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THE BIOCHEMISTRY OF THE EYE

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THE BIOCHEMISTRY OF THE EYE

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TO

DR. SAMUEL CLARK HARVEY

AND

DR. WILLIAM HOLLAND WILMER

PREFACE

For over a hundred years, the chemistry of the ocular tissues has been studied sporadically, and until forty years ago, ocular chemistry was nothing more than a series of observations and experiments which frequently had no obvious connection with one another. Since Mörner, in his classical studies on the chemistry of the refractive media, began the new era of intensive investigation, the voluminous literature has accumulated until it is difficult to follow the trend of chemical thought in the welter of experimental data, theory, hypothesis, and speculation, and finally we recall,

> *Polonius.* What do you read, my lord?
> *Hamlet.* Words, words, words.

The purpose of this monograph is to attempt to review briefly the results of the past work on the chemistry of the eye. It is obvious that if each writer were considered in detail, the purpose of this monograph would be lost in a volume of discussion and mass of miscellaneous data. Numerous articles of trivial value have been dismissed; others of unequal value have been mentioned. In many cases, no comment has been made, either because many writers have given little or no descriptions of their experimental procedures or because only abstracts of the articles were available. Analytical values have been recorded as much as possible in tabular form as averages. Data taken from the results of the chemical investigation of over 86,000 bovine eyes in the Wilmer Institute are also included. Occasional reference is made to the relation of the chemistry to the anatomy, physiology and pathology of the eye. It is unfortunate that much of the research of the eye has centered on a few tissues, and as a result the knowledge of the others is scanty. It is hoped that fertile mating of the disconnected facts will yield a progeny of suggestive problems for research.

In the future, chemical ophthalmology should begin with the study of the chromosomes in the germ cell. Chromosomes are derived from chromatin which appears to be composed mostly of protamine nucleate, a protamine combined with nucleic acid. Since the chromatin of the chromosomes determines the form and function of the eye, and as Leathes says, "even the color of the eyelashes," the vital significance of its chemistry is easily understood in relation to the embryology and genetics of the eye.

With the advance in chemical ophthalmology, there will be a tendency to regard the living eye not as a static organ composed of different tissues having fixed chemical structure or composition, but mainly as a functional organ of multiple chemical processes, for the essential characteristic of life is incessant change. Mathematics will be used to simplify and clarify the explanation of these complex chemical reactions with their limiting conditions and their changes in energy. Moreover, statistical analyses will be employed to correlate normal chemical processes with the abnormal in human eyes, to which direct chemical procedures are inapplicable. Chemical ophthalmology will thus rapidly become more quantitative and less qualitative in nature, for as Plato tersely remarks: "If arithmetic, mensuration and weighing be taken away from any art, that which remains will not be much."

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INTRODUCTION

Biochemistry of the eye is a special subordinate branch of ophthalmology which deals with the chemical properties and reactions of the eye and its adnexa. As biochemical investigation progresses, the subject tends to reach out further into medicine, surgery, biology, physics, physiology and pathology.

To understand ocular biochemistry, a knowledge of general biochemistry and its related subjects is required. Such knowledge is presupposed in this monograph. It is unnecessary to refer to general treatises and monographs on these subjects for they are well known to biochemists and are easily obtained by ophthalmologists. A general conception, however, of the biochemistry of the eye may be given here.

In a general sense, the material basis of the eye is the same as it is for the other tissues of the body. It consists of proteins, lipids, carbohydrates, organic water soluble extractives, inorganic substances, and water.

The proteins of the ocular tissues are like those found in other parts of the body. The albuminoids or scleroproteins are diverse insoluble proteins which make up the supporting structure of the eye. Collagen, which forms the chief protein of the white fibrous connective tissue and the ground substance of cartilage and bone, is found mainly in the sclera and corneal struma and in small amounts in the choroid and iris. Along with collagen occurs elastin, the protein of the elastic fibers of connective tissues. Keratin, another albuminoid, which is commonly produced by modified epithelial cells has not been discovered as a constituent in the ocular tissues, but neurokeratin which forms the basis of the neuroglial framework of the central nervous system has been isolated from retinal tissue. The proteins of the lamina vitrea, Descemet's membrane, lens capsule, and the vitrein of the vitreous humor are similar to those found in other cuticular membranes of the body. These proteins may be modified mucoids. The albuminoid of the lens substance is apparently a protein peculiar to the lens.

Glycoproteins consist of mucins, which act as protective or lubricating agents in the form of secretions found on mucous membranes, and of mucoids which may be extracted from various tissues such as tendons, cartilage, and bone. In some tissues, mucoids act as a cementing substance which holds cells or fibers together. Mucin is found in the conjunctival secretion and mucoid is present in all of the ocular tissues including the vitreous humor.

Globulins, albumins, nucleoproteins and simple proteins, which are the chemical foundations of all cells, are obtained from ocular tissues. In blood-free tissues, the amounts of these proteins tend to vary with the number of cells. Nucleoproteins are found in the cellular layers of epithelia of the iris, cornea, lens, and retina. Protein derivatives, such as proteoses, peptones, peptides and amino acids, are detected in every tissue. The protamines and histones of the eye await discovery. Of the ocular proteins, mucoids of the cornea, sclera and vitreous humor, and proteins of the lens are most completely characterized.

