

---

# Comparison of amino acid transport in ocular structures of rabbits made diabetic by alloxan and pancreatectomy

*D. V. N. Reddy, V. Everett Kinsey, and G. Nathorst-Windahl*

*Whether alloxan or diabetes is responsible for the lowered concentration of amino acids in aqueous humor and lenses of rabbits made diabetic by this drug was investigated. The rate of accumulation of <sup>14</sup>C-cycloleucine (l-amino cyclopentane carboxylic acid) in both posterior aqueous and lens was depressed to essentially the same degree in rabbits made diabetic by pancreatectomy or by alloxan. The uptake of cycloleucine in lenses cultured in the presence of alloxan was unaffected. It is concluded that some factor associated with diabetes, probably elevated level of glucose, is responsible for the altered rate of accumulation across both the ciliary epithelium and the lens rather than a direct toxic effect of alloxan.*

The rates of accumulation of two model amino acids,  $\alpha$ -amino isobutyric acid ( $\alpha$ -AIB) and l-amino cyclopentane carboxylic acid (cycloleucine), and the steady state concentration of most naturally occurring amino acids are decreased in the aqueous humors and lenses of rabbits made diabetic with alloxan. The reduction in concentration has been ascribed to some factor associated with diabetes.<sup>1</sup> However, it is possible that alloxan, or a derivative, may exert a direct toxic action on the

mechanisms, especially those in the ciliary processes, responsible for accumulation of the amino acids.

The present investigation is designed to distinguish between the two possibilities by (1) comparing the effect of diabetes produced by pancreatectomy and by alloxan on the rate of accumulation of <sup>14</sup>C-cycloleucine in posterior aqueous humor and lens, and (2) determining the effect of alloxan on the accumulation of this model amino acid in cultured lenses.

## Methods

Albino rabbits weighing about 3.5 Kg. were pancreatectomized under sterile conditions by a method not described heretofore. The operation was performed in two stages to reduce the mortality. Before each of the operations, animals were fasted for 10 to 14 hours and then anesthetized with Numal\* and ether. A midline in-

\*5-Allyl-5-isopropylbarbituric acid.

---

From the Kresge Eye Institute of Wayne State University School of Medicine, Detroit, Mich., and Patologiska Institutionen, University of Uppsala, Uppsala, Sweden.

This study was supported in part by Research Grants B-2885 and B-1100 from the National Institute of Neurological Diseases and Blindness, United States Public Health Service, and by the United States Atomic Energy Commission, Contract No. COO-152-50.

cision was made in the abdomen and the spleen was gently pulled out of the cavity. To mobilize this organ, the two rami gastrici of the arterial lienogastrica were divided. Then, without damaging the wall of the gut, the pancreatic tissue was gently removed from the left part of the mesocolon. The stomach was raised, and the retrogastric portion of pancreas covering the large abdominal arteries and veins was carefully dissected free. A small lobe of pancreatic tissue in the angle between the vena porta and vena lienogastrica and extending to the vena cava was dissected free with great care, to avoid hemorrhage. Starting at the vena lienogastrica and proceeding along the lienal vessels, the pancreatic tissue from the splenic vessels was then dissected. After the spleen was returned to its normal position, the proximal part of the duodenum was turned over to the left so that the portion of pancreas between the vena cava caudalis and the vena porta could be exposed and removed. Finally, the pancreatic tissue in the proximal duodenal mesentery, along with a

portion close to the ductus choledochus, was dissected free.

During the second stage of the operation, performed after an interval of at least three weeks, the abdominal cavity was again entered. The distal part of the duodenum was pulled out, and a thin ligament between the colon and duodenum was divided so that the distal part of the latter organ could be freed. The borderline to the proximal duodenal loop and mesentery was identified, and pancreatic tissue in this area was dissected free, whereupon the remaining part of the pancreas was removed. Finally, the pancreatic duct was sectioned between ligatures a few millimeters from the opening of the duct into the duodenum.

Mild diabetes, with occasional periods of moderate ketonuria, develops when 3 to 6 per cent of pancreatic tissue is left in situ. Total, or approximately total, pancreatectomy gives rise to severe diabetes, with pronounced lipemia, ketonemia, and acidosis.

In the present study, approximately

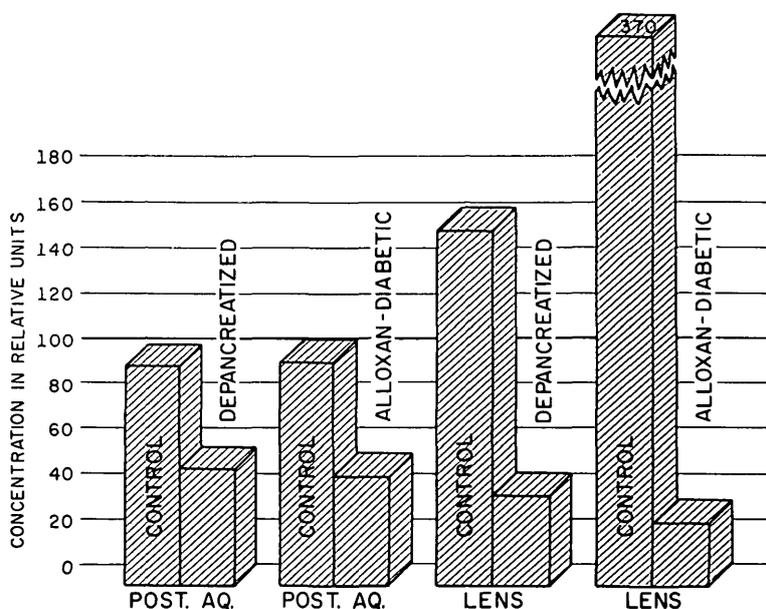


Fig. 1. Relative concentration of  $^{14}\text{C}$ -cycloleucine in posterior aqueous and lenses of normal, alloxan-diabetic, and depancreatized rabbits 48 hours after parenteral administration. All values are expressed as percentage of concentration in plasma water.

