INHIBITION OF SUGAR METABOLISM IN THE LENS*

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OF all the theories advanced to explain the pathogenesis of cataract, the one which seems to have withstood the test of time most satisfactorily is that which ascribes the opacification to a defect in the carbohydrate metabolism of the lens. The most adequate experimental method of obtaining proof of such a theory is to block the sugar metabolism in the lens and observe the results.

This was undertaken by Nordmann and Dillschneider (1951) in their first series of experiments. Interrupting the sugar metabolism by glyceraldehyde, they observed the formation of opacification in the lens, associated with a fall in the production of lactic acid and in the accumulation of energy-rich compounds (adenosine triphosphate) (ATP). We have followed up these experiments using a series of inhibitors—mono-iodacetic acid, sodium fluoride, phloridzine, sodium malonate, and fluoro-acetic acid. This paper gives the results of these experiments, combined with those obtained by Nordmann and Dillschneider.

Methods

During each experiment we compared the two lenses of the same calf, maintained in a perfusion medium; the control lens was kept in a tyrode glucose medium (1 in 1,000), a solution comparable to the aqueous humour; the other lens was kept in the same solution with the addition of the inhibitor, without altering the osmotic pressure, the pH, or the rH of the solution (Figure). The medium, sterilized by tyndalization, was maintained at 37° C. throughout the experiments.

FIGURE.—*Cataract by arrest of the sugar metabolism of the lens.*—The photograph was taken after 24 hrs' perfusion; the lens on the left was in the control solution, that on the right in sodium fluoride (2/1,000). The difference in transparency between the two lenses is well shown. The experimental cataracts produced with the other inhibitors were strictly comparable.

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Chemical Methods.—The determination of lactic acid followed two methods:

(1) that of Lieb and Zacherl (1932), modified by Fromageot and Heltz, with the apparatus of Fuchs (1933);

(2) that of Barker and Summerson (1941).

ATP was estimated by the method of Lohmann (1928), modified by Cori. Micro-estimations of citric acid were prepared by the method of Weil-Malherbe and Bone (1949).

Results

(1) GLYCERALDEHYDE (0.4 to 1.5 g./l.)

(a) *Action on Transparency of Lens.*—With these quantities of glyceraldehyde, lenses maintained in the medium for more than a day clouded and turned a yellowish colour.

(b) *Action on Carbohydrate Metabolism of Lens.*—The results of each experiment are seen in Table I. In contrast with the control lens, a fall in the concentration of lactic acid and of ATP was observed in each lens in the medium containing glyceraldehyde. The quantity of lactic acid in the medium remaining after the perfusion of the cataractous lens was also less than that in the medium containing the control lens.

TABLE I
EFFECT OF GLYCERALDEHYDE ON CATABOLISM OF THE PERFUSED LENS

Experiment No.	Type of Lens	Duration of Perfusion (days)	Quantity of Glyceraldehyde (g./l.)	Quantity of Lactic Acid (mg./100 g. fresh lens)	Quantity of Lactic Acid (mg./100 ml.) in Liquid in which Lens soaked for 4 hrs	Phosphate readily Hydrolysable by ATP (mg./100 g. fresh lens)
1	Calf	3	0 1.5	139.5 100.0 = -29 per cent.	6.95 2.54 = -64 per cent.	9.00 0
2	Calf	3	0 0.4	92.0 76.6 = -17 per cent.	3.36 1.09 = -68 per cent.	8.42 0
3	Calf	2	0 0.4	103.5 91.2 = -12 per cent.	4.70 1.38 = -71 per cent.	11.20 3.00 = -74 per cent.
4	Calf	2	0 0.4	131.0 114.0 = -13 per cent.	1.29 1.11 = -14 per cent.	6.00 1.35 = -78 per cent.
5	Calf	1	0 0.6	116.0 113.0 = -3 per cent.	3.01 0.46 = -85 per cent.	11.30 1.10 = -90 per cent.
6	Young Ox	10	0 1.5	81.0 50.0 = -39 per cent.	5.38 2.06 = -62 per cent.	not measured
7	Young Ox	2	0 0.6	66.0 70.7 = + 7 per cent.	3.12 1.84 = -41 per cent.	8.00 0.90 = -89 per cent.

In some of the experiments the irrigation was stopped after 10 hrs. At this time the two lenses were equally transparent, but the results seen in Table II show that there was a considerable diminution of ATP in the lens in the medium containing glyceraldehyde, and of lactic acid in the lens and surrounding medium.

