tions have been made indirectly on the biological activity of the adductor muscle components. This muscle consists, as is well known, of a large semi-translucent motor striated component and a smaller opaque so-called "catch" muscle. Graphs obtained by Galtsoff of the movements of the American oyster, *O. virginica,* are shown in Fig. 1, A. Somewhat similar figures (see Fig. 1, B) were obtained by Marshall Webb for the European oyster, *O. edulis.* Complete or almost complete closure is shown at infrequent intervals; this is the common natural movement when the animal closes the shell suddenly to extrude rejected food-material.[5] Nelson obtained his graphs from oysters actually immersed in the sea and interpreted the frequent partial closures as rejection of filtered sediment and inferred therefore that the oysters were feeding actively. Galtsoff obtained his graphs from oysters kept in glass-fronted tanks under temperatures when feeding does not occur and observed that rhythmic partial closure was not accompanied by rejection of unwanted filtrates or excreta. He suggests these partial rhythmical closures may be caused by various stimuli, *e.g.,* mechanical, changes in illumination, changes in pH, gas content, presence of certain chemicals. It seems unlikely, however, that stimuli of this kind can have operated in all the experiments. I have myself observed a quick partial closing which had the effect and no doubt the design of shaking ropes of mucus from the edge of the gill on to the mantle, but this again can hardly explain all the rhythmic partial closures obtained by Galtsoff. A more probable suggestion is that when partial closing does not occur from any other cause, it may be a relief contraction effected by the motor component to reduce fatigue in the catch component of the muscle. If partial closing occurs from whatever stimulus, fatigue is probably automatically relieved. The catch muscle is usually regarded as operating in the closed condition. Its major work is, however, that of maintaining the shell in a variable but fixed open condition, since oysters are open to some extent the greater part of their life. Biologically, therefore, the function of this muscle is mainly that of position fixing. There is the objection to this suggestion regarding fatigue that so far no evidence could be obtained by either Parnas[6] or Bethe[6] of work done by the position-fixing muscle, but it is perhaps more reasonable to doubt the evidence than the work done, for some minimum quantity of energy is required throughout the life of almost all bivalves to hold the shell against the maximum opening pull of the ever-operating hinge ligament, and it is probable that in no bivalve does the shell ever gape so widely in life as in death. Anaerobic respiration occurs in certain bivalves,[7] the European oyster[8] and without doubt also in the American oyster (see 9, Table 3), so that small variations in metabolism are intrinsically difficult to detect; nevertheless, further investigations on this subject by the ingenious experimental methods used especially by American workers may be expected to yield critical information.

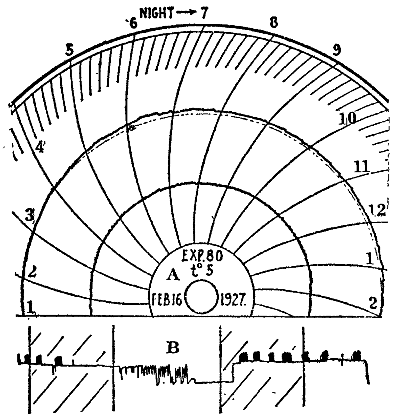

FIG. 1. A. Records by Galtsoff of shell-movements of *O. virginica;* B. Records by Marshall Webb of shell-movements of *O. edulis.*

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ON THE SITE OF ACTION OF ACETYLCHOLINE AND ITS SIGNIFICANCE

SINCE acetylcholine is closely identified with the cholinergic agent elaborated during autonomic nerve function, its properties assume particular importance and its site of action especial significance. It has been commonly assumed, though never completely demonstrated, that acetylcholine acts directly on the peripheral tissues innervated by parasympathetic nerves.

The current assumption concerning the direct action of acetylcholine has recently been questioned by Armstrong,[1] who has made the interesting observation that when the embryo fundulus heart is aneural its threshold for acetylcholine is higher than physiological limits. Moreover, when functional innervation of the heart does occur, the heart then responds to minute amounts of acetylcholine. From this it was concluded

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[5] J. H. Orton, *Jour. Mar. Biol. Assoc.,* 9: 1, 1913, Plymouth.

[6] See Bayliss, "Principles of General Physiology," p. 538, 1927.

[7] J. B. Collip, *Jour. Biol. Chem.,* 49: 2, 297, 1921, and bibliography.

[8] J. H. Orton, *Fishery Investig.,* England, II, Vol. 6, 3, p. 65, 1924.

[9] P. S. Galtsoff and D. V. Whipple, *Bull. Bur. Fish.,* U. S. A., Doc. No. 1094, 1931.