Lipids act as stores of potential energy and as essential lipids which have some intimate correlation with cellular processes. They are distributed throughout the ocular tissues. In general, the amount of lipids varies with the number of cells except in adipose tissue. Extra-cellular lipids in structural tissues tend to increase with age. Lecithins, kephalins and cerebrosides are vital constituents of the cell and are particularly abundant in nervous tissues. The retinal neuroepithelium and the corneal epithelium possess the greatest amount of lipid. From different tissues of the eye the usual tissue lipids are obtained,—namely, stearic, palmitic, oleic, linolenic acids and their glycerides, arachidonic, lignoceric, butyric and acetic acids, lecithins, kephalins, sphingomyelins and cerebrosides, free and esterified cholesterol, cetyl alcohol and polyenes (carotenoids, vitamin A). The biological significance of many of these substances in the eye is yet to be discovered.

Carbohydrates occur in the tissue as free and compound substances. Glucose is distributed throughout the ocular tissues and the intraocular humors as a substance taking part in the active cellular metabolism. Glycogen is found in the photoreceptive cells of the retina of certain species of animals. Carbohydrates or their derivatives are also a part of the molecular structure of the cerebrosides, as galactose, and of the mucins and mucoids, as glycuronic acid, chitosamine, and chondrosamine.

In the eye, the usual tissue extractives are found, for example, urea, purines, creatine, creatinine, carnosine, anserine, carnitine, choline, acetylcholine, organic phosphates, glutathione, and ascorbic, citric, lactic and amino acids. More is known about the water soluble organic extractives of the intraocular humors than of the tissues. Lipases, esterases, dehydrogenases, oxidases and other enzymes are present in various tissues and in intraocular humors.

The quantitative analysis of inorganic substances, such as sodium, potassium, calcium, magnesium, chlorides, sulfates, and phosphates, indicates that these substances are interrelated, especially in the intraocular humors and the lens. These inorganic substances are in part combined with organic constituents and may or may not be ionized, depending upon the nature of the substance. Potassium and phosphates are relatively more abundant in the cells than in the intraocular fluids while the opposite is true of sodium and chloride. The ionic distribution of inorganic substances between the aqueous humor and the blood tends to satisfy the requirement of the equilibrium theory of Donnan.

Knowledge of the physical chemistry of the tissues is useful in the interpretation of physical changes. The study of ionic equilibria, isoelectric points of proteins, potential differences, osmotic pressures, hydrogen ion concentrations, and oxidation and reduction potentials gives an insight into the properties of the ocular colloidal systems. All of the ocular structures are colloidal systems. Colloidal phenomena deal with the state of matter and not with a kind of matter. Since the reactions and interactions which are called life take place in a colloidal system, much of the ultimate source of vital energy arises from the effect of interfaces and films. This phase of ocular physical chemistry needs investigation. The study of the mechanism of thermodynamic and electrical changes on a mathematical basis in the living eye will probably undergo great development in the near future, for then the function of the eye as a whole will be better correlated with the activity of its various parts.

Since the eye is a singular organ designed for a particular purpose, some of its tissues have distinct properties which are not held in common with other organs. The cornea, lens and vitreous humor are extraordinarily clear structures which retain their embryological transparency. The cornea is an avascular tissue which obtains its nourishment from the intraocular fluid and a part of its supply of oxygen from the atmosphere. The lens is an isolated transparent colony of cells without a nerve or blood supply. In contrast to most tissue, it grows peripherally. As a result, the difference in age between the central and peripheral part of the lens increases gradually. The choroid is a tissue which aids in the nourishment of the retina by means of its great vascularity. This tissue, the iris, and the retinal pigmented epithelium are noted for their melanotic pigmentation which darkens the optical chamber of the eye. Through the action of muscle fibers, the anterior surface of the iris acts as a variable filtering disc. Seemingly, the ciliary body affects the inflow and composition of the aqueous humor while the canal of Schlemm and iris take care of most of the outflow of aqueous humor. The vitreous humor is an optically clear acellular gel composed of a delicate fibrillar network of vitrein which holds a hydrophilic mucoid. The only other similar structure is the gel of the umbilical cord, although in the strict sense those two tissues are not strictly comparable. The retina is a photoreceptive organ whose reactions are obscure. Its rod-shaped cells contain a photosensitive substance, visual purple, which evidently is a carotenoid-protein compound and which plays a part in the photopic visual process. The retinal cone-shaped photoreceptive cells possess indistinct chemical characteristics. In the retinal cells of some animals globules of carotenoids have an unknown physiological action. It is easily seen that the eye has many unique chemical and physiological qualities which no other organ possesses.

