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THE CHOLINESTERASE OF THE RETINA.

By *Roger Weekers.**)

From 1930 to 1936, Velhagen found several facts indicating the presence in the retina of a substance which has the chemical and biological properties of acetylcholine (12). More recently, Bakker demonstrated, by culture experiments, the ability of isolated retina to liberate acetylcholine (1).

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Anfinsen published an article on »Distribution of cholinesterase in the bovine retina«, February 1944 (Journ. Biolog. Chem. *142*, 267). Only an abstract of this paper came to our knowledge after the writing of the present publication (Amer. Journ. Ophth. 1944, 27, 1057). This abstract follows:

»That chemical substances liberated at autonomic nerve endings may act as mediators of the nervous impulse is supported by a large body of evidence. The rapidity of conduction in nerve tissue suggests that if this is so, high concentrations of cholinesterase must be present at localized points in order to remove the mediator, acetylcholine. Gross distribution of cholinesterase in the central nervous system has been determined. Retina ideally contains synaptic structures in reasonably isolated and compact form. Studies of cholinesterase activity of selected layers of bovine retina demonstrate the presence of concentration of cholinesterase for the most part in the synaptic layers as contrasted with the nuclear, rod and nerve fiber layers. Although this localization can be demonstrated, other factors are still necessary before accepting completely synaptic transmission in terms of acetylcholine cholinesterase system«.

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Research dealing with the variations of acetylcholine content, in relation to functional activity of the retina, led to contradictory results. Chang, Hsich, Lee and Li state that there is a decrease of acetylcholine when the retina is maintened in darkness (3); Lange, on the contrary, believes that there is an increase of acetylcholine under those conditions (6). Nakashima and Murata found only slight fluctuations in the choline concentration of the retina under the effect of light and darkness (10).

The part played by acetylcholine in retinal metabolism is unknown. Several hypotheses may be suggested. It is possible that acetylcholine regulates the size of the retinal vessels and, as a consequence, the blood pressure, inside these vessels (Villaret, Justin-Besançon, Schiff-Wertheimer, Gallois (13), Frommel, Bischler (5)). This substance perhaps controls the migration of retinal pigment under the influence of light (von Studnitz, Kosaroff (11)).

It is quite conceivable that acetylcholine is the chemical mediator of synaptic conduction in the retina. The problem is of physiological interest since the mode of conduction in the central nervous system is unknown. It is also of clinical interest because some defects in the visual fields and latency in the dark adaptation might be due to an alteration of the nerve conduction inside the retina (Evans (4), Weekers (14)).

The presence of cholinesterase is essential to the action of acetylcholine in tissue metabolism. Nachmansohn (9), Weve and Fischer (15) demonstrated recently the presence of cholinesterase in the retina. Nachmansohn proved that the cholinesterasic activity of the retina is greater than the one of the optic nerve (9). In this paper, we confirm the data of these authors and study biological properties of retinal cholinesterase extracted from the bovine retinas and optic nerves.

Technical procedure.

Normal eyes of cattle are removed after slaughter of the animal; dissection of retinas and optic nerves is started one or two hours later. The retinas are used either whole or ground with sand. The tissue is extracted by water at 4° C. or at room temperature during a period varying from 30 minutes to 24

hours. The suspension of retinal tissue is heated to 37° C. and acetylcholine is added. Destruction of acetylcholine by retina is observed after a few seconds when the tissue is minced and after a few minutes when the tissue is used whole. To obtain destruction of acetylcholine by optic nerve, this tissue must be thoroughly minced and kept in contact with acetylcholine for a longer period than retinas. The enzymic reaction is stopped by addition of trichloracetic acid. The liquid is filtered. One drop of methyl red is added to an aliquot fraction of the filtrate. This fraction is then neutralised with NAOH/N and completed to a known volume. An aliquot fraction of the treated sample is immediately withdrawn and added, without delay, to a bath of Ringer's solution containing a *rectus abdominis* muscle of frog (*Rana temporaria*). — The ensuing contraction of the muscle is registered. The dilutions and the volumes of the aliquot fractions are calculated in such a way that, at most, 10 gamma of acetylcholine are added to the Ringer's solution. Acetylcholine is kept in contact with the muscle for 3 minutes, then washed away.

