Noninvasive Glucose Monitoring of the Aqueous Humor of the Eye: Part II. Animal Studies and the Scleral Lens

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We have discussed the nature of a scleral lens that will allow us to follow changes in aqueous humor glucose levels in animals by a method based on optical rotation and a technique described in an earlier paper. We have shown how this lens can be micro-miniaturized and can be used in humans as a non-invasive glucose monitor. We have described preliminary experiments designed to show the correlation between the blood glucose assay (BGA) and the aqueous humor glucose concentration as determined by chemical assay (AGA) and by optical rotation determination (ARD). The last mentioned has been obtained by paracentesis directly into a microcell used in conjunction with instrumentation capable of measuring optical rotations as low as 0.0013° (4.5″) corresponding to 20 mg/dl glucose with a sensitivity of 0.0001° (0.36″). The variability among normal rabbits as a function of individuality and diurnal changes is described, and the correlation beteen AGA and ARD shown to be essentially 1.0. Such rabbits are examined when undergoing very rapid decreases in BGA (insulin treatment) or very rapid increases in BGA (bolus of glucose). The AGA and ARD are shown to lag behind the BGA, and this is discussed in terms of the rate of change of BGA with respect to time and its concomitant change in AGA/ARD as well as a simple procedure that would materially reduce this lag. DIABETES CARE 5: 259–265, MAY–JUNE 1982.

There is an urgent need for better control of the blood sugar in diabetes. If strict control of diabetic glucose levels in patients proves to be as effective as in experimental animals, improvement in control may be expected to result in a significant reduction in the vascular complications of diabetes. Diabetic kidney disease is a major cause of death, currently ranked fifth in leading causes of death in the United States. Diabetes has been well established as a major risk factor in myocardial infarction. The changes in myocardial metabolism have been well documented in diabetes, but it is not possible to determine if strict control of the blood glucose will reverse these changes unless we have a means at hand to better monitor changes in blood glucose. Development of an acceptable glucose monitor would be expected to allow this improvement in the control of diabetes, using, in addition, insulin pumps which are now readily available.

Improvement in the control of diabetes, especially the avoidance of both hypoglycemia and hyperglycemia, would be more readily attainable if fluctuations of blood glucose were continuously measurable. Since safety and convenience are prerequisite to acceptance of any device for monitoring blood glucose, the feasibility of using the eye as a window to the blood composition has been undertaken.

The studies proposed here will clarify the feasibility of monitoring changes in blood glucose noninvasively by optical analysis of a well defined body fluid, the aqueous humor of the eye. As a consequence of this study, a usable contact lens has been designed and built for animal testing with dogs and rabbits, both nondiabetic and diabetic, so that the response of the monitor to hypoglycemia and hyperglycemia may be tested.

Many of the changes in metabolism induced by the diabetic state have been well established for myocardium, kidney tubules, blood cells, lung alveolae, etc. We propose to monitor the changes in blood glucose noninvasively by optical analysis, in vivo, of the aqueous humor, through the development of instrumentation, the methods of which have already been demonstrated in our laboratories.[1]

We propose the miniaturization of this instrument so that the diabetic may wear it continuously and comfortably. The response of this sensor to hypoglycemia and hyperglycemia

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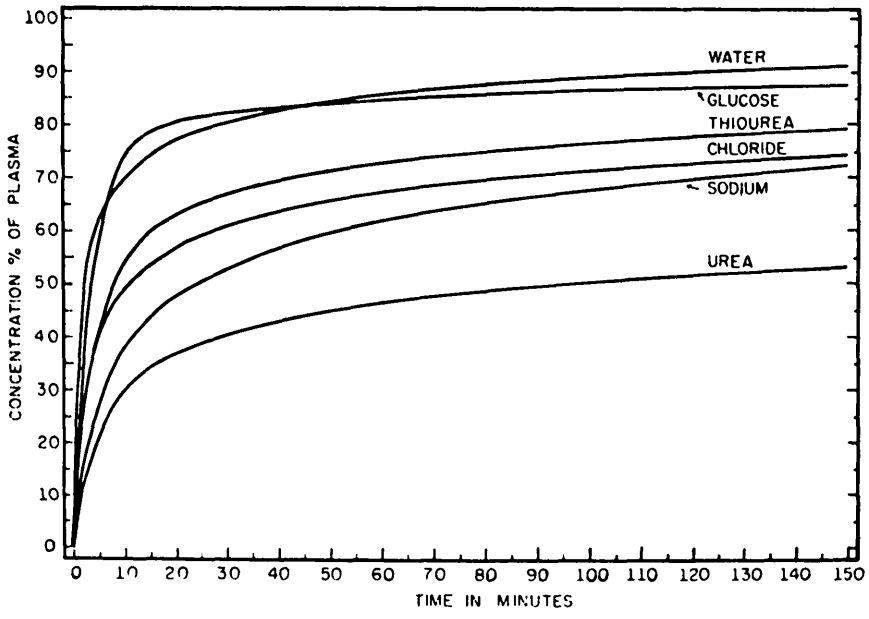

