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METABOLISM OF THE RETINA

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DURING the past few years the interest in retinal metabolism has been greatly intensified. Many investigations were made on the retina as part of a war emergency program. Because of this practical interest in a narrow but important subdivision of ophthalmology, this survey of the literature was made.

A study of the metabolic processes in the retina has been the basis of much research, particularly within the last ten years. This is not surprising, as such work may have a double goal—first, to provide additional information on the problem of visual mechanisms and, second, inasmuch as the retina shows metabolic activity greater than that of almost any other tissue, to study the chain reactions of intermediate metabolism. The extensive literature, dealing with many phases of the problem, is difficult to put together into a single picture, and therefore one must present many observations alone and await further investigation to understand fully their significance. In this paper no attempt is made to discuss the mechanism of the visual pigments, although their reactions are certainly a part of retinal metabolism. The discussion is limited chiefly to carbohydrate metabolism, with reference to a possible fat and protein metabolism. It is to be remembered that the process observed in a complex organ such as the retina may represent the summation of metabolic processes connected with the visual mechanism, the transmission of impulses along nerve fibers and across the synapses and the basal metabolism of the cells involved.

METHODS AND MATERIAL

These studies were made on retinas obtained from the eyes of various animals, principally the frog, rat, rabbit and ox. In many cases unexplained species variation may add to the difficulty in comparing results. Studies of oxygen utilization, carbon dioxide production, aerobic and anaerobic glycolysis and ammonia produc-

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tion were made with the Warburg manometer, employing the entire retina, sliced or minced retinas, retinal extracts or, occasionally, special slices including only certain regions or layers of the retina. The effect of various added substrates or poisons, as well as the production of final or intermediate products, was determined with this method. The Thunberg method was employed for determination of dehydrogenases. Measurements of the p_H were usually made with the hydrogen electrode. Acetylcholine was determined by bioassay, using the magnitude of muscular contraction as compared with a standard. The effects of light and dark adaptation were found by illuminating one eye of an animal, the lids being held open and the eye irrigated, while the other eye was blindfolded to serve as the control, and then quickly placing the retinas in the manometer vessels. The effect of illuminating and darkening the vessels was also investigated.

Determinations on the human retina were limited to a few cases in which the eye was enucleated, usually for glaucoma or tumor, and to the indirect observations in which the effect of anoxia and carbon dioxide excess on the dark adaptation and the visual acuity of volunteer subjects was noted.

RESULTS

Hydrogen Ion Concentration.—The early work of Chodin [1] in 1878, and of Kühne [2] and Cahn,[3] shortly thereafter, showed little change in the slightly alkaline retina on exposure to light; yet Angelucci,[4] at about the same time, first demonstrated the shift toward the acid side on illumination, which fact has subsequently been often confirmed. Lodato,[5] in 1900, stated that the greatest acidification was given by light of short wavelength and also demonstrated how thermal, electrical or chemical irritation could produce acidity. Majima,[6] however, showed that yellow and green light were more effective. Re [7] produced acidity by stimulation of the optic tract. Quantitative studies by Nakashima [8]

1. Chodin, A.: Ueber die chemische Reaktion der Netzhaut und des Sehnerven, Sitzungsb. d. k. Akad. d. Wissensch. Math.-naturw. Cl. **76**:121, 1878.

2. Kühne, W.: Chemische Vorgänge in der Netzhaut, in Hermann, L.: Handbuch der Physiologie, Leipzig, F. C. W. Vogel, 1879, vol. 3, p. 234.

3. Cahn, A.: Zur physiologischen und pathologischen Chemie des Auges, Ztschr. f. physiol. Chem. **5**:213, 1881.

4. Angelucci, A.: Histologische Untersuchungen über das retinale Pigmentepithel der Wirbelthiere, Arch. f. Physiol., 1878, p. 353.

5. Lodato, G.: Imutamenti della retina sotto l'influenza della luce, dei colori e di altri agenti fisici e chimichi, con speciale riguardo alla reazione chimica: contributo alla fisiologia della retina, Arch. di ottal. **7**:335, 1900; abstracted, Klin. Monatsbl. f. Augenh. **38**:365, 1900.

6. Majima, K.: Studien über die Struktur der Sehzellen und der Pigmentepithelzellen der Froschnetzhaut, Arch. f. Ophth. **115**:286, 1925.

7. Re, F.: Sulle modificazioni fisiche e chimiche della retina per l'eccitazione elettrica dell'encefalo, mesencefalo e chiasma, Arch. di ottal. **12**:147, 1904; abstracted, Arch. d'opht. **26**:123, 1906.

8. Nakashima, M.: Beiträge zur Kenntnis des Sehpurpurs, XIII. Internationaler Ophthalmologenkongress, Amsterdam, 1929; abstracted, Zentralbl. f. d. ges. Ophth. **22**:772, 1930.

showed the p_H of the dark-adapted retina to be about 7.3 and that of the light-adapted retina to be about 7.0.

That this change may be related to the activity of the visual cells rather than of the nerve elements and may vary with the type of cell was shown in 1937 by von Studnitz,[9] who found no change in the p_H of the guinea pig retina, which contains only rods, while the cat and fish retinas, which contain also cones, showed a shift to the acid side. The acidity increased with time, corresponding to the regeneration of visual substances. The production of acid increased with elevation in temperature, as well as with greater intensity of light. After fifteen minutes of illumination acidification decreased (probably through the exhaustion of visual substance reserves) but rose again after twenty to twenty-five minutes (probably because of supplementary visual substances). The curve of acid production at different wavelengths of light for the reptile corresponds to the absorption curve of the cone substance, being greatest with yellow light. Von Studnitz' further work[10] showed little change in p_H on illuminating the retinas of selachians and cephalopods, which contain only rods. He expressed the belief that acidification of the retina leads to contraction of the cones and expansion of pigment. Dittler,[11] in 1907, showed that an aqueous extract of a light-adapted retina caused the contraction of cones in a dark-adapted retina, similar to the stimulation of light.

Organic Phosphate.—A study of the phosphoric acid and phosphoric esters in the retina, particularly because of their importance in intermediate carbohydrate metabolism, may provide important information concerning the sources of energy for visual processes. Lange and Simon,[12] in 1922, showed that frog retina contains 0.08 per cent phosphoric acid. A phosphate ester apparently present in the outer segments of the rods of the frog and carp was hydrolyzed in alkaline solution into phosphoric acid and an organic substance. An increase of phosphoric acid in the vitreous of a removed eye was noted on high illumination; a partial reformation of the phosphate ester took place only if the retina was removed with the pigment epithelium; yet no phosphate was obtained from the pigment epithelium alone on illumination. The organic phosphate compound was not hexose phosphate, but probably

9. von Studnitz, G.: Die retinale Säurebildung, Arch. f. d. ges. Physiol. **238**:802, 1937.

10. von Studnitz, G.: Ueber die chemische Reaktion der Selachier- und Cephalopodenretina, Ztschr. f. vergl. Physiol. **19**:615, 1933.