[1] P. B. Armstrong, *Journal of Physiology,* 84: 20, 1935.

that acetylcholine exerts its effect on the parasympathetic ganglia or the post-ganglionic nerves and not on the cardiac muscle itself.

Unfortunately, in the adult heart it is impossible to remove the outlying parasympathetic ganglia so that the Armstrong experiment can not be repeated with adult tissue. The mammalian eye, however, is suitable for the investigation of this question, since the parasympathetic ganglion (ciliary) can be readily removed. Engelhart[2] has shown that the iris in the cat contains a cholinergic substance resembling acetylcholine and that oculo-motor nerve stimulation increases the cholinergic substance within the iris. Constriction of the pupils simultaneously with an increase in cholinergic substance suggests that the two changes are related.

Hence, in an attempt to demonstrate the actual site of action of acetylcholine in the eye, the following experiments on cats were done. The ciliary ganglion was removed and subsequently identified by histological examination. In four animals immediately after the removal of the ciliary ganglion a slit was made in the margin of the cornea and some of the fluid of the anterior chamber allowed to escape. This usually caused the dilated pupil to become slightly narrower. When equilibrium had been reached a needle was inserted in the anterior chamber and 0.1 cc 1/1,000,000 solution (0.1 microgram) of acetylcholine bromide solution was injected. Constriction of the pupil occurred within 15 to 30 seconds and was complete within a minute. As a control procedure 0.1 cc of Ringer solution was injected several times. The Ringer solution and needle produced slight or no effect on the size of the pupil. These experiments demonstrate that acetylcholine is effective in the eye when directly applied to the iris in the absence of the ciliary ganglion.

To ascertain whether the constriction of the pupil was due to the action of acetylcholine on the radial muscle or the parasympathetic nerve endings the post-ganglionic parasympathetic nerve fibers were allowed to degenerate following ciliary ganglion removal in three additional cats. The pupil of the unoperated side served as a control. After 6 to 12 days the dilated pupil on the deganglionated side showed no constriction after conjunctival instillations of 1 per cent. physostigmine solution, and the above-described procedure of slitting the cornea, controlling with Ringer solution, and injecting 0.1 microgram acetylcholine into the anterior chamber of the eye, was repeated. Construction of the pupil was again prompt and complete, except where it was partially impeded by connective tissue adhesions. After atropinization, acetylcholine no longer had any effect. In one cat the eye was removed and the iris studied *in vitro*. Though of a similar nature, the results were less satisfactory than those of the *in vivo* experiments.

These experiments demonstrate that, at least in so far as the iris of the cat is concerned, the action of acetylcholine is peripheral to the postganglionic fibers, and presumably a direct one on the radial muscles. This is of interest in relation to the experiments of Armstrong indicating that embryonic muscle does not acquire its sensitiveness to acetylcholine until after cholinergic nerves have reached it. Generalizing from both types of experiment, it would appear that the physiological properties of the muscle (adrenergic or cholinergic) are bestowed upon it by the proximity of specific nerve fibers and that, once acquired, the specific sensitiveness is retained, at least for a time, after degeneration of the nerve fibers. Since it is probable that many smooth muscle fibers do not receive direct nerve connections, it is necessary to assume that the nerve has a sphere of influence in functional differentiation which extends beyond its terminations. This influence may be analogous to the effect of neighboring cells on structural differentiation, of which there are many examples in experimental embryology.[3]

These preliminary observations permit of no conclusion as to whether the response to acetylcholine is retained permanently in the absence of the parasympathetic nerve supply.

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PIGMENTATION IN BLACK-HAIRED RATS[1]

IT is a known fact that when young black rats of the hooded variety are put on a sole diet of whole milk the black hairs become gray.[2] However, I have observed with all-black rats that this does not occur simultaneously all over the body of the animal but in a certain sequence. At the end of the third week of the milk diet the graying of hair first becomes noticeable on the front legs (outer parts), shoulders, around the eyes and around the nose. Next the hair on the belly, the lower part of neck, around the mouth, on the inner and outer parts of all four legs begins to

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[2] E. Engelhart, *Plüger's Archiv*, 227: 220, 1931.

[3] See H. Spemann and Hilde Mangold, *Arch. f. Entw-mech.*, 100: 599, 1923–24; C. L. Yntema, *Journal of Experimental Zoology*, 65: 317, 1933.

[1] Paper Number 311 of the Miscellaneous Journal Series of the Minnesota Agricultural Experiment Station.

[2] Bakke (*La Medicina Italiana*, 9: 574–576, 1930) observed that hairs of black rats which were fed basal diet and his vitamin B concentrate ("prodotto X") turned silver-gray. Feeding whole grain to the rats restored the coloration back to normal.