FIG. 1. Calculated rates of entry to various substances across the blood-aqueous barrier in the rabbit eye. Constant plasma level of 100%.[3]

induced in normal and diabetic dogs is to be tested under the influence of various dietary sugars, fat, protein, ethanol, carbohydrate, and selected pharmaceuticals. Once this monitor has been proven to be acceptable, it will be micro-miniaturized and coupled to already established insulin pumps through telemetry, so that the effect of strict blood sugar control on the changes in diabetic metabolism, with special reference to those tissues most affected by diabetes, may be determined.

Three questions need to be addressed to underline the rationale for our approach. First, the question might arise as to the ease in wearing a scleral contact lens with a hard limbal ring and a soft lens flange. Since the cornea has more sensitivity than the conjunctiva, it is actually quite easy to wear a scleral lens.[2] The patient should be quite comfortable with a lens of reasonable proportions. Second, the question arises as to the time of equilibrium between blood glucose and aqueous humor glucose. Figure 1 gives theoretically calculated curves[3] in which a sudden increase of various substances in the circulating blood is shown to reflect itself in a more or less rapid increase in the posterior chamber of the eye. It can be seen that glucose is transported into the aqueous humor particularly rapidly, being above 89% complete in 10 min and above 94% complete in 20 min. Inasmuch as blood glucose levels in the diabetic are likely to change considerably more slowly than this, transport into the eye should not be the rate-determining step. Diffusion from the posterior to anterior chamber is indeed rapid,[4] but is further addressed experimentally in the present paper. Third, we must be concerned with the possibility of other optically active substances in the aqueous humor interfering with the determination of glucose. Although the aqueous humor of the primate[5] contains substances, other than glucose, which are optically active, these substances have relatively low concentrations and rotations of opposite signs so that net rotations may well be negligible. Preliminary experiments in our laboratories have indicated a good relationship between the optical rotation observed in the aqueous humor and the blood glucose concentration, in spite of the presence in the aqueous humor of fructose, galactose, lactate, and sorbate.[6] We have attempted in the present work to establish to what extent other substances in the aqueous humor interfere with this correlation.

EXPERIMENTAL METHODS

To establish the relationship between the blood glucose concentration and the aqueous glucose concentration, we used rabbits (3.6–5.0 kg) that were kept on a normal rabbit chow and water ad libitum. Six such rabbits were used in our first experiment in which, under a general anesthetic of 2.0 ml of Ketamine and 0.5 ml of Rompun, blood was drawn from an

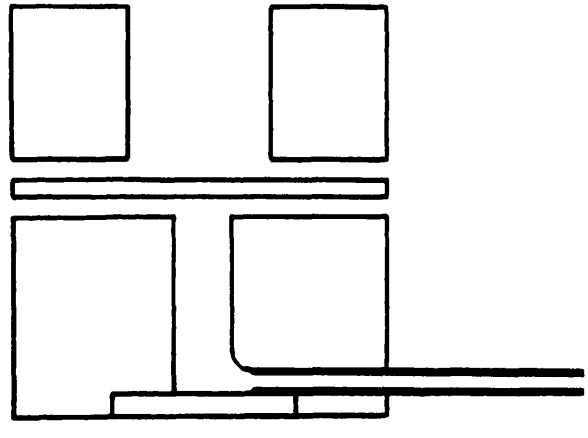

FIG. 2. Micro-cell, capacity 0.08 ml, with integrated 30-gauge needle for anterior chamber paracentesis and optical rotation determination.

