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INOSITOL IN THE OCULAR TISSUES

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The knowledge of the chemical composition of most tissues is yet unsatisfactory. A few organs have been studied intensively, and their composition is relatively well known; on the contrary, for many other organs systematic data are still lacking. To undertake a study of physiologic chemistry before having a sufficient knowledge of "anatomic chemistry" results in incomplete or even false interpretations.

The analyses of the ocular tissues are fairly complete. This is particularly true for the lens. For 100 Gm. of lens, the composition of about 98.5 Gm. is known. The remaining 1.5 Gm. contains substances which, acting as catalysts, play an important role in spite of their low concentrations. Other substances enter the lens by diffusion; they do not act as metabolites, and their role is unimportant. But although the unknown fraction of the lens is a small part of the total weight, investigation of it may reveal the presence of substances reaching high concentrations, even higher than that of dextrose. Inositol is such a substance and is the subject of this article. This investigation, however, has not been limited to the lens but has been extended to all ocular tissues.

Inositol (cyclohexanhexol) was discovered in 1850 by Scherer. Because of its sweet taste, this substance was for a long time considered as a sugar and was called inosite or *Muskelzucker*. It is known to be widely distributed in both animal and plant tissues. A great interest arose in this substance when it was discovered that phytin was the phosphoric ester of inositol and that the enzyme, phytase, occurred in many animal tissues. Later, inositol was found in the urine of persons with diabetes insipidus and diabetes mellitus. The discovery of inosituria resulted in contradictory interpretations. It is actually well demonstrated that any polyuria, with or without glycosuria, is accompanied by inosituria. Inosituria is a consequence of the polyuria, not of the glycosuria.

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The role of inositol in the tissues, although still actually unknown, seems to be important in the biologic processes. Its presence and its distribution in the ocular tissues have not yet been the subject of experimental investigation. For this reason this work has been undertaken.

EXPERIMENTAL PROCEDURE

Normal eyes from 3 or 4 year old cattle were immediately removed after slaughter of the animal; dissection of the ocular tissues was started as soon as possible. An unavoidable delay of a few hours was, however, necessary before the extraction was begun. Since inositol is stable, its destruction was unlikely; on the contrary, a slight increase arising from postmortem changes is possible.[1]

The method of Young [2] for the determination of inositol was used. Samples of corneal epithelium and conjunctiva weighing from 20 to 30 Gm., of vitreous weighing from 500 to 1,000 Gm. and of other tissues weighing from 60 to 150 Gm. were used.

The samples were extracted during two periods of twenty-four hours with a 70 per cent aqueous solution of acetone. For the first extraction, sufficient acetone was added to make a 70 per cent aqueous solution, depending on the amount of water in the tissue.

The acetone of the extract was removed by evaporation on the water bath. The remaining aqueous solution was extracted twice with ether and then evaporated to 100 cc. on the water bath. Extraction with acetone and ether gives better results than hydrolysis with hot alkali because it is more specific. All the tissues were extracted in this manner with the exception of the vitreous humor, which was desiccated before extraction.

Many interfering substances were removed by precipitation with mercuric sulfate in acid solution. After neutralization, the solution was filtered. The mercury was removed by means of hydrogen sulfide and filtration.

Barium hydroxide and 95 per cent ethyl alcohol were used to precipitate inositol. The precipitate was separated by centrifugation and was hydrolyzed by heating after acidification. The barium was removed by filtration, and the clear filtrate was reduced to a small volume. The inositol was precipitated by means of acetone and ether.

The precipitate formed under these conditions was not pure inositol. A certain amount of salts passed during these different procedures, depending on the tissue. The percentage of inositol was determined by a method of titration which is based on a reduction of potassium iodomercurate in alkaline solution. For this titration, an aliquot of solution of the precipitate containing 2 to 5 mg. of inositol was used. After the addition of sulfuric acid, an excess of iodine was added and titrated with thiosulfate.

RESULTS

The accompanying table shows the distribution of inositol in ocular tissues.

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1. Winter, L. B.: Inositol Metabolism in the Mammalian Heart, Biochem. J. **28**:6, 1934.