TABLE II

EARLY CHEMICAL MANIFESTATIONS OBSERVED IN LENSES EXPOSED TO THE ACTION OF GLYCERALDEHYDE

Experiment No.	Type of Lens	Length of Perfusion (hrs)	Quantity of Glyceraldehyde (g./l.)	Quantity of Lactic Acid (mg./100 g. fresh lens)	Quantity of Lactic Acid (mg./100 ml.) in Liquid in which Lens soaked for 4 hrs	Phosphate readily hydrolysable by ATP (mg./100 g. fresh lens)
1	Young Ox	10	0 0.6	78.8 47.6 = -40 per cent.	3.20 2.12 = -34 per cent.	9.57 7.32 = -24 per cent.
2	Young Ox	10	0 0.6	62.2 37.2 = -41 per cent.	3.02 2.04 = -33 per cent.	6.13
3	Young Ox	10	0 0.6	—	—	9.93 4.66 = -53 per cent.

(2) MONO-IODACETIC ACID (4 g./l.)

(a) *Transparency*.—Clouding of the lens first appeared soon after subcapsular opacities were observed with the slit-lamp, after 2 hrs' perfusion.

(b) *Chemistry*.—The lactic acid content in the lens and surrounding medium was considerably diminished in contrast with that in the control. The concentration of ATP in the cataractous lens was also lowered. The amount of phospho-creatine, which was measured only once, was 7.80 mg./100 g. fresh tissue in the control lens, and 2.79 mg. in the cataractous lens (Table III, overleaf).

(3) SODIUM FLUORIDE

(a) After only 4 hrs a slight subcapsular clouding was noted with the slit lamp.

(b) A considerable fall in the concentration of lactic acid in the lens and surrounding medium was noted, as well as a diminution of ATP in the lens (Table IV, overleaf).

The total potassium in the control lens (measured once) was 2.61 mg.; in the cataractous lens it was 1.86 mg.

(4) PHLORIDZINE (2 and 1 g./l.)

(a) The same phenomena were observed as in the preceding experiments, except that the opacities appeared a few hours later.

TABLE III
EFFECT OF MONO-IODACETIC ACID ON CATABOLISM OF THE PERFUSED LENS

Experiment No.	Type of Lens	Length of Perfusion (hrs)	Quantity of Mono-iodacetic Acid (g./l.)	Quantity of Lactic Acid (mg./100 g. fresh lens)	Quantity of Lactic Acid (mg./100 ml.) in Surrounding Medium	Phosphate readily Hydrolysable by ATP (mg./100 g. fresh lens)
1	Calf	18	0 4	not measured	1.530 0.319 = -80 per cent.	7.100 5.194 = -27 per cent.
2	Calf	18	0 4	91 82 = -10 per cent.	4.375 1.629 = -63 per cent.	9.336 2.066 = -78 per cent.
3	Calf	8	0 4	83 67 = -20 per cent.	2.681 1.754 = -35 per cent.	10.458 7.602 = -28 per cent.
4	Calf	6	0 4	78 56 = -28 per cent.	3.866 1.010 = -74 per cent.	8.020 4.035 = -50 per cent.

TABLE IV
EFFECT OF SODIUM FLUORIDE ON CATABOLISM OF THE PERFUSED LENS

Experiment No.	Type of Lens	Length of Perfusion (hrs)	Quantity of Sodium Fluoride (g./l.)	Quantity of Lactic Acid (mg./100 g. fresh lens)	Quantity of Lactic Acid (mg./100 ml.) in Surrounding Medium	Phosphate readily Hydrolysable by ATP (mg./100 g. fresh lens)
1	Calf	18	0 2	86 73.5 = -15 per cent.	5.32 1.47 = -73 per cent.	6.560 5.060 = -23 per cent.
2	Calf	18	0 2	104 89 = -15 per cent.	3.08 1.73 = -44 per cent.	6.260 2.353 = -63 per cent.
3	Calf	8	0 2	78 58 = -26 per cent.	1.250 0.670 = -47 per cent.	8.158 6.362 = -22 per cent.

(b) Only the concentrations of lactic acid and ATP in the lens were measured; the results, similar to those in the previous experiments, are seen in Table V (opposite).

(5) SODIUM MALONATE (2 g./l.)

(a) The lesions produced were similar to those found in the four previous experiments, but they appeared very much later—after 24 hours' perfusion.