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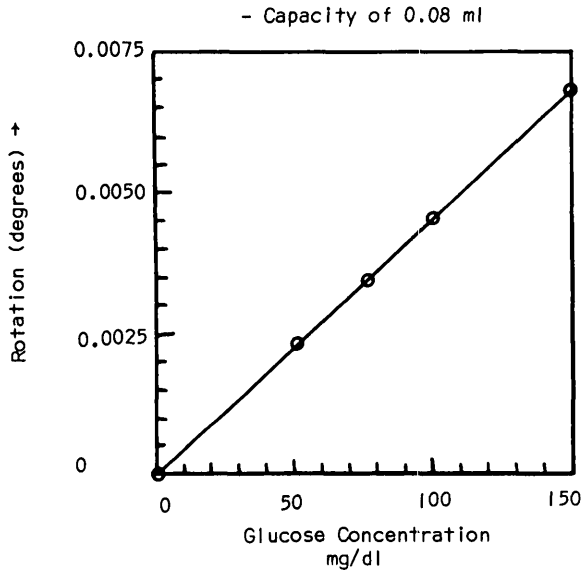

FIG. 3. Calibration of microcell with standard glucose solutions.

ear vein for blood glucose assay (BGA) in duplicate by the glucose-oxidase-peroxidase-o-dianisidine method.[7] The procedure was performed on undeproteinized plasma, which in fact gives values 10 mg/dl higher than when applied to whole blood,[8] but we have chosen to refer to the values as BGA. Blood samples were drawn from these rabbits at times spread over a 10-h period. At the time of blood withdrawal, a sample of aqueous humor was taken by paracentesis directly into a microcell (Figure 2) whose total capacity is 0.08 ml. This microcell was designed for use with the ultra-sensitive polarimeter described previously,[1] and Figure 3 shows the calibration of the cell using known concentrations of glucose. A determination of the optical rotation of the aqueous humor was made within 1 min of sampling in the expectation that if the active transport of glucose across the blood-aqueous barrier were specific for one glucose anomer, subsequent mutarotation might become evident from time-dependent changes in angle of rotation. No such firm evidence for such mutarotation was evident, although at times as much as a 10% change in angle of rotation was seen to occur over a 20-min period. However, this was at times an increase, at other times a decrease, and frequently no change at all. This aspect of the problem, as small as it is, will be pursued more carefully.

After optical rotation determination (ARD) on the aqueous humor, the sample was used for an aqueous glucose assay (AGA) in duplicate by the same technique as described for the blood sample. Since the blood-aqueous barrier in the eye is frequently broken down after a traumatic event such as paracentesis, so that serum proteins may appear in the aqueous humor, each eye was used only once for sampling. The results of these determinations are shown in Figure 4. It must be emphasized that the data shown in Figure 4 cannot be interpreted simply as showing the diurnal variations of the glucose levels since six rabbits are involved and therefore variations of individuality in glucose levels are included. However, to the extent that these are rabbits of the same breed and age, as well as healthy and in a similar nutritional state, some significance can be placed upon the variation of glucose levels throughout the day. We feel that the exceptionally high values of BGA and AGA at 12:15 p.m. are due