Results.

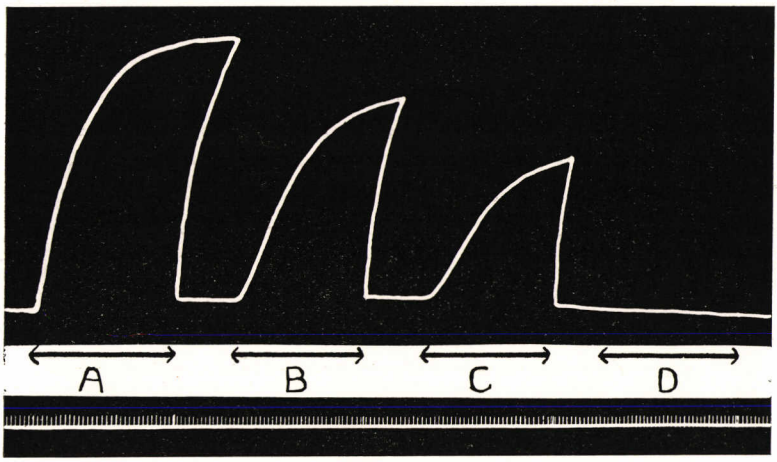
A. — *Presence of cholinesterase in the retina.* —

There is a ferment, in the retina, the activity of which results in the transformation of acetylcholine into a substance (presumably choline) which does not induce contraction of the rectus muscle.

Exper. 4; — *25. V. 1944.* —

Mince 7,4 gm. retinas with 0,1 gm. sand in a mortar. Put in 4 conical flasks 500 mgm. of the minced tissue + 8 cc. water. Keep for 30 minutes at room temperature. Heat to 37° C. Add, to each flask, 1 cc. water containing 200 gamma acetylcholine. After 0, 10, 20 and 30 seconds, respectively, add 1 cc. 33 % trichloracetic acid to each flask. Filter. Take off 5 cc. and neutralise. Complete to 10 cc. with Ringer's solution. Without delay, add 1 cc. to an eserined and oxygenated Ringer's bath containing a frog's rectus. Graph. 1.

Retinal cholinesterase is rather stable, not being altered after 24 hours in cold room at 2° C. To obtain a complete extraction of the ferment and, as a consequence, a very active



Graph. 1; Exper. 4.—

Destruction of acetylcholine by ground retina.

Records of the contraction induced in a frog's *rectus abdominis* muscle by

A. — 10 γ acetylcholine left 0 sec. in contact with ground retina.
B. — 10 γ acetylcholine left 10 sec. in contact with ground retina.
C. — 10 γ acetylcholine left 20 sec. in contact with ground retina.
D. — 10 γ acetylcholine left 30 sec. in contact with ground retina.

Below: time recorded every 6 seconds.

preparation, the tissue should be thoroughly minced and extracted with water for several hours.

B. — *Activity of cholinesterase in the peripheral and central retina.* —

Histological and physiological research has demonstrated that the structure and functions of the central and peripheral parts of the retina are different. In the center, more specifically in the macula, one cone is connected with a single bipolar cell which is in relation with a single ganglion cell. At the periphery, on the contrary, each ganglion cell receives the nerve impulses from several bipolar celles and each bipolar cell is linked with several photoreceptor cells, mostly rods. This structure results in an unequal number of synapses in equal areas taken from either the peripheral or the central

parts of the retina. This is why we tried to compare the activity of cholinesterase in different portions of the tissue. Unfortunately, for technical reasons, it was not possible to dissect equal areas in different portions of the retina. We have been compelled to compare the activity of the ferment extracted from equal weights of peripheral and central parts. To this technical procedure, however, it may be objected that the retinal membrane because of the increased number of nerve fibers, is thicker in the center than in the periphery.

Our work has led to negative results. In five experiments the cholinesterasic activity of the peripheral portions of the retina was equal to that of the central portions. In two experiments, it was lower. In two experiments, it was higher.

