

genetic. Here again the compensatory process frequently overshoots and produces a hyperglycemia in the sense that the blood sugar rises temporarily above the fasting level. This does not happen in all animals. In our experiments, the hypoglycemia which occurs following the administration of glucose elicited hyperglycemia in 11 out of 27 normal, and in 9 out of 21 hypophysectomized dogs. Insulin-hypoglycemia was followed by hyperglycemia in 16 out of 24 normal, and in 9 out of 21 hypophysectomized animals. The extent of the hyperglycemia shows considerable individual variations; in a few cases it represents a rise of no more than about 5 mg. % above the fasting level.

The question may be raised as to whether such apparently trivial differences are sufficiently beyond the limits of analytical errors as to deserve consideration. The analytical technique employed in this work warrants an answer in the affirmative. True sugar values were determined by the copper-iodometric method with the Shaffer-Somogyi reagent No. 50⁶ in filtrates prepared by Somogyi's copper precipitation method.⁷ This procedure, in the hands of various workers in this laboratory, yields duplicate values which, as a rule, check within 1 mg. %; the maximum discrepancy between duplicate determinations is 2 mg. %.

Thus if blood sugar values showed differences of more than 1 to 2 mg. %, these were safely beyond the limits of experimental errors and represented actual changes in the blood sugar level.

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Development of Autonomic Innervation Correlated with Reactivity of the Fetal Pig Iris.

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The results of recent studies of the responses of developing organs to certain choline esters have suggested an important rôle of the nerves in the initiation and progressive development of these reactions. Armstrong¹ has shown that when the embryonic Fundulus heart is differentiated to the adult morphology but is, as yet, without

⁶ Shaffer, P. A., and Somogyi, M., *J. Biol. Chem.*, 1933, **100**, 695.

⁷ Somogyi, M., *J. Biol. Chem.*, 1931, **90**, 725.

¹ Armstrong, P. B., *J. Physiol.*, 1935, **84**, 20.

nerves, injections of large amounts of acetylcholine do not inhibit contractility. After the heart has become morphologically innervated, the injection of a smaller amount of the choline compound produces a definite vagomimetic effect, as shown at first by diastolic arrest of the auricle and later by diastolic arrest of both the auricle and the ventricle. Armstrong has suggested that acetylcholine probably produces its vagus effect by stimulating the vagus postganglionic fibers and that it is effective before the intramyocardial synapses are functional. In a study, the results of which have been reported, we² observed that instillation of carbaminoyl choline chloride on the eye of the fetal pig kept at room temperature elicited no constriction of the iris or only a slight constriction up to a certain stage of development. After that, the response became more marked for a certain period of development, until a maximum level of constriction was reached. It was suggested that probably a choline-labile receptive substance was beginning to form through contact of nerves with muscles in fetuses when little or no constriction was observed, and that this receptive substance might be one of several structures which develop gradually during the period of increase in the constriction observed. It had been planned² to determine by histological studies what relation the beginning of drug action has to the development of the parasympathetic nerves and the sphincter muscle; especially, what relation the response of the receptive substance to choline esters has to the differentiation of the nerve terminations.

In most fetuses less than 100 mm. in length, no evidence of muscle histogenesis is to be observed. In fetuses just over 100 mm. in length, myoblasts of the sphincter muscle appear in the connective tissue at the pupillary margin of the iris just external to the pars iridica of the retina, where these myoblasts originate. In all fetuses 125 mm. in length a definite muscle mass has been formed. Differentiation progresses until in fetuses 175 to 200 mm. in length a thin but definite sphincter muscle is present in which the muscle cells appear completely formed. In fetuses 200 to 300 mm. in length growth in the individual muscle fibers and in the sphincter muscle, itself, is the most marked change.