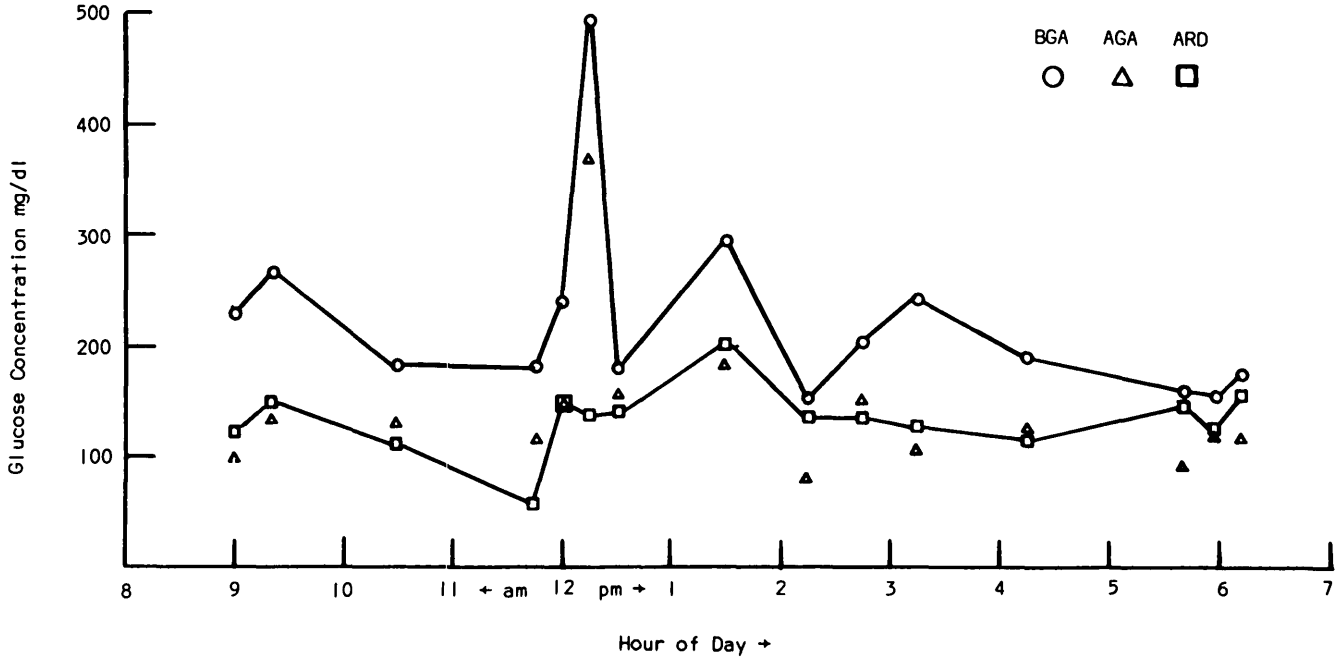

FIG. 4. Diurnal and individual variation in the rabbit of blood glucose levels by chemical assay (BGA) and of aqueous glucose levels by chemical assay (AGA) and by optical rotation (ARD).

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TABLE 1
Variability of glucose determinations among rabbits

Rabbit	BGA/ AGA	AGA/ ARD	Rabbit	BGA/ AGA	AGA/ ARD
84 LE	2.365	0.793	89 RE	1.962	0.564
84 RE	1.977	0.886	87 LE	1.333	1.117
80 RE 1st	1.368	1.167	97 RE	2.327	0.806
91 RE	1.602	1.982	88 LE	1.500	1.068
88 RE	1.342	2.592	60 RE	1.314	0.952
87 RE	1.174	1.076	97 LE	1.483	0.742
80 RE 2nd	1.578	0.917			

RE, LE = right eye, left eye. BGA = blood glucose assay. AGA = aqueous glucose assay. ARD = aqueous rotation determination.
Mean BGA/AGA = 1.644 ± 0.364(SD): less highest and lowest values = 1.624 ± 0.304.
Mean AGA/ARD = 1.085 ± 0.531(SD): less highest and lowest values = 1.009 ± 0.333.
Mean BGA = 223 ± 86 mg/dl: less highest and lowest values = 207 ± 42 mg/dl.
Mean AGA = 141 ± 69 mg/dl: less highest and lowest values = 129 ± 27 mg/dl.
Mean ARD = 136 ± 31 mg/dl: less highest and lowest values = 136 ± 14 mg/dl.

to an excited rabbit's high hormonal activity, but the observed relatively normal ARD remains unexplained and needs further investigation.

Table 1 shows these data listed as the ratios BGA/AGA and AGA/ARD for individual rabbits with mean values of the separature BGA, AGA, and ARD determinations and ratios given with the standard deviations. It is clear that: (1) the blood glucose assay shows the widest variations, even when the highest and lowest values are omitted as lying outside of the statistically expected variation; (2) the ratio BGA/AGA in our hand is far larger than that reported by Kinsey[9] who gives a value of 1.235. However, no standard deviation is given nor yet the number of determinations and time span; and (3) the AGA/ARD mean ratio differs little from 1.0, especially if the highest and lowest values are neglected.