total pancreatectomy was performed on two animals, and a sham operation on two litter mate controls. All animals were sent via air freight from Sweden to Detroit. One of the pancreatectomized rabbits had been diabetic for 72 days, and the other for 155 days before they were injected with labeled cycloleucine. After the second stage of the operation, depancreatized animals were given 2 to 12 units of insulin daily. During the last week of the experiment they were given 4 units daily. Throughout the experimental period their diet consisted of 100 Gm. each of hay, crushed oats, and turnips and they were given free access to water. Under these conditions the diabetic animals maintained constant weight and a blood sugar level of 400 to 500 mg. per cent. At the termination of the experiment, the lenses were examined under a dissecting microscope and three of them were found to have narrow bands of opacity around the periphery. These lenses weighed between 502 and 506 mg.; the fourth, which was clear, weighed 520 mg.

Methods for measuring uptake of  $^{14}\text{C}$ -labeled cycloleucine by the lens *in vitro* have been described previously.<sup>2, 3</sup> The distribution of cycloleucine in plasma, posterior aqueous, and lens of control and depancreatized animals was determined 48 hours following a single injection of the labeled compound, a procedure which was shown to maintain an essentially constant level of the amino acid in the plasma for this period of time.

### Results

Fig. 1 shows the concentration of  $^{14}\text{C}$ -cycloleucine in aqueous humor of the posterior chamber and lens relative to the concentration in plasma, in both control and depancreatized rabbits. For comparison, analogous data for "alloxan-diabetic" rabbits and corresponding controls taken from a previous study,<sup>1</sup> are also included. In all instances, the data were obtained 48 hours following parenteral administration of the test substance, and the concen-

tration of radioactivity in the plasma was set arbitrarily at 100 relative units.

The concentration of cycloleucine in the posterior aqueous of depancreatized rabbits, like that in alloxan-diabetic animals, is reduced to approximately 50 per cent of that in the control group. Likewise, the concentration of cycloleucine is reduced in lenses of animals made diabetic by both procedures, pancreatectomy or alloxan, although the reduction appears to be proportionately greater in the case of alloxan-diabetic animals. The lower concentration of cycloleucine in the controls of the depancreatized group may be due to the fact that lenses in this group were from rabbits 2 to 5 months older than the controls of the "alloxan-diabetic" animals.

Accumulation of  $^{14}\text{C}$ -cycloleucine in normal rabbit lenses cultured for 24 hours in media containing various concentrations of alloxan was not affected by concentrations as high as  $10^{-3}\text{M}$  (Table I).

### Discussion

The similarity of the results obtained with depancreatized rabbits and those made diabetic with alloxan suggests that reduction in accumulation of  $^{14}\text{C}$ -labeled cycloleucine in both the aqueous of the posterior chamber and the lens is caused by some factor, or factors, associated with diabetes rather than by the toxic action of alloxan. This conclusion is strengthened by failure to find any reduction in the rate of accumulation of cycloleucine in lenses

**Table I.** Accumulation of  $^{14}\text{C}$ -cycloleucine in rabbit lenses cultured for 24 hours in media containing various concentrations of alloxan

Concentration of alloxan (M)	Ratio of concentration of $^{14}\text{C}$ -cycloleucine Lens water Initial medium
0	15.0 ± 2.3 (9)
$10^{-5}$	14.8 ± 1.6 (7)
$10^{-4}$	15.0 ± 2.2 (8)
$10^{-3}$	13.3 ± 2.4 (7)

cultured in the presence of alloxan (Table I).

Experiments which showed the rate of accumulation of  $\alpha$ -AIB in cultured lenses to be reduced by concentrations of glucose in excess of 300 mg. per cent<sup>4, 5</sup> suggest that reduction in the level of amino acids in lenses of diabetic rabbits is caused by the elevated level of glucose in the ocular fluids. Similar effects were noted with high concentrations of other sugars, i.e., galactose and xylose. The high concentrations of sugars seem to affect both the pump concerned with active transport of amino acids across the lens epithelium and their rate of leakage out of the lens. At the present time, however, it is not known whether reduction in transport of amino acids across blood aqueous barrier in diabetic animals is brought about by

mechanisms analogous to those occurring in the lenses of these animals.

#### REFERENCES

1. Reddy, D. V. N., and Kinsey, V. E.: Transport of amino acids into intraocular fluids and lens in diabetic rabbits, *INVEST. OPHTH.* **2**: 237, 1963.
2. Merriam, F. C., and Kinsey, V. E.: Studies on the crystalline lens. I. Technic for in vitro culture of lenses and observations on the metabolism of the lens, *Arch. Opth.* **43**: 979, 1950.
3. Kinsey, V. E., and Reddy, D. V. N.: Studies on the crystalline lens. X. Transport of amino acids, *INVEST. OPHTH.* **2**: 229, 1963.
4. Reddy, D. V. N.: Amino acid transport in the lens in relation to sugar cataracts, *INVEST. OPHTH.* **4**: 700, 1965.
5. Kinoshita, J. H., Merola, L. O., and Hayman, S.: Osmotic effects on the amino acid concentrating-mechanism in the rabbit lens, *J. Biol. Chem.* **240**: 310, 1965.