(b) Only the ATP content in the lens was measured; the fall in the concentration in the lens was similar to that in earlier experiments (Table VI, opposite).

TABLE V
EFFECT OF PHLORIDZINE ON CATABOLISM OF THE PERFUSED LENS

Experiment No.	Type of Lens	Length of Perfusion (hrs)	Quantity of Phloridzine (g./l.)	Quantity of Lactic Acid (mg./100 g. fresh lens)	Phosphate readily Hydrolysable by ATP (mg./100 g. fresh lens)
1	Calf	24	$\left\{ \begin{array}{c} 0 \\ 2 \end{array} \right.$	$\begin{array}{c} 100 \\ 72 = -28 \\ \text{per cent.} \end{array}$	$\begin{array}{c} 7.500 \\ 5.760 = -24 \\ \text{per cent.} \end{array}$
2	Calf	36	$\left\{ \begin{array}{c} 0 \\ 1 \end{array} \right.$	$\begin{array}{c} 108 \\ 92 = -15 \\ \text{per cent.} \end{array}$	$\begin{array}{c} 9.840 \\ 3.290 = -67 \\ \text{per cent.} \end{array}$
3	Calf	48	$\left\{ \begin{array}{c} 0 \\ 1 \end{array} \right.$	$\begin{array}{c} 130 \\ 76 = -34 \\ \text{per cent.} \end{array}$	$\begin{array}{c} 6.382 \\ 4.386 = -32 \\ \text{per cent.} \end{array}$

TABLE VI
EFFECT OF SODIUM MALONATE ON CATABOLISM OF THE PERFUSED LENS

Experiment No.	Type of Lens	Length of Perfusion (hrs)	Quantity of Sodium Malonate (g./l.)	Phosphate readily Hydrolysable by ATP (mg./100 g. fresh lens)
1	Calf	36	$\left\{ \begin{array}{c} 0 \\ 2 \end{array} \right.$	$\begin{array}{c} 5.13 \\ 3.21 = -38 \\ \text{per cent.} \end{array}$
2	Calf	36	$\left\{ \begin{array}{c} 0 \\ 2 \end{array} \right.$	$\begin{array}{c} 9.03 \\ 6.27 = -31 \\ \text{per cent.} \end{array}$
3	Calf	36	$\left\{ \begin{array}{c} 0 \\ 2 \end{array} \right.$	$\begin{array}{c} 10.74 \\ 4.89 = -44 \\ \text{per cent.} \end{array}$
4	Calf	36	$\left\{ \begin{array}{c} 0 \\ 2 \end{array} \right.$	$\begin{array}{c} 7.71 \\ 5.96 = -23 \\ \text{per cent.} \end{array}$

(6) FLUORO-ACETIC ACID (1 and 2 g./l.)

(a) The opacities were first observed after about 24 hrs' perfusion with sodium malonate.

(b) The ATP content of the cataractous lens showed a definite diminution compared with the transparent control lens (Table VII, overleaf).

The concentration of *citric acid* was also measured during five of these experiments, both in the lens and in the surrounding medium. The quantities found in the control and cataractous lenses were equal; in the surrounding medium, on the other hand, the citric acid in the solution containing fluoro-acetic acid was found in a higher concentration than in the control solution.