11. Dittler, R.: Ueber die chemische Reaktion der isolierten Froschnetzhaut, Arch. f. d. ges. Physiol. **120**:44, 1907.

12. Lange, H., and Simon, M.: Ueber Phosphorsäureausscheidung der Netzhaut bei Belichtung, Ztschr. f. physiol. Chem. **120**:1, 1922.

phosphocreatine. Tawara [13] found two types of organic phosphate in the retina which liberated inorganic phosphate on illumination, one of which appeared to be phosphocreatine, the other a soluble phosphate ester. Kuzuya,[14] also working with the rabbit retina, demonstrated an acid-soluble and an acid-insoluble phosphate compound. He also investigated the changes in the amount of these different phosphate fractions with the growth of the rabbit.[15]

Nakashima and Arata [16] considered the possibility that phosphoric acid arose from the splitting of phosphocreatine in a manner analogous to that occurring in muscle but presented evidence against the photolysis of phosphocreatine. They showed that it is the Kjeldahl nitrogen (choline, carnosine) which increases on illumination and proposed the theory that phosphoric acid arises through the photolysis of retinal lipids. They stated the belief that phosphocreatine nitrogen, which increased with damage to the tissue and was inhibited by light, occurred through the autolysis of the retina.

Takamatsu [17] demonstrated that the isolated retinas of frogs and white rats produced phosphoric acid in Ringer's solution (isotonic solution of three chlorides U.S.P.) even in the dark, probably as the result of autolysis. Within limits, the increased production of phosphoric acid on illumination varied with length of exposure. That this process was reversible was shown by the fact that on darkening the once lighted retina production of phosphoric acid could again be demonstrated on illumination. Illumination of the retina of the white rat produced a reduction of phosphatide content. He further showed [18] that in frogs and rabbits poisons which produced acute amaurosis or amblyopia, such as methyl alcohol and quinine, decreased the phosphate ester content.

13. Tawara, M.: The Effect of Light upon the Phosphoric Acid Fraction of the Retina, Acta Soc. ophth. jap. **42**:1503, 1938; abstracted, p. 106.

14. Kuzuya, N.: Veränderung des Gehalts von verschiedenen Phosphorsäurefraktionen in Hell- und Dunkelnetzhaut, Acta Soc. ophth. jap. **41**:637, 1937; abstracted, p. 51.

15. Kuzuya, N.: Veränderung des Gehaltes der Netzhaut an verschiedenen Phosphorsäurefraktionen mit dem Wachstum, Acta Soc. ophth. jap. **41**:335, 1937; abstracted, p. 28.

16. Nakashima, M., and Arata, Y.: Azotometria der Netzhaut, Acta Soc. ophth. jap. **40**: 1586, 1936; abstracted, p. 97.

17. Takamatsu, T.: Photochemische Studien der Netzhaut: II. Ueber die Phosphorsäurebildung und die Phosphatidveränderung der Netzhaut bei Lichtung, Acta Soc. ophth. jap. **38**:1035, 1934; abstracted, p. 71.

18. Takamatsu, T.: Photochemische Studien der Netzhaut: III. Ueber den Einfluss der Gifte (Methylalkohol, Chinin) auf die Phosphorsäurebildung der Netzhaut bei Belichtung, Acta Soc. ophth. jap. **39**:598, 1935; abstracted, p. 54.

Von Studnitz [19] assumed that light decomposed the cone substance, with the production of phosphoric acid, expansion of pigment and contraction of cones, and as proof he showed that the injection of this acid into the dark-adapted retina produced a configuration of the cones and pigment for light, while injection of alkali into light-adapted retinas resulted in a configuration for the dark. There was a parallel change in the light absorption curves with injection of acid and of alkali. He expressed the belief that the cones alone show motility.

The increase in electrical conductivity of a solution of visual purple in the light and the decrease in the dark, as measured by Lasareff,[20] was probably due to the reversible hydrolysis of organic phosphate.

Evidence would indicate, however, that the acid reaction on illumination previously mentioned is probably due to the production of lactic acid in glycolysis rather than to the aforementioned liberation of phosphoric acid.

Oxidation-Reduction Potential.—Nakashima [21] found that the oxidation-reduction potential of suspensions of frog retinas increased on illumination and decreased in the dark, while he and Hayashi [22] showed that at the same time the consumption of oxygen was unchanged. Iron, enzymes and lipid apparently took part in the transfer of electrons, as the potential was decreased by potassium cyanide and the prolonged action of toluene and was increased by iron salts. The change did not depend on the presence of visual purple.

Respiration and Glycolysis.—The unique respiratory activity of the retina has led to its extensive study. Warburg,[23] in 1927, showed that the retina had the highest rate of respiration and of anaerobic glycolysis of any tissue studied and an extremely high anaerobic glycolysis, which even surpassed that of most tumors. Kubowitz,[24] Negelein [25] and

19. von Studnitz, G.: Vom Energieumsatz in der Netzhaut, Naturwissenschaften **22**:193, 1934.

20. Lasareff, P.: Sur le changement de la conductibilité électrique du pourpre visuel au cours de l'éclairge, Compt. rend. Acad. d. sc. **181**:476, 1925.

21. Nakashima, M.: Ueber das Oxydations-Reduktions-Potential der Netzhaut, Ber. ü. d. Versamml. d. deutsch. ophth. Gesellsch. **47**:369, 1929.

22. Nakashima, M., and Hayashi, K.: Sur le potentiel d'oxydo-réduction de la rétine, J. Biochem. **17**:315, 1933.

23. Warburg, O.: Ueber die Klassifizierung tierischer Gewebe nach ihrem Stoffwechsel, Biochem. Ztschr. **184**:484, 1927.

24. Kubowitz, F.: Stoffwechsel der Froschnetzhaut bei verschiedenen Temperaturen und Bemerkung über den Meyerhofquotienten bei verschiedenen Temperaturen, Biochem. Ztschr. **204**:475, 1929.

25. Negelein, E.: Ueber die glykolytische Wirkung embryonalen Gewebes, Biochem. Ztschr. **165**:122, 1925.

Oguchi,[26] working with frogs, and Nakashima,[27] using fish, demonstrated that in these cold-blooded animals the anaerobic glycolysis increased with the temperature and was much greater than the aerobic glycolysis up to 37 C., after which the aerobic glycolysis predominated. This effect of temperature was confirmed for mammalian and avian retinas by Warburg and associates,[28] Krebs [29] and Schmitz-Moormann.[30] Oguchi [31] compared the rates of respiration and glycolysis in birds, fish and frogs and found values for both to be highest in birds and least in frogs. Warburg and associates [28] suggested that the lactic acid produced aerobically from glucose was the result of trauma to the retina. In studying the lactic acid production of the retina of growing chickens, Tamiya [32] obtained the highest values during the period of growth and a decrease on maturity.

There appears to be a distinct difference between warm-blooded and cold-blooded animals in the effect of light on respiration and glycolysis. Kiyohara [33] reported that the dark-adapted retina of the toad took up 3.9 cu. mm. of oxygen per milligram hour, while the light-adapted retina took up 2.8 cu. mm. Jongbloed and Noyons [34] showed the oxygen consumption to be 26.5 per cent higher and the carbon dioxide production 25 per cent higher in dark-adapted than in light-adapted retinas of the frog. Similar results were obtained in the frog by Noyons and Wier-

26. Oguchi, T.: Ueber den Stoffwechsel der Netzhaut: II. Der Stoffwechsel der Netzhaut der Reptilien; der Stoffwechsel der Netzhaut nach dem Tode des Froschen, Acta Soc. ophth. jap. **36**:1702, 1932.

27. Nakashima, M.: Stoffwechsel der Fischnetzhaut bei verschiedenen Temperaturen, Biochem. Ztschr. **204**:479, 1929.

28. Warburg, O.; Posener, K., and Negelein, E.: Ueber den Stoffwechsel der Carcinomzelle, Biochem. Ztschr. **152**:308, 1924.