Ocular pathology may be greatly advanced by the knowledge of the chemistry of the eye. Pathological ocular chemistry is nothing more than the variation from normal ocular chemistry. It is based primarily on the chemistry of the normal eye and secondarily, on that of the pathological eye and its relation to reactions of the diseased body. Much of the progress made in the pathological chemistry of the eye has been incidental or secondary to other investigations. For instance, xanthomatosis of ocular structures has been a part of the study of generalized xanthomatosis of diabetes; xerophthalmia and night blindness from avitaminosis A, and dermatitis with corneal ulcers from human avitaminosis B, were observed in the study of the effects of vitamin deficiencies. The subjects which have been investigated intensively by the ophthalmologists are the metabolism of the cornea, the formation of cataract and the ionic equilibrium in glaucoma.

Some empiricists have frequently voiced the opinion that biochemistry of the eye is a highly academic subject without practicality. The basis of normal and abnormal physiology of the eye is chemistry and physics and furthermore, the physiology of the eye is the foundation for the theoretical conceptions of medical and surgical ophthalmology. The biochemist develops his subject while the ophthalmologist applies the resulting biochemical theories to conquer diseases of the eye.

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CHAPTER I

THE CHEMISTRY OF THE EXTERNAL SECRETIONS AND CONJUNCTIVA

I. The External Secretions

A. *The Secretion of Lacrimal Glands: Tears*

Formation: The lacrimal secretion is formed by the superior, inferior and accessory lacrimal glands. The usual amount is sufficient to keep the eyeball moist. The water of the secretion is lost by evaporation. It is obvious that the secretion is increased by stimuli. Certain drugs, such as pilocarpine also increase it, while atropine diminishes it. Merz [39] found that after section of the cervical sympathetic nerves and extirpation of the superior cervical ganglion, the permeability of the glandular cells to chlorides is decreased. After extirpation of the superior and inferior glands, the conjunctiva and cornea are kept moist by the conjunctival mucous cells and by the secretion of accessory lacrimal glands. Extirpation of the lacrimal sac does not cause epiphora, unless the stimulation itself produces increased secretion, but according to Avižonis,[7] it frequently results in xerosis in the healthy middle aged patient. Allessandro [3] claims that in starved dogs the secretion is decreased or absent.

Secretion: Schirmer [50] believes that the total secretion of 3 to 4 c.c. per 24 hours reported by Magaard [35] and Ahlström,[1] is excessive and is the result of irritation, but that 0.5 to 0.76 c.c. is probably the normal amount of tears secreted during 16 hours while one is awake. Cerrano's [10] report of 12 c.c. in 24 hours is also abnormal. Kurose [29] obtained, per day, from healthy Japanese 6.6 c.c. of tears without irritation and 32.9 c.c. with conjunctival irritation.

Physical Properties: In tears produced by irritation, Cerrano [10] found a freezing point depression of 0.6000 to 0.9566° C.; specific gravity, 1.001 to 1.005; electrical conductivity, $K = (1950 \text{ to } 2272) 10^{-5}$; viscosity, 1.053 to 1.405; surface tension, 0.694 to 0.746. The last three properties were relative to water at 38° C. Rötth [48, 49] reported the refractive index of normal tears to be 1.3362 to 1.3374, and of tears caused by irritation to be 1.3361 to 1.3379. Massart[37] calculated the osmotic pressure of tears to be equivalent to a solution of 1.39 per cent sodium chloride.

Chemical Composition: Luzsa,[34] Charlton,[11, 12] Kusunoki,[30] Lipschütz,[33] Oguchi and Nakashima[43] observed that the pH of the normal tears was generally 7.0 to 7.4, but occasionally it was as high as 8.4. The pH of conjunctival fluid in a large number of diseased eyes varied from 6.4 to 8.4, and the purulent conjunctival exudates were usually below 7.0.[43] von Pelláthy and Schneider[44] noticed that tears from inflamed trachomatous eyes were acid. Being weakly acid, the conjunctival secretion, if it is increased, may reduce the alkalinity of the lacrimal secretion.

The first chemical analysis of tears was recorded in 1791 by Fourcroy and Vauquelin.[23] Table 1 gives the analysis of tears by Frerichs,[24] Arlt (Lerch),[6] Magaard[35] and Ridley.[47]

TABLE 1

THE CHEMICAL COMPOSITION OF THE TEARS

	Magaard[35]	Arlt[6]	Frerichs[24]	Ridley[47]
	grams per cent			
Water	98.1200	98.223	99.06–98.70	
Total solids			0.94– 1.30	1.8
Ash			0.42– 0.54	1.05
Organic substances	1.464			
Total N				0.158
Non-protein N				0.051
Urea				0.03
Protein		0.504	0.08– 0.10	0.669
Albumin				0.394
Globulin				0.275
Sugar				0.65
Mucus and fat		traces		
NaCl as chlorides		1.257		0.658
Na as Na_2O	0.4160	0.016	0.72– 0.88	0.60
K as K_2O				0.14
NH_3				0.005
Phosphates, etc.				
Epithelium			0.32– 0.14	
Freezing point depress.				—0.551° C.
Specific gravity		1.0086 (20° C.)		