In Figure 5, we show the results of determining values for BGA, AGA, and ARD on a normal rabbit given, at time zero, 10 U of crystalline Zn insulin. The BGA value is seen to fall rapidly from normal to immeasurably small within 2 h, while the AGA and ARD values, lying close together, lag behind. However, it should be pointed out that such a precipitous drop in blood glucose levels is probably unusual in the controlled diabetic and that for more gradual changes in BGA, values for AGA and ARD should not be far behind. Moreover, the average normal AGA and ARD indicate that there is an immediate and linear fall in their values from the time of injection, though more work on this is under way.

Figure 6 shows how glucose levels vary in a normal rabbit given a bolus of 10 g glucose, in solution, injected directly into the stomach. Again we see that while the AGA and ARD lag behind, by the lapse of 2 h they have caught up with the BGA. It is to be expected that with a less dramatic

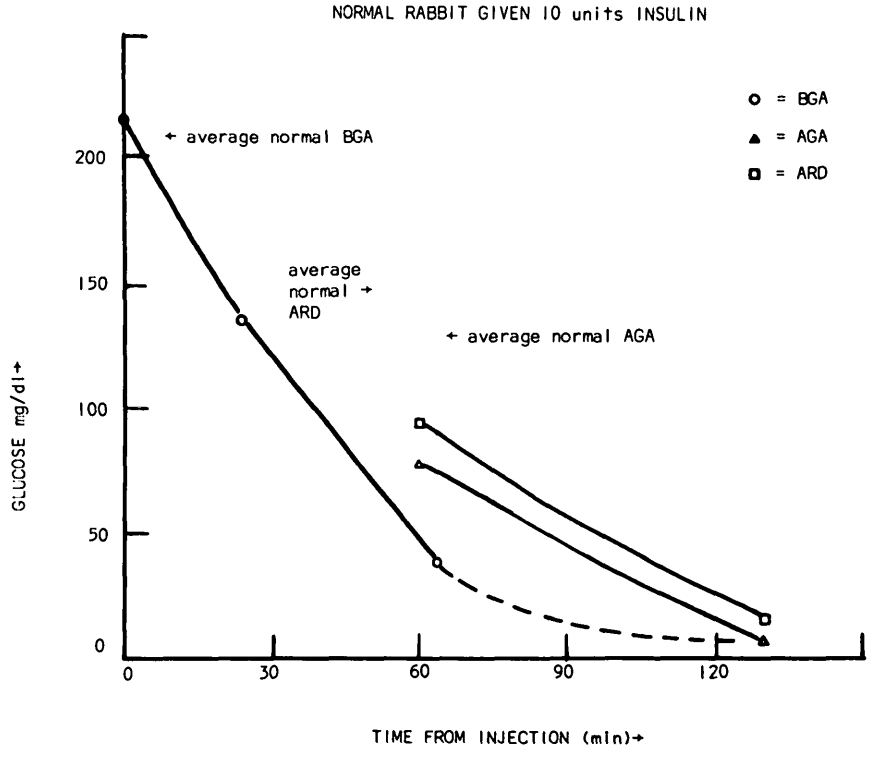

FIG. 5. Hypoglycemic reaction in the normal rabbit after injection of 10 U of Zn insulin. The changes in blood glucose (BGA) and aqueous glucose (AGA and ARD) as a function of time.

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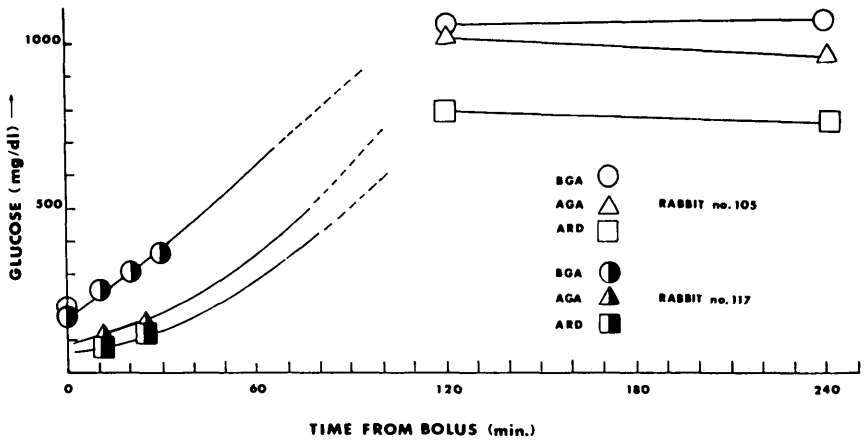