TABLE VII
EFFECT OF FLUOROACETIC ACID ON THE CATABOLISM OF THE PERFUSED LENS

Experiment No.	Type of Lens	Length of Perfusion (hrs)	Quantity of Fluoro-acetic Acid (g./l.)	Phosphate readily Hydrolysable by ATP (mg./100 g. fresh lens)	Quantity of Citric Acid (mg./100 g. fresh lens)	Quantity of Total Citric Acid (mg.) in Surrounding Medium
1	Calf	36	$\left\{ \begin{array}{l} 0 \\ 2 \end{array} \right.$	$\begin{array}{l} 9.96 \\ 7.83 = -22 \\ \text{per cent.} \end{array}$	—	—
2	Calf	36	$\left\{ \begin{array}{l} 0 \\ 2 \end{array} \right.$	$\begin{array}{l} 10.27 \\ 6.27 = -39 \\ \text{per cent.} \end{array}$	—	—
3	Calf	36	$\left\{ \begin{array}{l} 0 \\ 1 \end{array} \right.$	$\begin{array}{l} 13.06 \\ 8.94 = -31 \\ \text{per cent.} \end{array}$	—	—
4	Calf	48	$\left\{ \begin{array}{l} 0 \\ 1 \end{array} \right.$	$\begin{array}{l} 10.66 \\ 6.69 = -38 \\ \text{per cent.} \end{array}$	—	—
5	Calf	48	$\left\{ \begin{array}{l} 0 \\ 1 \end{array} \right.$	—	$\begin{array}{l} 4.50 \\ - \end{array}$	$\begin{array}{l} 5.00 \\ 7.20 = +31 \\ \text{per cent.} \end{array}$
6	Calf	48	$\left\{ \begin{array}{l} 0 \\ 1 \end{array} \right.$	—	$\begin{array}{l} 5.40 \\ 6.20 = +13 \\ \text{per cent.} \end{array}$	$\begin{array}{l} 6.90 \\ 8.10 = +15 \\ \text{per cent.} \end{array}$
7	Calf	48	$\left\{ \begin{array}{l} 0 \\ 1 \end{array} \right.$	—	$\begin{array}{l} 6.00 \\ 5.10 = -15 \\ \text{per cent.} \end{array}$	$\begin{array}{l} 7.20 \\ 9.00 = +20 \\ \text{per cent.} \end{array}$
8	Calf	48	$\left\{ \begin{array}{l} 0 \\ 1 \end{array} \right.$	—	$\begin{array}{l} 5.00 \\ 5.30 = +6 \\ \text{per cent.} \end{array}$	$\begin{array}{l} 6.60 \\ 7.50 = +12 \\ \text{per cent.} \end{array}$
9	Calf	48	$\left\{ \begin{array}{l} 0 \\ 1 \end{array} \right.$	—	$\begin{array}{l} 5.65 \\ 5.20 = -8 \\ \text{per cent.} \end{array}$	$\begin{array}{l} 7.20 \\ 9.20 = +22 \\ \text{per cent.} \end{array}$

Discussion

At which point in the carbohydrate cycle do these substances act?

- (1) The action of glyceraldehyde is not completely clear. It is possible that it inhibits the activity of hexokinase, which might activate the combination of l-glyceraldehyde and triose-phosphate, to form l-sorbose-1-phosphate. This substance is very similar to glucose-6-phosphate, which is formed by the phosphorylation of glucose in the presence of hexokinase, and it is to be remembered that synthetic l-sorbose-1-phosphate strongly inhibits the action of hexokinase. If this is true, it would mean that the action of glyceraldehyde affects the first stage of carbohydrate glycolysis.
- (2) Mono-iodacetic acid inhibits the action of triose-phosphate dehydro-

genase on the formation of 1-3-diphosphoglyceric acid from 3-phosphoglyceraldehyde.

(3) Sodium fluoride inhibits several reactions in the cycle:

(a) phosphatases, which are necessary for the phosphorylation of glucose;

(b) adenosine triphosphatase;

(c) enolase, the enzyme responsible for the formation of phosphopyruvic acid from 2-phosphoglyceric acid.

(4) The action of phloridzine in inhibiting the phosphorylation of glucose, necessary to allow the penetration of hexose into the lens, suggests that the lens derives its nourishment from an external source. In these circumstances the lens could survive, but only at the expense of its own glucose and those intermediary products which it contains, and only until these reserves were exhausted. It follows that the carbohydrate metabolism is disturbed at the commencement of the cycle.

(5) Sodium malonate inhibits succinic dehydrogenase and prevents the formation of fumaric acid from succinic acid. The aerobic cycle is therefore undisturbed until the succinic acid stage; this substance will accumulate and the catabolic process will cease.

(6) Fluoro-acetic acid inhibits isocitric dehydrogenase, preventing the formation of oxalosuccinic acid from isocitric acid. Citric acid accumulates and the subsequent reactions do not take place.

In the breakdown of sugars, the four first inhibitors act on the enzymes of the anaerobic cycle; with sodium malonate and fluoro-acetic acid, the block is at the level of the citric cycle.

Conclusions

The experiments undertaken have confirmed that a blockage of the breakdown of sugars is always followed by cataract. The first four substances used acted at different phases of the anaerobic cycle; sodium malonate and fluoro-acetic acid on the aerobic phase. Although their site of action is different, all these substances produced marked opacification of the lens. The cataract appeared most quickly when the block affected the enzymes at the commencement of the cycle; less quickly with the agents which disturbed the citric cycle. The positive results of these experiments indicate that the breakdown of glucose in the lens is similar to the process in other organs.

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