29. Krebs, H. A.: Ueber den Stoffwechsel der Netzhaut, Biochem. Ztschr. **189**:57, 1927.

30. Schmitz-Moormann, P.: Ueber den Glykogengehalt der Retina und seine Beziehungen zur Zapfenkontraktion, Klin. Monatsbl. f. Augenh. **78**:69, 1927.

31. Oguchi, T.: Ueber den Stoffwechsel der Netzhaut: I. Der physiologische Stoffwechsel der Netzhaut der verschiedenen Tierarten; der Stoffwechsel der Netzhaut nach dem Tode der Tiere, Acta Soc. ophth. jap. **36**:1203, 1932.

32. Tamiya, C.: Ueber den Stoffwechsel der Netzhaut in verschiedenen Stadien ihrer Entwickelung, Biochem. Ztschr. **189**: 114, 1927.

33. Kiyohara, K.: Ueber die Wirkung der Strahlen verschiedener Wellenlängen auf die Säuerstoffatmung der Netzhaut, Nagasaki Igakkai Zassi **9**:730, 1931; abstracted, p. 735.

34. Jongbloed, J., and Noyons, A. K. M.: Säuerstoffverbrauch und Kohlendioxydeproduktion der Freschretina bei Dunkelheit und bei Licht, Ztschr. f. Biol. **97**:399, 1936.

sma [35] and Lindeman.[36] Ikemune [37] found a 10 per cent greater carbon dioxide production from the frog retina in the dark than on illumination and noted that the production was greater for longer periods of dark adaptation. He [38] reported that the glucose concentration of light-adapted retinas averaged 91 mg. per hundred cubic centimeters, while for dark-adapted retinas it averaged 20 to 79 mg. per hundred cubic centimeters. He interpreted these results as showing that the resynthesis of visual purple in the dark occurred with the energy of glucose metabolism and that the increase in carbon dioxide production was related to the increase in glycolysis.

On the other hand, Galante [39] found that light increased the oxygen consumption of the dove and sparrow retina, and Takano [40] demonstrated that in the rat the rate of respiration, as well as the aerobic glycolysis, increased on illumination of the dark-adapted retina. He [41] found that small differences in the duration, intensity or color of the light had no effect on the change. Yet, also working with the rat, Campos [42] obtained higher oxygen consumption in the dark-adapted than in the light-adapted retina. Kodama [43] compared the respiratory rate in vessels in the light and in the dark, perhaps a different matter than comparing the respiratory activity of light-adapted and of dark-adapted eyes, and found that the oxygen consumption and aerobic glycolysis were much greater in the dark than in the light for the first thirty minutes but then fell, approaching that of the retina in the light.

35. Noyons, A. K. M., and Wiersma, C. A. G.: L'influence de la lumière sur la consommation d'oxygène de la rétine de l'œil de grenouille, Acta brev. Neerland **3**:156, 1933.

36. Lindeman, V. F.: The Respiratory Metabolism of the Frog Retina, Physiol. Zoöl. **13**:411, 1940.

37. Ikemune, I.: Einfluss des Lichtes auf die CO_2-Produktion der Netzhaut, Okayama-Igakkai-Zasshi **51**:529, 1939; abstracted, p. 538.

38. Ikemune, I.: Der Einfluss des Lichtes auf die Glykolyse in der Froschnetzhaut, Okayama-Igakkai-Zasshi **51**:539, 1939; abstracted, p. 543; Der Einfluss des Lichtes auf die Glykolyse in der Froschnetzhaut, Jap. J. M. Sc. III, Biophysics **5**:90, 1938.

39. Galante, E.: L'azione dell'eccitamento retinico sul ricambio gassoso, Ann. di clin. med. **20**:79, 1930.

40. Takano, M.: Ueber die Gewebsatmung der Netzhaut der Ratte, Acta Soc. ophth. jap. **38**:1307, 1934; abstracted, p. 85.

41. Takano, M.: Ueber die Gewebsatmung der Netzhaut der Ratte: II. Ueber die Einflüsse der kurzen Beleuchtung des farblosen Lichtes und der dauernden Beleuchtung des farbigen Lichtes auf die Gewebsatmung und aerobe Glykolyse der Netzhaut, Acta Soc. ophth. jap. **39**:162, 1935; abstracted, p. 17.

42. Campos, R.: Ricerche sul ricambo della retina, Ann. di ottal. e clin. ocul. **64**:456, 538 and 577, 1936.

43. Kodama, S.: Studien über den Gaswechsel der Netzhaut der Ratte, Tohoku J. Exper. Med. **28**:423, 1936.

The oxygen consumption was not proportional to the intensity of the light. In colored light the oxygen consumption and aerobic glycolysis were greatest in red, less in green and least in blue light. In the dark, in dextrose-free Ringer's solution the oxygen consumption was high but fell rapidly to zero. In 0.1 per cent dextrose the oxygen consumption was high at first but fell rapidly to a fifth of its original value, and the aerobic glycolysis was high and decreased but little. In 0.4 per cent dextrose the oxygen consumption and aerobic glycolysis were comparatively low at the start and decreased only slightly. The anaerobic glycolysis was equally high in Ringer's solution containing 0.1, 0.2 and 0.4 per cent dextrose.

Working with the peripheral and central parts of monkey eyes, Campos [44] showed that both parts of the lighted retina showed less oxygen consumption than the dark-adapted retina, the values rising during the experiment and approaching the value for the dark-adapted retina. Glycolysis of the macula of the illuminated eye was less than that of the dark-adapted eye, while the peripheral parts showed no difference on illumination. Campos [42] measured the exchange of gases in human eyes removed six to ten days after injury and development of glaucoma. There was a high rate of respiration and of aerobic and anaerobic glycolysis, particularly the former. There was no excess of glycolysis, and the Pasteur effect was low or absent. The peripheral retina showed more active respiration than the macula except in cases of absolute glaucoma. He also examined the retinas of albino rats and found slightly more active respiration but definitely lower aerobic glycolysis than in pigmented retinas.

Anoxia.—Santoni [45] noted the effect of interrupting retinal circulation by ligating the entire orbital contents of rabbits and rats for fifteen to ninety minutes. He found that the oxidizing power increased after interruption for fifteen to thirty minutes but decreased after sixty to ninety minutes. De Crecchio [46] produced anoxia by compressing the globe of rabbits for thirty to one hundred and eighty minutes; anaerobic glycolysis was decreased after sixty minutes but increased after ninety or one hundred and eighty minutes. They expressed the belief that the decrease in glycolysis and the increased respiratory rate indicated degenerative changes as a result of ischemia, with the freeing of substances such as fats.

44. Campos, R.: L'azione della luce sulla respirazione e sulla glicolise della retina, Boll. Soc. ital. di biol. sper. **11**:320, 1936.

45. Santoni, A.: Il ricambio della retina dopo interruzione della circolazione retinica, Ann. di ottal. e clin. ocul. **67**:299, 1939.

46. de Crecchio, A.: Il recambio della retina dopo compressione del bulbo, Ann. di ottal. e clin. ocul. **67**:739, 1939; abstracted, Arch. Ophth. **24**:1016 (Nov.) 1940.

The effect of anoxia in the human retina has been studied by many investigators. McFarland and Evans,[47] Gellhorn,[48] McDonald and Adler[49] and McFarland and Halperin[50] proved that lack of oxygen, excess of carbon dioxide or hyperpnea decreased visual acuity and dark adaptation. McFarland and Forbes[51] showed a similar effect of hypoglycemia. Vitamin A deficiency did not alter the effect of lack of oxygen. It undoubtedly acts by a different mechanism. It is probable that these changes were not concerned with the photochemical substances but involved the neural elements of the retina. In relation to avitaminosis A, however, de Leonibus[52] found that in albino rats placed on a deficiency diet until keratomalacia developed there was no change in oxygen consumption but that glycolysis was 8.6 per cent higher in the avitaminotic animals.

