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. Entwmech.*, 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.

change color together with a wide strip of hair on the back, leaving black hair only on the top of the head and on the area around the base of the tail. Finally the last-mentioned parts also become gray.

If such rats are returned to the stock diet their hair generally regains the original coloration. First the skin under the gray hair becomes blue-black and then a deposition of the pigment in the hair takes place, accompanied by a gradual disappearance of the blue-black color of the skin which becomes white again. Nevertheless, the tip and the lower third part of the hair remain gray for a certain period of time. In all cases the dark coloration of the skin precedes the darkening of the hair. (This is in line with Saccardi's histological studies on production of pigment in rabbits.)[3] The hairs become dark in the same sequence as they turned gray. First lower neck, portion around eyes, belly and outer parts of the extremities, then the whole back and finally the top of the head and the region around the tail become black. In rats whose diet was changed when blackish spots still remained on the top of the head and on the area around the base of the tail the color in these places was intensified simultaneously with its restoration at the parts where it normally appeared first. These changes were found very easy to follow when rats were made hairless by a diet consisting of one part by weight of honey and one part by volume of milk, although in this case the sequence was somewhat changed.

In order to ascertain whether the hemoglobin level bears any relation to the darkening of the skin, hemoglobin reading was taken simultaneously with the first appearance of the skin pigmentation. The coloration of the skin of a rat which had 8 gms of hemoglobin per 100 cc of blood appeared two days after the rat was put on the stock diet. A rat which had 3.4 gms of hemoglobin per 100 cc of blood showed first signs of darkening six days later when the hemoglobin reading was equal to 7.5 gm per 100 cc. One may consider such a coincidence at least suggestive, if not significant, since iron was found in melanin compounds.[4] Pough[5] discovered that ferrous salts in small quantity hasten the production of melanine, and Cohen and Elvehjem[6] demonstrated the importance of copper in an increase of the oxidase test of the liver tissue of anemic rats.

Then the question arises as to why these changes do not occur simultaneously over all the body but are localized to certain areas, anterior parts being, it appears at least, preferential. In rats, the young are born white (red), no matter whether the parents are black or white. Pigmentation appears a few days later and starts on the anterior and lateral parts of the body, so far as the writer noticed. One is tempted to make the suggestion that this sequence in repigmentation is just a phylogenetic factor.

Not only does the hair of rats put on a milk diet become gray, but the front teeth become white as well. This is noticeable first at the end of the third week of the milk diet. The brown color of incisors begins to disappear from the base of the teeth and at the end of the fifth week of the milk diet they are usually completely white or only a small brown spot can be seen on the distal parts of the incisors. When such rats are put back on the stock diet their incisors become brown again in a 5 to 6 weeks' period. The brown color appears at the base and proceeds distally, which signifies that the pigment is deposited at the roots of the teeth.

In order to find out whether iron and copper (directly or indirectly) are responsible for the changes in the color of teeth of anemic rats a pair of such rats was changed to the iron-copper-milk diet. Unfortunately one of them died on the second day. The incisors of the other rat became normally brown nine weeks after the change of the diet. One may conclude that this experiment is suggestive enough to warrant further investigations.

It is interesting to note that when teeth first appear in a new-born rat, they are white and only later gradually become brown. It is possible that the brown pigment of incisors in Rodentia has something to do with the strength of these teeth in that family.

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[3] *Biochem. Ztschr.*, 132: 443–456, 1922.

[4] H. Wealsch, *Ztschr. Physiol. Chem.*, 213: 35–57, 1932.

[5] *Biochem. Jour.*, 26: 106–117, 1932.

[6] *Jour. Biol. Chem.*, 107: 97–105, 1934.

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