

TABLE I.
Relationship of Homoplastic Ovarian Transplantability to the Type of Ovariectomy in the Host.

Type of Ovariectomy	No. of attempted grafts	Unsuccessful grafts	Functional grafts
Unilateral	13	13	0
Incomplete bilateral	8	8	0
Complete bilateral	30	12	18

ing that principle can be successful. This has been confirmed by results of autoplasmic transplantations of parathyroids (Halsted),⁶ adrenal glands (Ingle and Higgins),⁷ thyroid (Ingle and Cragg),⁸ and ovaries (Lane and Markee),⁹ and homoplastic transplantations of adrenal glands (Pomerat *et al.*).¹⁰ In the

⁷ Ingle, D. J., and Higgins, G. M., *Endocrinology*, 1938, **22**, 458.

⁸ Ingle, D. J., and Cragg, R. W., *Endocrinology*, 1939, **24**, 550.

⁹ Lane, C. E., and Markee, J. E., *Growth*, 1941, **5**, 61.

¹⁰ Pomerat, C. M., Breckenridge, C. G., and Gordon, L., *Endocrinology*, 1944, **34**, 60.

present work homoplastic ovarian transplantations were successful only when there was a complete absence of host ovarian tissue. Therefore, the results are in accord with the concept that creation of a deficiency of the ovarian principle is necessary for the establishment and maintenance of homoplastic ovarian grafts.

Summary. Functional homoplastic ovarian grafts in mice became established only after complete bilateral ovariectomy in the host. This is in agreement with Halsted's "law of endocrine deficiency" since, in this series of experiments, the transplants were successful only in the absence of host ovarian tissue.

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Acetylcholine in the Light and Dark Adapted Frog Retina.

DEXTER M. EASTON. (Introduced by F. L. Hisaw.)

From the Biological Laboratories, Harvard University.

Like central nervous tissue of which it is a derivative, the vertebrate retina has been shown to contain acetylcholine (Ach)^{1,2,3,4} and cholinesterase.⁵ That Ach may affect the retina has also been demonstrated, von Studnitz and Kosarov⁶ having observed a contraction of the cones and an expansion of the rods

at concentrations of 10^{-4} and 10^{-8} Ach upon both light and dark exposure. In the excised eye, Therman² has found that the application of Ach causes an irreversible depression of excitation as measured by diminished retinal potentials (an effect enhanced by eserine).

Two attempts to demonstrate a possible functional significance of retinal Ach by comparing the amounts of Ach in frog retina under conditions of light and dark adaptation have resulted in completely contradictory results. Therman,² using the leech muscle and the frog heart as test objects, found under both conditions of adaptation 6 γ Ach/g of retina extracted with trichloroacetic acid. Lange,⁴ extracting with bicarbonate-free frog Ringer, and assaying with the leech muscle, found, after complete light adaptation of 10 frogs,

¹ Loewi, O., and Hellauer, H., *Arch. f. d. ges. Physiol.*, 1938, **240**, 769.

² Therman, P. O., *Acta Soc. Sci. fenn.*, Ser. B, 1938, **2**, 5.

³ Chang, H. C., Lee, L. Y., and Li, T. A., *Chinese J. Physiol.*, 1941, **16**, 373.

⁴ Lange, V., *Ztschr. f. Physiol. Chem.*, 1943, **279**, 73.

⁵ Anfinsen, C. B., *J. Biol. Chem.*, 1944, **152**, 267.

⁶ Studnitz, G. v. and Kosarov, W. G., *Z. f. vergl. Physiol.*, 1942, **29**, 418.

ACETYLCHOLINE CONTENT OF FROG RETINA

TABLE I.

Exp.	Time adapted, hr	Frog	Wt 2 retinas, mg	Total Ach, γ/g
Acetylcholine Content of Dark-Adapted Retinas.				
10-12	48	A	29	5.0
		B	23	4.0
10-9	115	A	37	2.8
		B	31	3.2
		C	22	3.0
10-4	36	A	28	4.0
		B	31	2.8
		C	29	2.0
9-30	42	A	23	4.0
		B	23	4.8
				—
				Avg 3.6
Acetylcholine Content of Light-Adapted Retinas.				
10-12	48	A	36	3.0
		B	28	3.0
10-11	20	A	35	(1*)
		B	31	5.0
10-4	2	A	36	3.0
		B	28	3.0
		C	28	3.0
10-3	2	A	33	2.8
		B	24	3.5
		C	24	2.5
9-30	1.5	A	29	4.0
		B	23	4.5
				—
				Avg 3.4

* Not included in the average.