With atrophy of the retina produced experimentally by cutting the optic nerve in cats, Adler[53] showed there was an increased concentration of sugar in the vitreous. The glycolytic activity of the atrophied retinas, however, was decreased. In this manner the hypothesis that the low concentration of sugar in the vitreous is due to the high rate of glycolysis in the normal retina was confirmed. Craig and Beecher[54] observed that twice as much lactic acid was produced by the rat retina in phosphate as in bicarbonate buffer. In phosphate buffer there was a 38 per cent lower rate of respiration in 10 per cent oxygen and a 51 per cent lower respiratory rate in 5 per cent oxygen than in 100 per cent oxygen. The rates of respiration and glycolysis showed a reciprocal relationship to each other as the oxygen tension varied. In bicar-

47. McFarland, R. A., and Evans, J. N.: Alterations in Dark Adaptation Under Reduced Oxygen Tensions, Am. J. Physiol. **127**:37, 1939.

48. Gellhorn, E.: The Effect of O_2 Lack, Variations in the CO_2-Content of the Inspired Air, and Hyperpnea on Visual Intensity Discrimination, Am. J. Physiol. **115**:679, 1936.

49. McDonald, R., and Adler, F. H.: Effect of Anoxemia on the Dark Adaptation of the Normal and of the Vitamin A-Deficient Subject, Arch. Ophth. **22**: 980 (Dec.) 1939.

50. McFarland, R. A., and Halperin, M. H.: The Relation Between Foveal Visual Acuity and Illumination Under Reduced Oxygen Tension, J. Gen. Physiol. **23**:613, 1940.

51. McFarland, R. A., and Forbes, W. H.: Effects of Variation in the Concentration of Oxygen and of Glucose on Dark Adaptation, J. Gen. Physiol. **24**: 69, 1940.

52. de Leonibus, F.: Il ricambio della retina in avitaminosi A, Ann. di ottal. e clin. ocul. **67**:512, 1939.

53. Adler, F. H.: The Metabolism of the Retina: Further Notes, Arch. Ophth. **6**:901 (Dec.) 1931.

54. Craig, F. N., and Beecher, H. K.: The Effect of Low Oxygen Tension on Tissue Metabolism (Retina), J. Gen. Physiol. **26**:467, 1943.

bonate buffer, on the other hand, there was no sign of change in respiration with the oxygen tension lowered from 95 to 5 per cent, but glycolysis was increased nearly to anaerobic levels. Dickens and Greville [55] found that the rat retina, as well as the brain, oxidizes fructose strongly, although their earlier work [56] would indicate that in the case of the retina there is not an intermediate conversion into lactic acid, as they noticed negligible production of lactic acid from fructose. Chase and Hagan [57] proved that lack of oxygen does not alter the changes in the absorption spectrum of visual purple, thus giving further evidence that lack of oxygen affects only the neural elements of the retina.

General Metabolism.—Much information about normal retinal metabolism, as well as facts concerning pathologic conditions, has been gained by studying the effect of various poisons on metabolic changes. Holmes [58] and Lenti [59] showed that fluoride inhibited lactic acid formation from glucose, prevented the disappearance of inorganic phosphate and allowed no accumulation of phosphate esters, thus indicating that fluoride inhibits the first stage of glucose breakdown. Dickens and Greville [56b] noted that the inhibition of anaerobic glycolysis in a tissue parallels the magnitude of the glycolysis, and thus retina, with the highest glycolytic activity, was inhibited by less than 1 millimol of fluoride. Kerly and Bourne [60] found that in fluoride-poisoned extracts there was some formation of an ester which was hydrolyzed with difficulty from glucose and that its formation was increased by addition of pyruvic acid.

Lenti [59] also showed that a two-hundreth molar solution of iodoacetate inhibited glycolysis but that phlorhizin did not. On the contrary, de

55. Dickens, F., and Greville, G. D.: The Metabolism of Normal and Tumour Tissue: III. Respiration in Fructose and in Sugar-Free Media, Biochem. J. **27**:832, 1933.

56. Dickens, F., and Greville, G. D.: (*a*) The Metabolism of Normal and Tumour Tissue: VI. The Conversion of Fructose and Glucose to Lactic Acid by Embryonic Tissue, Biochem. J. **26**:1251, 1932; (*b*) VII. The Anaerobic Conversion of Fructose into Lactic Acid by Tumour and Adult Normal Tissues, ibid. **26**:1546, 1932.

57. Chase, A. M., and Hagan, W. H.: The Photochemical and Thermal Reactions of Visual Purple in Absence of Oxygen, J. Cell. & Comp. Physiol. **21**:65, 1943.

58. Holmes, B. E.: Inhibition by Fluoride of Glucose Breakdown in Tumour Tissue and Retina Extracts, Biochem. J. **34**:926, 1940.

59. Lenti, C.: Glycolysis in the Retina, Arch. di sc. biol. **25**:455, 1939; abstracted, Chem. Abstr. **34**:5903, 1940.

60. Kerly, M., and Bourne, M. C.: Glycolysis in Retinal Extracts, Biochem. J. **34**:563, 1940.

Conciliis [61] found that 0.02 to 0.01 millimol of phlorhizin had a strong inhibiting action on glycolysis, proportional to the concentration; there was little effect on the respiration, the action apparently being specific for glycolysis. Bisulfite and hydrazine inhibited glycolysis without the formation of methylglyoxal, triose or pyruvic acid. Süllmann and Vos [62] found phosphorylation and lactic acid formation are inhibited by glyceraldehyde and one-fortieth molar maleic acid. Kerly and Bourne [60] and Lenti [59] also observed the inhibitory effect on glycolysis of glyceraldehyde. Greville [63] noticed that in tissue with pure carbohydrate respiration, such as the retina, the respiration was inhibited by malonate but fumarate only partially relieved the inhibition.

Oguchi [64] showed that poisons, such as methyl alcohol, quinine and illuminating gas, which injure vision, also reduce the oxygen consumption 25 to 50 per cent in vitro. Wolff [65] reported that quinine if injected into the vitreous, and thus brought in direct contact with the retina, rapidly inhibited respiration but that subcutaneous injections did not inhibit oxygen consumption, even though complete amaurosis occurred. Only after repeated doses was any effect noted. Califaro [66] demonstrated how retinal glycolysis was inhibited by the agents which inhibit muscular glycolysis. Oguchi [67] showed that, while after the injection of 0.1 mg. of thyroxin per hundred grams of body weight the respiratory activity of the retina increased, the injection of one-tenth this amount produced a fall in the respiratory rate up to 20 per cent and a fall in glycolysis up to 30 per cent.

For the rat retina Laser [68] showed that carbon monoxide did not inhibit the oxygen uptake even in high concentration; however, it inhibits the Pasteur effect, aerobic equaling anaerobic glycolysis.

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61. de Conciliis, N.: Ricerche sulla fosfatasi retinica, Sperimentale, Arch. di biol. **88**:793, 1934.

62. Süllmann, H., and Vos, T. A.: Der glykolytische Kohlenhydrataffau in Extraken der Retina, Enzymologia **6**:246, 1939.

63. Greville, G. D.: Fumarate and Tissue Respiration: I. The Effect of Dicarboxylic Acids on the Oxygen Consumption, Biochem. J. **30**:877, 1936.