FIG. 6. Hyperglycemic reaction in the normal rabbit after injection of 10 g glucose. The changes in blood glucose (BGA) and aqueous glucose (AGA and ARD) as a function of time.

infusion of glucose, the lag would be considerably less. This figure shows that, in a second experiment, over the short initial period of time after injection, the rise in aqueous humor glucose is indeed immediate.

To compare the relationship between BGA, AGA, and ARD in diabetic subjects, we treated a rabbit with repeated doses, over a 19-day period, of intravenously-injected alloxan, 100–240 mg/kg body weight. At the end of 11 days, the rabbit was acceptable as diabetic, having a BGA that rose from 137 mg/dl to 400 mg/dl, and a mean BGA of 253 ± 75 mg/dl over a period of 7 days.

The rabbit was then given a bolus of 10 g glucose injected directly into the stomach. Figure 7 shows how the BGA rose rapidly over a period of 2 h and appeared to be continuing to rise, while the AGA and ARD paralleled it closely. There is no reason to believe that the AGA and ARD does not show an immediate rise parallel to that of the BGA from the time of injection, though the data is insufficient at present to prove this. The value of BGA/AGA for the normal rabbit of 1.62 places the AGA for this diabetic rabbit at 253 mg/dl, a value not inconsistent with parallelity.

DISCUSSION

We have shown, in these preliminary results, that the AGA and ARD are a proper measure of the aqueous humor glucose concentration, being statistically indistinguishable from each other. These data are consistent with the curves of Kinsey and Reddy[3] for diffusion of glucose from the posterior to the anterior chamber after a rapid change in blood glucose. Although such rapid changes would not be expected in the human diabetic under usual circumstances, it may become desirable to speed diffusion by a method currently practiced in our clinics. This is done by performing a series of laser iridotomies, a procedure that has become accepted throughout the major ophthalmologic patient facilities of the world. It routinely reduced the time for glucose equilibration between the posterior and anterior chambers by a factor of 15.[10]

It is also quite clear that changes in the anterior chamber glucose begin simultaneously with those in the blood and the first derivative of this change with respect to time may in itself represent the means of triggering an infusion of insulin at

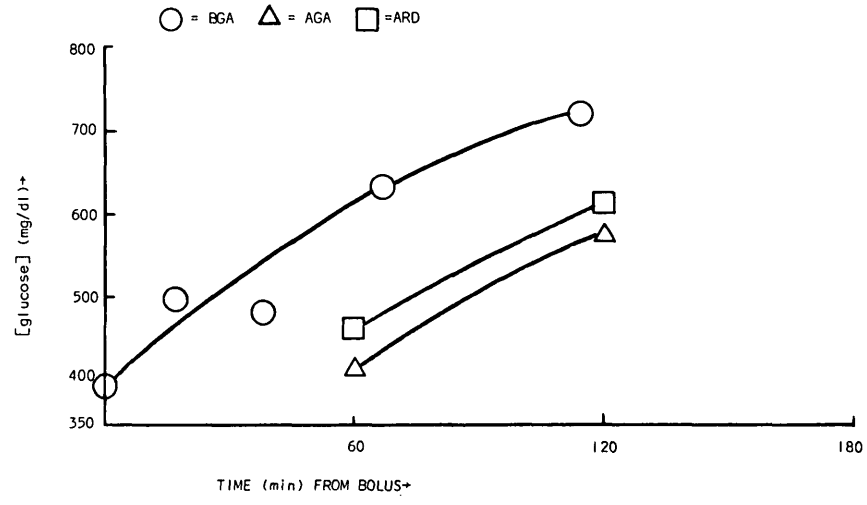

FIG. 7. Hyperglycemic reaction in the alloxan diabetic rabbit after injection of 10 g glucose. The changes in blood glucose (BGA) and aqueous glucose (AGA and ARD) as a function of time.