2. Young, L.: Determination of Inositol in Animal Tissues, Biochem. J. **28**: 1435, 1934.

The high concentrations of inositol in the lens and in the optic nerve permitted the isolation of the substance in almost pure form. The inositol obtained from the lens at the second crystallization had a melting point of 220.5 C. (uncorrected); that of the optic nerve had a melting point of 222 C. (uncorrected). The melting point of a commercial inositol was 223 C. (uncorrected). The inositol isolated from the lens was optically inactive like that isolated from the brain.[3]

The amount of inositol in the vitreous humor was small. As a rule, the figures obtained by titration were lower than 2 mg. in 100 Gm. of vitreous humor. It was questionable whether this slight reduction was due to inositol or to compounds liberated during the procedure. An attempt to isolate it was unsuccessful. Inositol in the vitreous humor, if present, was probably a product of diffusion and not a metabolite.

Inositol in Ocular Tissues in Milligrams per Hundred Grams of Tissue

Whole Cornea		Corneal Epithelium		Corneal Stroma		Conjunctiva		Iris	
Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry
8.0	42.9	26.5	132.5	5.3	28.2	6.05	33.4	22.0	118.0
6.5	34.8	17.2	86.6	5.1	27.2			27.5	147.5
		15.9	76.6					35.0	187.5

Lens		Vitreous Humor		Retina		Choroid*		Sclera		Optic Nerve	
Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry
150	395	Traces	—	13.4	107.5	35.5	206.8	14.0	50.5	103	342
172	450	Traces	—	16.6	133.0	32.5	187.8	10.5	37.8	96	321
154	405	Traces	—	17.0	136.2	29.2	168.5	13.5	48.6	89	298
126	330	Traces	—	14.5	116.3					111	372
177	465	Traces	—							90.5	303
149	392										

* The retinal pigment epithelium was removed with the choroid.

COMMENT

An excellent survey of the literature up to 1926 has been made by Needham.[4] In spite of rather large amounts of information, knowledge concerning metabolism of inositol is still scanty.

Since inositol is widely distributed in animals and plant tissues, the amount daily ingested in the food is relatively high. However, inositol as such is destroyed by the intestinal bacteria, and only a small fraction is absorbed through the intestinal walls. The inositol is likely absorbed in the form of phytin.

3. Momose, G.: On the Inositol of Brain and Its Preparation, Biochem. J. **10**:120, 1916.

4. Needham, J.: Die physiologische Bedeutung der Cyclosen, Ergebn. d. Physiol. **25**:1, 1926.

Inositol in the blood reaches only low concentrations.[5] The concentrations in tissue are much higher and cannot be explained by simple diffusion. From beef brain, Thudichum [6] has isolated 10 Gm. of pure inositol from 50 pounds (22.679 Gm.) of tissue, or 44 mg. per hundred grams. For human brain, the figures given by the same author are higher: 193 mg. per hundred grams in the gray matter and 217.2 mg. per hundred grams in the white matter. Since this early publication, the data found in the literature show a wide range of variation depending on the method used and the experimental conditions. According to recent determinations, inositol can reach concentrations higher than 170 mg. per hundred grams in the brain. About 85 mg. per hundred grams is found in fresh heart muscle, and about 125 mg. per hundred grams is found in the same tissue some hours after death. The amount of inositol in skeletal muscle seems to be much lower.[7]

Furthermore, previous investigations show that the tissues are able to synthesize inositol, for neither suppression of inositol from the food for a long period nor polyuria with inosituria is able to decrease the amount of inositol in the animal tissues. Since the injection of dextrose increases the amount of inositol in the incubating egg and since the injection of insulin decreases the sugar in the blood and increases the inositol in the tissues, it may be supposed that dextrose plays a role in the synthesis of inositol.[8]

The question arises as to the nature of the inositol in the tissues. Since the concentration of inositol increases with the autolysis, the hypothesis of a precursor of inositol, inositogen, has been suggested.[9] It is possible that the inositol itself is partly free and partly bound with a molecule containing phosphorus. The present methods do not allow more than an estimation of these two fractions.[1]

The final fate of inositol is unknown. It has been suggested that inositol is changed into glycogen, dextrose or perhaps into lactic acid in the vertebrate cardiac muscle.[10] However, inositol in the food does

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5. Needham, J.: Studies on Inositol: II. The Synthesis of Inositol in the Animal Body, Biochem. J. **18**:891, 1924.