64. Oguchi, T.: Ueber den Stoffwechsel der Netzhaut: IV. Ueber den Einfluss der Gifte (Methylalcohol, Chinin und Leuchtgas) auf den Stoffwechsel der Netzhaut, Acta Soc. ophth. jap. **36**:1997, 1932; abstracted, p. 155.

65. Wolff, E.: The Effect of Quinine on the Oxygen Consumption of the Dog's Retina, Tr. Ophth. Soc. U. Kingdom **56**:162, 1936.

66. Califaro, L.: Ricerche sulla glicolisi della retina, Atti d. Accad. Lincei **25**:93, 1937.

67. Oguchi, T.: Ueber den Stoffwechsel der Netzhaut: V. Ueber den Einfluss des Thyroxins auf den Stoffwechsel der Netzhaut, Acta Soc. ophth. jap. **37**:103, 1933; abstracted, p. 6; VI. Weiteres über den Einfluss des Thyroxins auf den Stoffwechsel der Netzhaut, ibid. **37**:956, 1933; abstracted, p. 72.

68. Laser, H.: Tissue Metabolism Under the Influence of Carbon Monoxide, Biochem. J. **31**:1677, 1937.

Elliott and Baker [69] found that the oxidation-reduction potential indicator 2,6-dichlorophenolindophenol in 1.3×10^{-3} concentration produced almost complete inhibition of respiration in the presence or absence of dextrose. According to Oyama,[70] the optimum p_H for respiration was 8.0 to 8.5. He also showed that the oxygen consumption of the temporal and that of the nasal half of the retina were the same. Between p_H 6.88 and 7.88 he found that the oxygen consumption increased with increasing alkalinity.[71]

The respiratory rate of rat retina was about twice as high in bicarbonate Ringer's solution as in phosphate Ringer's solution. Laser [72] also found that hydrocyanic acid inhibited the respiration of the retina in phosphate Ringer's but not in bicarbonate Ringer's solution. Craig and Beecher [73] observed that the metabolism of rat retina was sensitive to the concentration of the carbon dioxide–bicarbonate buffer system. Increasing the carbon dioxide concentration from 1 to 5 per cent at constant p_H almost doubled both the oxygen consumption and the glycolysis; increasing it from 5 to 20 per cent had no effect on glycolysis but depressed the oxygen uptake from 31 to 19. In mediums containing dextrose and 1 per cent carbon dioxide–bicarbonate buffer the addition of succinate increased the oxygen uptake from 12 to 16 without affecting glycolysis; succinate, however, had no effect on a medium containing dextrose and phosphate.

Shaffer, Chang and Gerard,[74] in studying the influence of blood constituents on tissue metabolism, reported that in dog retina the oxygen consumption was 187 per cent greater in serum than in Ringer's solution; in other tissues this increase is related to serum proteins. Ideta [75] observed the effect of sugar concentration on the metabolism of rabbit retina and found that the respiratory rate increased

69. Elliott, K. A. C., and Baker, Z.: The Effects of Oxidation-Reduction Potential Indicator Dyes on the Metabolism of Tumour and Normal Tissues, Biochem. J. **29**:2396, 1935.

70. Oyama, N.: Einfluss der Wasserstoffionenkonzentration auf den Säuerstoffverbrauch der Hellnetzhaut von Kaninchen in vitro, Tohoku J. Exper. Med. **35**:576, 1939.

71. Oyama, N.: Einfluss des Natriumbikarbonats auf den Säuerstoffverbrauch der Hellnetzhaut von Kaninchen in vitro, Tohoku J. Exper. Med. **37**:78, 1939; abstracted, Chem. Abstr. **34**:2051, 1940.

72. Laser, H.: Metabolism of Retina, Nature, London **136**:184, 1935.

73. Craig, F. N., and Beecher, H. K.: The Effect of Carbon Dioxide Tension on Tissue Metabolism (Retina), J. Gen. Physiol. **26**:473, 1943.

74. Shaffer, M.; Chang, T. H., and Gerard, R. W.: The Influence of Blood Constituents on Oxygen Consumption in Nerve, Am. J. Physiol. **111**:697, 1935.

75. Ideta, K.: Studien über die Gewebsatmung der Netzhaut des Kaninchens in Vitro: Einfluss des Zuckers im Serum, Jap. J. M. Sc., III, Biophysics **5**:105, 1938.

with increasing sugar concentrations up to a maximum at 0.5 per cent, while glycolysis was greatest at a concentration of 0.2 to 0.4 per cent. Oguchi [76] also noted that the metabolism of rabbit retina was greater in serum than in Ringer's solution. Aerobic glycolysis was present in serum, but the Pasteur effect was prominent. In both mediums the temperature coefficient increased slowly with time, this increase, the author stated, indicating tissue damage.

Considerable information on the chemical changes which take place in retinal metabolism has been gained in recent years by groups of investigators who have studied the formation and effect of intermediary products. In 1935 Possenti [77] observed that the aerobic and anaerobic glycolysis of the retina was higher than that of any other normal tissue and was comparable to that of tumor tissue. The production of lactic acid from dextrose was higher than that from hexosediphosphoric acid. From α-glycerophosphoric acid and phosphoglyceric acid, although they were actively metabolized, the lactic acid production was very small and was not influenced by mixing α-glycerophosphoric acid and phosphoglyceric acid or by the addition of pyruvic acid to α-glycerophosphoric acid. Hexosediphosphoric acid, α-glycerophosphoric acid and phosphoglyceric acid showed an induction period for formation of lactic acid, probably due to dephosphorylation. They found no liberation of phosphoric acid from the retina during glycolysis.

Süllmann and Vos [62] asserted that an extract of ox retinas inactivated by dialysis was able to produce lactic acid after the addition of muscle adenylic acid. Cozymase alone could not activate it. Manganese, magnesium and pyruvic acid increased the formation of lactic acid. While dextrose, mannose and fructose were equivalent as substrates, galactose yielded only small amounts of acid. Hexosediphosphate gave much less lactic acid than did dextrose, but dextrose-1-phosphate gave as much. Inorganic phosphate was found necessary for glycolysis. Nicotinic acid, nicotine or nicotinamide had no stimulatory effect on metabolism. When inosinic acid was used instead of adenylic acid, there was no phosphorylation or lactic acid production. In a subsequent study Süllmann [78] found that inosinic acid could function in retinal extracts as a coenzyme in the phosphorylation of both glucose and glycogen, although not as effectively as adenylic acid.

76. Oguchi, T.: Experimentelle Studien über den Einfluss des verschiedenartigen Bedingungen auf den Stoffwechsel der Netzhaut: I. Der physiologische Stoffwechsel der Kaninchennetzhaut in Ringerlösung und im Serum, Acta Soc. ophth. jap. **40**:1568, 1936; abstracted, p. 96.

77. Possenti, G.: Prime ricerche sulla glicolisi retinica, Riv. di pat. sper. **15**:183, 1935.

78. Süllmann, H.: Ueber die CO-Enzymwirkung von Inosinsäure beim Glucose- und Glykogenabbau in Extrakten der Retina, Helvet chim. acta **23**:606, 1940.

Mixtures of the acids were not as effective as adenylic acid alone, but inosinic acid did not inhibit adenylic acid.

Süllmann and Brückner [79] showed that glycolytic extracts of ox retinas break down glycogen chiefly via phosphorolysis. The addition of dextrose-1-phosphoric acid inhibited the phosphorylation of dextrose and of glycogen. In experiments with dextrose plus glycogen, dextrose plus dextrose-1-phosphoric acid and glycogen plus dextrose-1-phosphoric acid there was no additional lactic acid formation. Retinal extracts were able to form glycogen from dextrose-1-phosphoric acid, this reaction being accelerated by muscle adenylic acid. These authors also studied the transformation of dextrose-1-phosphoric acid to hexosediphosphoric acid.