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a rate determined by the rate of change observed in the anterior chamber. These results have encouraged us to proceed with an experimental lens for use in animal experiments, and such a lens has already been designed and built (Figure 8) such that fiber optics may be used with a remote light source and remote photodetector. This will allow us to assess the effect of the optical activity of the collagen of the cornea on the state of polarization of the incident light. Although the cornea is approximately 0.52 ± 0.04-mm thick,[11] it is composed of approximately 15% collagen[12] which, in the undernatured state, has high specific rotation.[13] However, it may be taken that the composition of the cornea remains constant and that it will make a constant contribution to the optical rotation. This rotation can readily be compensated for by appropriate adjustment of the relative orientations of Polarizer and Analyzer.

We will thus monitor continuously the anterior chamber glucose concentration noninvasively as a function of a programmed blood glucose change, in vivo, by i.v. infusion of a glucose solution or rapid acting insulin of low concentration. We can then say whether the absolute value of the anterior chamber glucose concentration or the first derivative of its concentration with respect to time will be the measure of required insulin. This information may in the final analysis be relayed by telemetry to the already available insulin pump. That such a telemetric method is available and can be miniaturized to fit into a contact lens has already been demonstrated in its use for continuous monitoring of intraocular pressure.[14] We anticipate that both the electro-optic and telemetric components will be incorporated eventually into a scleral contact lens which the diabetic patient may wear comfortably at all times and thus remain under strict blood glucose control. In Figure 9, we show two views of such a scleral lens, in which micro-miniaturization and integrated circuitry could allow for a totally self-contained glucose monitor.

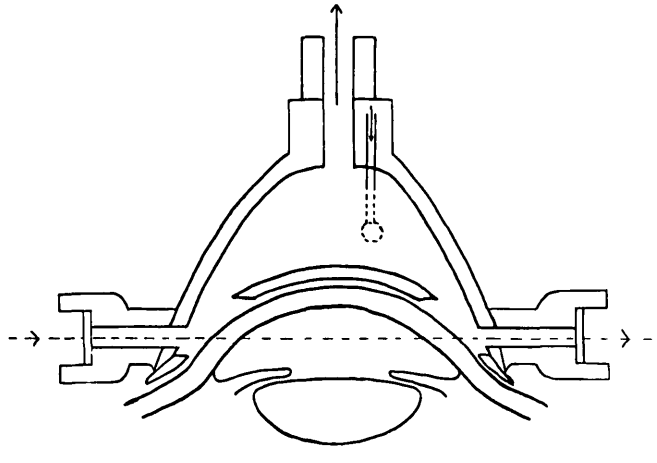

FIG. 8. Diagrammatic representation of experimental scleral lens for animal studies. The path of the incident light traversing the anterior chamber is shown, as well as the exit port for suction placement and the entry port for bathing fluid.

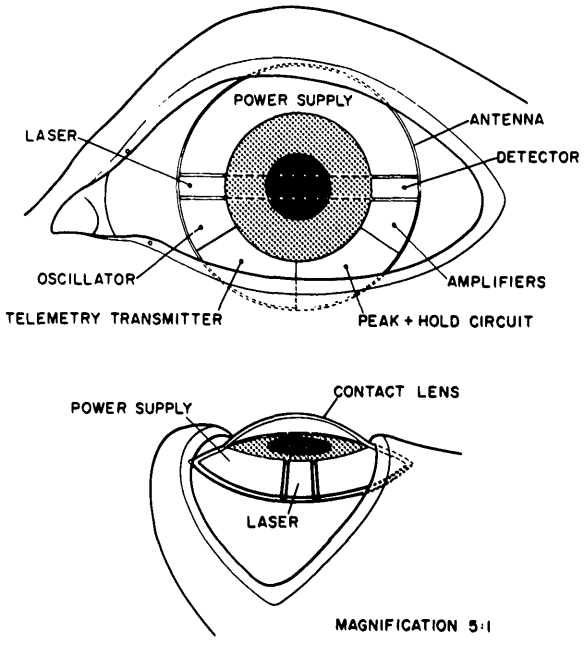

FIG. 9. Two views of a proposed scleral lens for human use as a noninvasive glucose monitor, showing various electronic and electro-optic units.

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