30 γ Ach/g of tissue in the 20 pooled retinas; after complete dark adaptation of an equal number, 300 γ Ach/g.

The following experiments were performed in an attempt to resolve these differences.

Methods. The eyes of dark-adapted frogs (*Rana pipiens*) were prepared throughout under ruby light, those light-adapted were prepared under bright light. The cornea with the lens was cut from the excised eye; the remaining portion was then immersed in eseriniz (ca. 1:50,000 by weight) bicarbonate-free Ringer solution, the more easily to tease out the retina. Blotting the retinas with filter paper, before weighing, removed excess moisture. The retinas from each frog were ground in eseriniz Ringer solution, and the volume of the suspension was increased to secure a final proportion of 10 mg of tissue per cubic centimeter of fluid. Total Ach was determined

one hour or more after the addition of ca. 0.1 cc of normal HCl. The suspension was brought to pH 5-6 with normal NaOH before testing. Ach determinations were made with the heart of *Venus mercenaria*, according to the suggestion of Prosser.⁷ Comparative experiments on other material reveal that the *Venus* heart is more sensitive than the leech muscle, and equally as accurate a test object for the quantitative determination of Ach.⁸ The sensitivity of the heart enables one to make several determinations of Ach in such small amounts of tissue as 2 frog retinas. One cubic centimeter of the suspension was added to the heart bath of oxygenated sea water (total volume 10 cc), and the resultant depression matched, after a 15-minute recovery period, with that of a

⁷ Prosser, C. L., *Biol. Bull.*, 1940, **78**, 92.

⁸ Welsh, J. H., unpublished observations.

known concentration of Ach, or with another retinal extract.

Results. In one series of experiments (Table I) 2 or 3 frogs were dark-adapted for an approximately equal length of time in each experiment. Two or 3 were also light-adapted for the same or different lengths of time. In 3 of the experiments, determinations of Ach in both light and dark retinas were made on the same heart.

The values of total Ach given in Table I are of the same order of magnitude as those reported by other authors except Lange. Chang *et al.*³ found about 2 γ Ach/g of tissue in the normal dog retina extracted with alcohol. After an eye had been blindfolded for 7 to 220 days they found an Ach content averaging 1.3 γ /g. This was interpreted as being a consequence of "functional disuse." During the first few days of blindfolding, when any effect would presumably be that of "dark adaptation," the values were not significantly different from "normal." Loewi and Hellauer¹ reported 5-6 γ of Ach/g of cattle retina.

The data in Table I indicate: (1) fairly good correspondence in any one experiment between individuals similarly adapted; (2) length of period of adaptation does not consistently alter the Ach content; (3) the difference in Ach content of light- and dark-adapted retinas is not significant.

During the course of a given assay, the sensitivity of the heart may decline. The last of eight or ten records is not, therefore, directly comparable with the first, although absolute values, as determined by direct match-

ing with known Ach concentrations, will generally agree fairly closely. In order to bring out more clearly any light-dark differences, another series of experiments was conducted, in each of which 2 pooled dark-adapted retinas were matched several times, at different dilutions, against 2 pooled light-adapted retinas. Several direct matchings brought out no more variation than was found between individuals exposed to the same conditions of adaptation.

These experiments, though constituting an affirmation of Therman's failure to find any differences in Ach content of light and dark retinae, do not necessarily prove that changes in retinal sensitivity may not be associated with changes in Ach content. The range of experimental error of the assay method is no less than 10%, even under the best conditions, and a difference of such magnitude might very well be of physiological significance.

Summary. Extraction of frog retinas in acidified Ringer solution reveals an average Ach content of 3.4 γ /g in light-adapted and 3.6 γ /g in dark-adapted frogs. Direct comparison of Ach activity in extracts of light- and dark-adapted retinas, without reference to absolute magnitudes, also reveals no difference greater than the error of the method. The lack of significant difference is in accord with the work of Therman, but not with that of Lange who finds a tenfold increase upon dark adaptation.

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