Kerly and Bourne [60] noted that aqueous extracts of ox retina converted dextrose, glycogen or hexosediphosphate into lactic acid on the addition of adenosine triphosphate or adenylic acid. Adding phosphate or magnesium increased formation of lactic acid from dextrose, and the yield of hexosediphosphate was increased by magnesium but not by phosphate. After dialysis of the extract there was no formation of lactic acid from dextrose unless magnesium and phosphate were added; for formation from glycogen only phosphate, and from hexosediphosphate only magnesium, was required. In the experiments adenylic acid could replace adenosine triphosphate but with less yield; with added phosphate, however, there were greater yields of lactic acid with adenylic acid. Cozymase could not replace either adenosine triphosphate or adenylic acid. In extracts incubated with dextrose there was no evidence of phosphoric-carbonic esters. With adenylic acid used as the coenzyme there was a transfer of inorganic phosphate to an ester, probably adenosine triphosphate. With hexosediphosphate as the substrate almost all was broken down to inorganic phosphate. The incubation of dextrose with hexosediphosphate gave only slightly more lactic acid than dextrose alone.

In studying the phosphorylating glycolysis of various tissues, Meyerhof and Perdigon [80] found that the oxidation-reduction process between pyruvic acid and triose phosphate, formed from hexosediphosphate, was rapid in the retina, especially if hexosemenophosphate and creatine were present. The addition of a coenzymatic system, such as cozymase and adenosine triphosphate, increased the speed of glycolysis.

79. Süllmann, H., and Brückner, R.: Glykogenolyse und Glykogenbildung in Extrakten der Retina, Enzymologia **8**:167, 1940.

80. Meyerhof, O., and Perdigon, E.: Sur la glycolyse phosphorylante des tissus animaux, Enzymologia **8**:353, 1940.

Greig, Munro and Elliott [81] discovered that the oxidation in the retina was different from that in most other tissues studied in that pyruvate did not seem to be removed by the succinate-fumarate-malate-oxalacetate series. Lactate and pyruvate were oxidized rather rapidly. Succinate was oxidized only slightly to fumarate, and there was an equilibrium established between fumarate and malate, but with no further oxidation. Fumarate caused an unexplained lowering of the respiratory quotient. Acetate, formate, β-hydroxybutyrate, citrate and α-ketoglutarate were not oxidized to any extent. This would indicate that oxidation probably does not take place through the Krebs citric acid cycle, although Krause and Stack [82] noted a fairly high citric acid content in the retina, which might suggest this scheme. The manometric work of Greig and associates [81] showed that some acid intermediate other than pyruvate was formed from lacate during the first stage of the experiment and that later, when lactate was no longer oxidized, the unknown acid acted as a substrate.

Possenti [83] found no, or only traces of, carboxylase in the retina and suggested that, instead of carbon dioxide being split from pyruvic acid to yield acetaldehyde, the Törniessen scheme was employed, whereby two pyruvates form a diketo fatty acid, which splits into succinic and formic acids. Meyerhof and Lohmann [84] demonstrated the presence of zymohexase, which splits hexosediphosphate in the retina; and Elliott and Greig,[85] the presence of complete succinic oxidase, indophenol oxidase and succinic dehydrogenase systems in the retina. The oxygen uptake was increased to a maximum through the addition of cytochrome-C.

In the retina, Weil-Malherbe [86] showed that *l*-glutamic acid did not affect anaerobic gycolysis, in contrast to its inhibitory action in the

81. Greig, M. E.; Munro, M. P., and Elliott, K. A. C.: The Metabolism of Lactic and Pyruvic Acids in Normal and Tumour Tissue: VI. Ox Retina and Chick Embryo, Biochem. J. **33**:443, 1939.

82. Krause, A. C., and Stack, A. M.: Citric and Malic Acids of the Ocular Tissues, Arch. Ophth. **22**:66 (July) 1939.

83. Possenti, G.: Ricerche sul ricambio dell'acido piruvico nella retina, Riv. di pat. sper. **15**:229, 1935.

84. Meyerhof, O., and Lohmann, K.: Ueber die enzymatische Gleichgewichtsreaktion zwischen Hexosediphosphorsäure und Dioxyacetonephosphorsäure: III. Ueber Abfangen der Triosephosphorsäure mit Bisulfit und die Verbreitung des Ferments; "Zymohexase," Biochem. Ztschr. **273**:413, 1934.

85. Elliott, K. A. C., and Greig, M. E.: The Distribution of the Succinic Oxidase System in Animal Tissues, Biochem. J. **32**:1407, 1938.

86. Weil-Malherbe, H.: Observations on Tissue Glycolysis, Biochem. J. **32**: 2257, 1938.

brain. Santoni [87] found that glutamic acid increased respiration. Greig and Munro [88] demonstrated that pyrophosphate had practically no effect on the normal respiration of the retina but considerably increased the rate of oxidation of glucose and lactate. By studying frozen-dried sections of the retina, Anfinsen [89] was able to show that the adenosine diphosphate was greater in the synaptic layers and the ganglion cell layer and less in the rods and cones.

In studying the oxidation-reduction systems in the retina, Süllmann and Schmid [90] found the ascorbic acid content to be 15 mg. per hundred grams (determined by titration) and that there was no difference between the light-adapted and the dark-adapted eye. Deproteinized retinal extracts gave a positive nitroprusside reaction, indicating the presence of sulfhydryl groups. Kodamari [91] noticed that the normal light-adapted rabbit retina contained 147.2 mg. of glutathione but that the dark-adapted retina contained 32 per cent less. Bleeding, cyanide, cocaine, ligation of the bile ducts and avitaminosis A, all conditions which decrease dark adaptation, increased the glutathione content of the retina.

Von Euler and Adler [92] noted the high content of flavin (evidently riboflavin) in the retina. Gourévitch [93] expressed the belief that the flavin content of rat retina corresponded in general to the intensity of the residual respiration. Süllmann [94] showed that added lactoflavin caused a loss of pyruvic acid when the mixture was illuminated.

87. Santoni, A.: Ulteriori ricerche sul metabolismo della retina; metabolismo degli aminoacidi, Rassegna ital. d'ottal. **9**:81, 1940; abstracted, Am. J. Ophth. **23**: 1290, 1940.

88. Greig, M. E., and Munro, M. P.: Some Effects of Pyrophosphate on the Metabolism of Tissues, Biochem. J. **33**:143, 1939.

89. Anfinsen, C. B.: The Distribution of Diphosphopyridine Nucleotide in the Bovine Retina, J. Biol. Chem. **152**:279, 1944.

90. Süllmann, H., and Schmid, A. E.: Ueber die Oxydoreduktionssysteme der Netzhaut, Ophthalmologica **103**:150, 1942.

91. Kodamari, S.: Ueber Glutathion in der Netzhaut von Kaninchenaugen: I. Ueber den Gehalt von Glutathion in der Netzhaut bei normalen Kaninchen und einige daran anschliessende Experimente, Acta Soc. ophth. jap. **39**:2053, 1935; abstracted, p. 147; II. Ueber den Gehalt von Glutathion in der Netzhaut und einiger anderer Organe bei Kaninchen unter verschiedenen Bedingungen, ibid. **40**: 2444, 1936; abstracted, p. 159.