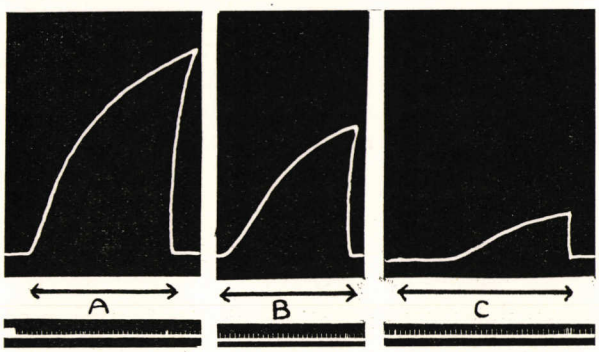
C. — *Comparison between the cholinesterasic activity of retina and optic nerve.* —

The retina has a great number of synapses, the optic nerve has none. For this reason, it might be interesting to compare the distribution of cholinesterase in the retina and in the optic nerve. This comparison is significant only when the extraction of the ferment in both tissues is complete. That is why we have thoroughly minced the retinas and the optic nerve and brought the duration of extraction for both tissues up to 24 hours. These experiments demonstrate that the enzymic activity in optic nerve is about a hundred times smaller than in retina.

Exper. 21; — 7. XI. 44.—

Mince retinas and optic nerves. Put in 4 small conical flasks 500 mgm. of the minced retinas + 8 cc. water; in 4 other flasks 500 mgm. of the minced optic nerve + 8 cc. water. Keep at 4° C. during 24 hours. Heat to 37° C. Add to each flask 1 cc. water containing 200 gamma acetylcholine. Incubate 20, 30, 40, 60 seconds respectively for the retina and 20, 30, 40, 60 minutes for the optic nerve. Add 1 cc. 33 % trichloracetic acid. Follow the procedure as in exper. 4 — Graph. 2 (only a part of the graph. is given).

The destruction of acetylcholine brought in contact with the minced optic nerve is very slow. However, control experiments prove that this destruction is not a spontaneous hydro-



Graph. 2; Exper. 21.—

A comparison of cholinesterasic activity of retina and optic nerve.

Records of the concentration induced in a frog's *rectus abdominis* muscle by

A. — 10 γ acetylcholine left in contact with ground optic nerve during 1800 sec.

B. — 10 γ acetylcholine left in contact with ground retina during 20 sec.

C. — 10 γ acetylcholine left in contact with ground optic nerve during 2400 sec.

Below: time recorded every 6 seconds.

lysis of acetylcholine, but is due to the presence of a small amount of cholinesterase in the nerve.

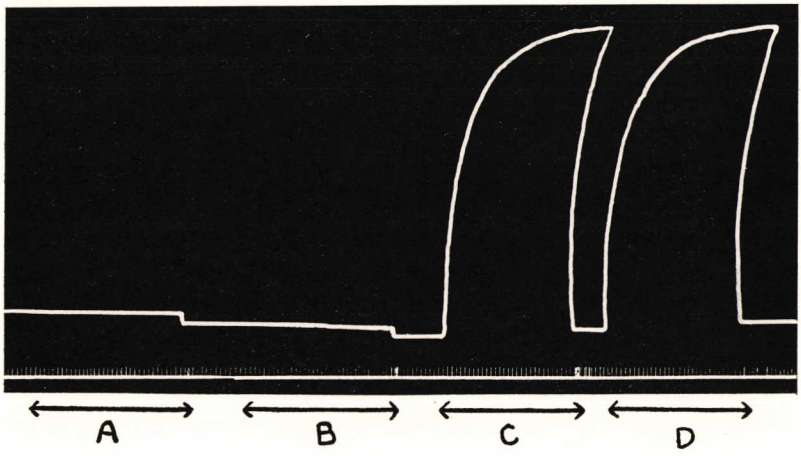
D. — *Inhibition of retinal cholinesterase by eserine.* —

In another series of experiments, we checked the inhibitory action of eserine on the retinal cholinesterase. When added to the ground tissue at a dilution of 1/50,000 eserine sulfate completely inhibits the activity of the ferment. We did not investigate the action of eserine in lower concentration. Eserine also prevents the destruction of acetylcholine when injected in the vitreous body (0,5 mgm. of eserine sulfate injected 2 to 3 hours before the experiment in the vitreous body of an enucleated cattle eye).

