment were based chiefly on the drug's efficacy in preventing tissue polyol accumulation, a goal implicit in the hypothesis being tested. Dr. Robison's incredible assertion that cataractogenesis in our dogs was not inhibited by Sorbinil treatment is baseless and untrue. Lens observations in our dogs confirm a well-known inhibition of cataract by the drug and will be published after completion of studies of greater priority on retinopathy and other complications in our animals. Moreover, his claim that "aldose reductase inhibitors have been shown to prevent. . . cataract in all other animal models" is contradicted by his subsequent sentences acknowledging that cataract was, in fact, not prevented in the only other long-term study of aldose reductase inhibition in dogs (3). Cataract in that study and in ours was inhibited, but not as completely as often has occurred in rats.

The adequacy of our methods for study of retinopathy in animal models has been demonstrated heretofore in numerous publications, workshops, and conventions. In this paper on retinopathy, we evaluated previously reported anatomic features in a quantitative fashion with the intent of drawing statistical inferences. Also, the manuscript's referees presumably felt that photos would be an unnecessary expense.

The final paragraph of Dr. Robison's letter claims that retinopathy has been prevented by aldose reductase inhibitors in many more rats than the 3 galactose-fed rats we mentioned. However, when he alludes to the "prevention of various stages of retinopathy. . . in the galactose-fed rat" by these drugs, he is mistaking early changes, such as basement membrane thickening, for overt retinopathy. Although he claims that the drugs also have prevented lesions, such as microaneurysms, in galactose-fed rats, the evidence he cites consists of a 1990 abstract that makes absolutely no mention of any effect of drugs, and in the several years after that abstract no supporting evidence has appeared in any peer-reviewed publication. Reported effects of aldose reductase inhibitors on microangiopathy (i.e., basement membrane thickening) are not as generalized as he suggests. In contrast to the reported inhibition of capillary basement membrane thickening in the rat retina, thickening in the glomerulus is reportedly not inhibited by these drugs in either diabetic or galactosemic rats. Unfortunately, no information has been published on the basement membrane of dogs in which retinopathy has been said by