92. von Euler, H., and Adler, E.: Ueber das Vorkommen von Flavinen in tierischen Geweben, Ztschr. f. physiol. Chem. **223**:105, 1934.

93. Gourévitch, A.: La distribution de la flavine dans les tissus des mammifères, en relation avec leur respiration résiduelle en présence des cyanures, Compt. rend. Acad. d. sc. **204**:526, 1937.

94. Süllmann, H.: Sensibilisierende Wirkung des Lactoflavins bei der photochemischen Umwandlung von Brenztraubensäure, Klin. Wchnschr. **7**:1157, 1938.

Theorell [95] asserted that the destruction of flavin or its ester proceeds more rapidly in the presence of oxygen and more rapidly in alkaline than in neutral mediums. Stern, Melnick and DuBois [96] studied the nature of Pasteur enzyme in the retina and showed it to be a pheohemin protein, similar to the respiratory enzyme in yeast and in Acetobacter but differing in its affinity for carbon monoxide and oxygen and in its absorption spectrum.

Respiratory Quotient.—Dickens and Šimer,[97] together with other investigators, concluded that the respiratory quotient of the retina was 1 and thus placed retina in the group of tissues, including brain, chorion and embryo, in which the metabolism consists strictly in the oxidation of carbohydrates. This view has been recently opposed by Elliott and Baker,[98] who obtained a value of 0.91 for rat retina and of 0.86 for rat brain. Possenti [83] obtained a respiratory quotient of 0.8 to 0.9 in Ringer's solution, which became over 1.0 when pyruvate was added.

Fat Metabolism.—An explanation for a respiratory quotient below 1.0 may be given by recent work, which suggests the part played by a fat metabolism. Sgrosso [99] found in ox retina high quantities of a lipase for the low neutral fats but not for the high neutral fats. Santoni [100] showed that ox or rabbit retina oxidized numerous fatty acids, with the exception of formic, caproic and oleic acid. These acids inhibited the respiration. It was found that the methyl esters of the fats had a greater capacity for oxidation than the free fats. He suggested that these esters may be those normally found in the cells rather than the esters produced by the action of enzymes.

Formation of Ammonia.—Warburg, Posener and Negelein [28] noticed that large quantities of ammonia were formed during anaerobic glycolysis in the retina and brain, and even larger quantities during aerobic

95. Theorell, H.: Quantitative Bestrahlungsversuche an gelbem Ferment, Flavinphosphorsäure und Lactoflavin, Biochem. Ztschr. **279**:186, 1935.

96. Stern, K. G.; Melnick, J. L., and DuBois, D.: Nature of the Pasteur Enzyme, Science **91**:436, 1940. Stern, K. G., and Melnick, J. L.: The Photochemical Spectrum of the Pasteur Enzyme in Retina, J. Biol. Chem. **139**:301, 1941.

97. Dickens, F., and Šimer, F.: The Metabolism of Normal and Tumour Tissue: II. The Respiratory Quotient, and the Relationship of Respiration to Glycolysis, Biochem. J. **24**:1301, 1930.

98. Elliott, K. A. C., and Baker, Z.: The Respiratory Quotients of Normal and Tumour Tissue, Biochem. J. **29**:2433, 1935.

99. Sgrosso, S.: Ricerche sui fermenti lipolitici della retina, Rinasc. med. **11**: 334, 1934.

100. Santoni, A.: Sulla capacità del tessuto retinico di ossidare alcuni acidi grassi ed esteri metilici di acidi grassi "in vitro," Ann. di ottal. e clin. ocul. **67**:845, 1939; abstracted, Am. J. Ophth. **23**:729, 1940.

glycolysis. Rösch and te Kamp [101] found that retina gave off over 50 per cent of ammonia from muscle adenosine-5-phosphoric acid, 20 per cent from yeast adenosine-3-phosphoric acid, some from adenosine but none from adenine. Light-adapted retina gave 5.22 mg., and dark-adapted retina 0.74 mg., of ammonia per hundred grams. Lighting the dark-adapted retina increases the ammonia content at least 72 per cent. Stutzke [102] obtained increases of 200 to 500 per cent in ammonia content on illuminating the dark-adapted retina but no increase in the light-adapted retina. Dextrose lactate or pyruvate inhibited this evolution of ammonia. Rösch [103] observed that visible light and roentgen rays of long medium wavelength, but not ultraviolet rays, caused the evolution of ammonia, in proportion to the intensity of light. Dickens and Greville [104] found production of ammonia to be small in the retina, but in the absence of dextrose or fructose it became large relative to the respiratory rate, partly because of the large fall in the latter.

Glycogen.—The presence of glycogen in the retina has been a subject of some dispute. Ehrlich,[105] in 1833, detected glycogen in frog retina, and Luna,[106] Müller [107] and others found it in other animals. Best [108] and Matsuoka [109] claimed that the normal retina was free of glycogen, but Schmitz-Moormann,[110] with careful staining, demonstrated glycogen in the inner segment in the base of the cones and in the myoid

101. Rösch, H., and te Kamp, W.: Ueber Ammoniakbildung bei Belichtung der Netzhaut, Ztschr. f. physiol. Chem. **175**:158, 1928.

102. Stutzke, S.: Ueber die Ammoniakbildung in der Netzhaut, Klin. Wchnschr. **15**:524, 1936.

103. Rösch, H.: Weitere Untersuchungen über die Ammoniakbildung in der Netzhaut, Ztschr. f. physiol. Chem. **186**:237, 1930.

104. Dickens, F., and Greville, G. D.: The Metabolism of Normal and Tumor Tissue: IX. Ammonia and Urea Formation, Biochem. J. **27**:1123, 1933.

105. Ehrlich, P.: Ueber das Vorkommen von Glykogen im diabetischen und im normalen Organismus, in Frerich, F. T.: Ueber den plötzlichen Tod und über das Coma bei Diabetes, Ztschr. f. klin. Med. **6**:3, 1833.

106. Luna, E.: Ricerchi istologische e istochimiche sulla retina dei vertebrati, Monitore zool. ital. **22**:119, 1911.

107. Müller, C.: Das Glycogen der Retina des Frosches, Ztschr. f. d. ges. Anat. **81**:220, 1926.

108. Best, F.: Demonstration mikroskopischer Präparate vom diabetischen Auge, Ber. ü. d. Versamml. d. deutsch. ophth. Gesellsch. **32**:315, 1906; Die Bedeutung des pathologischen Glykogengehaltes, Zentralbl. f. allg. Path. u. path. Anat. **18**:465, 1907.

109. Matsuoka, J.: Ueber das Glykogen in der Netzhaut, Nippon Gankwa Gakkawi Zasshi **23**:1919; cited by Schmitz-Moormann.[110]

110. Schmitz-Moormann, P.: Ueber der Glykogengehalt der Retina und seine Beziehungen zur Zapfenkontraktion, Arch. f. Ophth. **118**:506, 1927.

but not in the rods. Fontana [111] saw glycogen granules in the pigment epithelium, in the cones and in the external limiting membrane. There was no glycogen in the pigment epithelium if the eyes were kept in the dark, but much in bright light; the other deposits of glycogen remained unchanged. Brammertz,[112] on the other hand, detected in the eye of the housefly an increase of glycogen in the dark-adapted eye and a decrease in the light-adapted eye.