Exper. 9; — 8. VI. 1944.—

Remove one pair of cattle eyes. 2 hours and 40 minutes before the experiment inject 0,5 cc. NaCl, 8 % in the vitreous of the first eye; 0,5 cc. NaCl containing 0,5 mgm, eserine sulfate in the vitreous

of the second eye. Dissect the retinas, wash them in Ringer's solution. Both retinas have approximatevely the same weight (790 mgm.). Put each of them without being ground in a 50 cc. conical flask containing 9 cc. NaCl 0,8 %. Heat to 37° C. Add to each flask 1 cc. water containing 100 gamma acetylcholine. Stir. Take off 1 cc. from each flask after 20 and 30 minutes. Add to this sample 0,1 cc. 33 % trichloracetic acid and one drop of methyl red. Neutralize with NaOH/N. Introduce the neutralized solution in the Ringer's bath containing the muscle. Graph. 3. —



Graph. 3; Exper. 9.—

Inhibitory action of eserine upon the activity of retinal cholinesterase.

Records of the contraction induced in a frog's *rectus abdominis* muscle by

A. — 10 γ acetylcholine left in contact with normal whole retina during 20 minutes.

B. — 10 γ acetylcholine left in contact with normal whole retina during 30 minutes.

C. — 10 γ acetylcholine left in contact with eserined whole retina during 20 minutes.

D. — 10 γ acetylcholine left in contact with eserined whole retina during 30 minutes.

Below: time recorded every 6 seconds.

Discussion.

Recent researches suggest that acetylcholine might play an important role in the metabolism of the central nervous system. It would be too long to give a historical review of this

question. Several examples will suffice. Bonnet and Bremer demonstrated that the injection of very small quantities of acetylcholine (less than I gamma) in the carotid artery modifies the electrical activity of the acoustic cortex of the cat (2). Martini was able to detect the presence of acetylcholine in the internal ear after stimulation by sound (8). Loewi and Hellauer found acetylcholine in the optic nerve (7). Nachmansohn found more cholinesterase in the synaptic area of the brain than in other portions of this organ and more cholinesterase in the retina than in the optic nerve (9). The question arises whether acetylcholine is a chemical mediator of central nerve conduction. The retina, an extracranial part of the brain, is, from different view points, a favorable material for this study. The number of its synapses in relation to its weight is very high. Its stimulation is easy and can be measured. Its vascular supply is autonomous. Unfortunately, its dimensions are small, its vessels are narrow and its circulation difficult to control.

The experiments mentioned in this paper are only preliminary. They deal with the presence and the biological properties of retinal cholinesterase.

The activity of retinal cholinesterase is high. In the conditions set forth in our experiments, 500 mgm. of tissue, (less than a bovine retina), are able to destroy 200 gamma of acetylcholine in a few seconds. This enzyme is rather stable, it is not altered when left for 24 hours in the refrigerator. Its distribution throughout the retina appears to be even at least when equal weights of tissue, taken either from the peripheral or the central parts, are compared. It is very suggestive to notice that the optic nerve, in which the neurons are uninterrupted, has approximatively a hundred times less cholinesterase than the retina which has a very high number of synapses.

When injected in the vitreous body, eserine inhibits retinal cholinesterase. We don't know, at présent, if repeated instillations of this drug in the conjunctival sac have the same effect. It would be interesting to know if any of the visual function is modified when retinal cholinesterase is inhibited.

The problem of synaptic conduction inside the retina is

worth studying from a clinical point of view. Evans recently suggested that defects in the visual field and latency in dark adaptation might result from interference with retinal nerve conduction (4). This hypothesis agrees with our observations in the post concussion syndrome (14). The campimetric defects detected by us in this syndrome might result from an obstacle in the nerve conduction in the retina. More knowledge of the mechanism by which the nerve impulse passes from one retinal neuron to the next might result in a better understanding of some retinal diseases.

Summary.

1) Cholinesterase is present in the retina.

2) Cholinesterase is about hundred times more concentrated in the retina than in the optic nerve.

3) Cholinesterase seems evenly distributed throughout the whole retina when equal weights of peripheral and central retina are compared.

4) The retinal cholinesterase is inhibited by injection of eserine in the vitreous body.

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