6. Thudichum, J. L. W.: Die chemische Konstitution des Gehirns des Menschen und der Tiere, Tübingen, Franz Pietzcker, 1901, pp. 40 and 276-278.

7. Gregory, R. A.: A Modification of Young's Method for Determination of Inositol in Animal Tissues, Biochem. J. **29**:2798, 1935. Winter.[1] Young.[2]

8 (*a*) Needham, J.: Studies on Inositol: III. The Metabolic Behaviour of I-Inositol in the Developing Avian Egg, Biochem. J. **18**:1371, 1924. (*b*) Needham, J.; Smith, W., and Winter, L. B.: Insulin and Inositol, J. Physiol. **57**:lxxxii, 1923.

9. (*a*) Rosenberger, F.: Weitere Untersuchungen über Inosit, Ztschr. f. physiol. Chem. **64**:341, 1910. (*b*) Needham, J.: Studies on Inositol: I. A Method of Quantitative Estimation, Biochem. J. **17**:422, 1923.

10. Boyland, E.: Chemical Changes in Muscle: II. Invertebrate Muscle; III. Vertebrate Cardiac Muscle, Biochem. J. **22**:362, 1928.

not increase the respiratory quotient,[11] and perfusion of tissue with inositol gives no formation of dextrose or lactic acid.[12] The experiments done with the Warburg apparatus are not consistent.[13]

It has been suggested that inositol has no other role than that of bringing phosphorus into the organism.[14] If this were true, why does the organism show an active synthesis of inositol and why is there a constant concentration of inositol in the tissue when the diet is free from inositol and or when there is prolonged polyuria with inosituria? Hypotheses on the subject will not add anything more, for experimental facts are missing.

The results of the investigations show that the concentration of inositol varies greatly with the type of ocular tissues. It is high in the lens and in the optic nerve. Further experimental work will perhaps give a clue to the relation of metabolism to these high concentrations.

CONCLUSIONS

Inositol was found in each of the bovine ocular tissues, namely: conjunctiva, corneal epithelium, corneal stroma, iris, lens, retina, choroid, sclera and optic nerve. The presence of inositol in the vitreous humor is questionable.

Its concentration is relatively constant in a definite type of tissue but varies broadly in different tissues. The concentration is remarkably high in the lens (150 mg. per hundred grams of wet tissue) and in the optic nerve (100 mg. per hundred grams of wet tissue).

The role played by inositol in these tissues is still unknown.

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11. Anderson, R. J.: The Utilization of Inosite in the Dog, J. Biol. Chem. **25**: 391, 1916.

12. (*a*) Oppenheimer, S.: Ueber die Milchsäurebildung in der künstlich durchströmten Leber, Biochem. Ztschr. **45**:30, 1912. (*b*) Griesbach, W., and Oppenheimer, S.: Ueber Milchsäurebildung im Blut, ibid. **55**:328, 1913. (*c*) Embden, G., and Griesbach, W.: Ueber Milchsäure und Zuckerbildung in der isolierten Leber: I. Ueber den Abbau der d-Sorbose; II. Ueber das Schicksal des d-Sorbits und einiger anderer Hexite, Ztschr. f. physiol. Chem. **91**:284, 1914.

13. (*a*) Das, N., and Guha, B. C.: The Biological Oxidation of Inositol, Current Sc. **3**:157, 1934; abstracted, Chem. Abstr. **29**:1110, 1935; (*b*) Die Unwandlung von Inosit durch Rattengewebe, Ztschr. f. physiol. Chem. **231**:157, 1935. (*c*) Young, L.: Inositol and the Respiration of the Brain, Proc. Soc. Exper. Biol. & Med. **35**:507, 1936.

14. Starkenstein, E.: (*a*) Die Beziehungen der Cyklosen zum tierischen Organismus, Ztschr. f. physiol. Chem. **58**:162, 1908; (*b*) Die biologische Bedeutung der Inosit Phosphorsäure, Biochem. Ztschr. **30**:56, 1911.

To consider inositol as a stable form of metabolite in the tissues which have no glycogen or a small amount of it is a good working hypothesis.

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