Best [113] found that ocular inflammations, subconjunctival injections of a 10 per cent solution of sodium chloride, subcutaneous injections of phlorhizin and diabetes increased the retinal glycogen. Majima [6] and Nakayasu [114] noted that epinephrine increased the glycogen. Nakayasu also found that the injection of 10 units of insulin per kilogram produced a fall or disappearance of glycogen in the retina, 5 units a slight reduction and ½ unit a rise. Thyroxin or thyroid extract produced a rise of glycogen, as also did solution of posterior pituitary U. S. P. Trematori [115] investigated the amylase of the retina and found it to have an optimum of p_H of 7.8 and an optimum sodium chloride concentration of 0.008 to 0.013 per cent. Fluoride completely inhibited, and hydrogen peroxide and formaldehyde partially inhibited, the amylase.

Carbon Dioxide Anhydrase.—Bakker [116] found a high concentration of this enzyme in the retina and lens, some in the cornea and choroid and none in the sclera. He assumed that tissues with a high aerobic glycolysis and a paucity of blood vessels need a high concentration to catalyze the rapid dissolution of carbon dioxide. He [117] showed that carbon dioxide anhydrase produced both a hydration and a dehydration of carbon dioxide, although in the retina dehydration occurs more readily than hydration. The reaction was activated in both directions by cysteine, histidine, histamine and glutathione.

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111. Fontana, G.: Ulteriori ricerche istologische sul glicogeno della retina di alcuni vertebrati allo stato normale ed in talune condizioni sperimentali, Rassegna ital. d'ottal. **4**:135, 1935.

112. Brammertz, W.: Ueber das normale Vorkommen von Glykogen in der Retina, Arch. f. mikr. Anat. **86**:1, 1914.

113. Best, F.: Beitrag zur Wirkung subconjunctival Injektionen, Arch. f. Augenh. **57**:173, 1907; footnote 108.

114. Nakayasu, S.: Ueber den Einfluss der verschiedenen Präparate der inneren sekretorischen Organe auf den Glykogenstoffwechsel in der Netzhaut, Acta Soc. ophth. jap. **37**:941, 1933; abstracted, p. 71.

115. Trematori, M.: L'amilasi della retina, Riv. biol. **20**:108, 1936.

116. Bakker, A.: Der Kohlensäureanhydrasegehalt verschiedener Augengewebe einiger Säugetiere, Ophthalmologica **102**:351, 1941.

117. Bakker, A.: Bewirkt die Kohlensäureanhydrase in den Augengeweben eine Hydratation von Kohlendioxyd oder eine Dehydratation von Kohlensäure? Ophthalmologica **103**:88, 1942.

Acetylcholine.—Lange [118] found that after dark adaptation 20 frog retinas (0.5 Gm.) contained 150 micrograms of acetylcholine, while after light adaptation the same number contained only 15 micrograms. Similar results on the effect of light were obtained by Chang, Hsieh, Lee and Li; [119] Nakashima and Murata,[120] and Chang, Lee and Li.[121] Bakker [122] demonstrated the formation of acetylcholine in the retina by noticing the action of an explanted iris when the retina was also explanted. Anfinsen [123] demonstrated that cholinesterase occurred chiefly in the synaptic layers of the retina.

Detached Retina.—By studying the special metabolism of the detached retina, some clues may be obtained as to normal retinal metabolism. Weve and Fischer [124] showed that, while normal retina in contact with the pigment epithelium has a low oxidation-reduction potential, the detached retina was more highly oxidized, this oxidation being evidently due to an alteration in its metabolism. On detachment it was no longer glycolysis, but respiration, which prevailed. Glycolysis and vision seemed to depend on the contact of retina with pigment epithelium. Fischer [125] showed the detached retina to be more acid. Weve and Fischer [126] demonstrated a high amylase content of the subretinal fluid, liberated as a result of autolysis of retinal cells and not due to any admixture of blood. Amylase was found to occur

118. Lange, V.: Ueber das Vorkommen von Azetylcholin im hell- und dunkeladaptierten Auge, Ztschr. f. physiol. Chem. **279**:73, 1943; abstracted, Chem. Abstr. **38**:3024, 1944.

119. Chang, H.; Hsieh, W. M.; Lee, L. Y., and Li, T. H.: Diminution of Acetylcholine Content of Retina After Prolonged Functional Disuse, Proc. Soc. Exper. Biol. & Med. **43**:140, 1940.

120. Nakashima, M., and Murata, G.: Ueber die Cholinmenge der Hell- und Dunkelnetzhaut, Acta Soc. ophth. jap. **43**:660, 1939; abstracted, p. 41.

121. Chang, H. C.; Lee, L. Y., and Li, T. H.: Tissue Acetylcholine: IX. Diminution of Acetylcholine of Retina During Functional Disuse, Chinese J. Physiol. **16**:373, 1941.

122. Bakker, A.: Ueber Acetylcholinbildung in der Retina, Arch. f. Ophth. **141**:326, 1939; abstracted, Chem. Abstr. **34**:3801, 1940.

123. Anfinsen, C. B.: The Distribution of Cholinesterase in the Bovine Retina, J. Biol. Chem. **152**:267, 1944.

124. Weve, H. J. M., and Fischer, F. P.: Le métabolisme de la rétine décollée, Ann. d'ocul. **175**:817, 1938.

125. Fischer, F. P.: Metabolismus der abgelösten Netzhaut, Nederl. tijdschr. v. geneesk. **83**:4187, 1939; abstracted, Zentralbl. f. d. ges. Ophth. **44**:395, 1939.

126. Weve, H. J. M., and Fischer, F. P.: Ueber die Amylase der subretinalen Flüssigkeit, Arch. f. Augenh. **110**:390, 1937.

only in the retina.[127] A high acetylcholine esterase content was observed in subretinal fluid.[128]

COMMENT

The foregoing evidence is not sufficient to allow one to outline an integrated framework of retinal metabolism; yet one may list, without attempting to connect, some of the important reactions in accordance with the weight of the evidence, as few facts stand completely unopposed.

On stimulation with light, acetylcholine is liberated in the retina, this perhaps being the substance which stimulates the nerve elements; phosphocreatine is converted into creatine and phosphoric acid, and adenosine triphosphate is split into the disphosphate and phosphoric acid, in addition to the fundamental conversion of rhodopsin into other products. The decomposition of phosphocreatine and adenosine triphosphate results in the liberation of energy, possibly both reactions occurring in a stepwise fashion. The energy, then, for the reformation of rhodopsin, phosphocreatine, adenosine triphosphate and perhaps other substances, as well as the basal energy requirements of the cells, comes largely from the oxidation of glucose. The effect of poisons and substrates and of added enzyme systems and the determination of intermediate products would indicate that glycolysis follows a scheme similar to that of muscle: glucose through the hexosemonophosphates to hexosediphosphate, thence to dioxyacetone phosphate and glyceraldehyde phosphate followed by oxidization to phosphoglycerate and then pyruvate, which yields lactate and is eventually oxidized to carbon dioxide and water. The intermediate substances are not all known, but it may be supposed that the entire Cori scheme is reproduced, and it seems likely that the Krebs citric acid cycle is employed. The Pasteur effect is present. Glycogen, which is probably stored in the normal retina, may supplement blood glucose. Certainly the metabolism, primarily glycolytic, may also involve the oxidation of fats and amino acids, with resulting respiratory quotients below 1.

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127. Weve, H. J. M.: Der Enzymgehalt der retroretinalen Flüssigkeit, Nederl. tijdschr. v. geneesk. **81**:3000, 1937; abstracted, Zentralbl. f. d. ges. Ophth. **39**:367, 1937.

128. Weve, H. J. M., and Fischer, F. P.: Ueber den Azetylcholinesterase-Gehalt der subretinalen Flüssigkeit bei rupturellen Netzhautablösungen, Ophthalmologica **96**:348, 1939.