Nerves are present early in the embryonic iris. Kuntz³ has found that the ciliary ganglion, from which parasympathetic fibers to the iris have their origin, is well developed in pig embryos of 15 to 20 mm. and that many of its constituent cells have become differ-

² Shaklee, A. O., Christensen, K., and Oppenheimer, H. E., *PROC. SOC. EXP. BIOL. AND MED.*, 1936, **34**, 225.

³ Kuntz, A., *J. Comp. Neur.*, 1913, **23**, 71.

entiated into neuroblasts. The reactivity of the fetal iris to the carbaminoyl choline chloride² and the histological picture of the innervation of the iris in fetuses of the same length is indicated in the following summary.

Fetuses 60 to 120 mm. in length. In the younger individuals of this group, the iris usually does not constrict; in the older individuals it constricts only slightly (about 3%). In the irises of fetuses 70 mm. or over in length which have been studied, the nerves are grouped at the periphery of the iris into bundles forming arcs which correspond to the major arterial circle. From these peripheral nerves, branches are given off radially which, especially in the younger fetuses, appear to accompany vessels toward the center of the iris. Smaller branches or individual fibers arising from these radial nerves reach the undifferentiated connective tissues where they form loose networks or accompany capillaries into the zone where histogenesis of the sphincter muscle occurs.

Fetuses 120 to 220 mm. in length. In this group the constriction of the iris gradually increases from about 3% to the maximum obtained, about 70%. The nerve development far beyond that observed in the preceding group which may be designated as the complete primordial development of the iris innervation. In the oldest fetuses of this group the innervation of the iris resembles that of the adult except as to quantity. In these fetuses simple branchings of slightly varicose fibers are found in contact with the sphincter muscle on its external border and scattered ramifying fibers appear within the muscle. Some of the fibers appear to end in beadlike terminations.

Fetuses 220 to 300 mm. in length (to the end of the period of gestation). Although the percentage of constriction is lower in some fetuses, 70% represents the maximum constriction found for the fetuses of this group that were tested. The branching fibers of nerves within the muscle become somewhat more abundant during the final stages of fetal differentiation. In particular, the nerve terminations become differentiated as in the adult with small loops or knoblike enlargements along their courses. The absence of a denser network of terminal endings in the late fetuses indicates that differentiation of terminal endings is not complete at birth. Many sphincter muscle fibers apparently have no direct contact with the terminal neural structure.

Summary. The microscopic study of the developing nerves of the iris indicates a very intimate relationship between the nerves and the differentiating sphincter muscle. The exact relationship be-

tween the initial innervation and the choline ester response has not been determined. More intimate morphological details of what occurs when the nerves make contact with the muscle fibers remain to be worked out. However, these findings suggest that the receptive substance may develop only after the nerves join the muscle fibers.

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Differential Survival in Isolated Strips of Frog Intestine.

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As part of the problem of survival of isolated tissue it became desirable to determine what elements within the tissue itself are responsible for the failure of spontaneous activity. Data are presented here correlating the physiological activity as measured by spontaneous movements with the histological picture in surviving strips of small intestine taken from the common leopard frog, *Rana pipiens* Shreber. In all, 50 frogs were used and over 250 series of activity records taken.

Segments one to 2 cm. in length, either intact or split lengthwise along the greatest curvature, were cut from the upper portion of the small intestine and transferred to shallow glass dishes containing 5 cc. of Ringer's fluid, buffered to pH 7.4-7.5 with carbonates and phosphates. No bacteriostatic substance was used, but the Ringer's fluid was changed twice daily. The strips were held at 5°C. except for an 80-minute period each day when each strip was mounted, by means of threads attached at the time of its removal from the frog, in a warm chamber filled with Ringer's fluid at 37.5°C. During this 80-minute period a continuous record of all spontaneous movements of the longitudinal muscle fibers was taken on an electric kymograph.

Frog intestine under such conditions survived for long periods as compared with the reported survivals of other smooth muscle preparations. One group of 16 frogs strictly comparable in all details shows the range of survival found in these tests, the minimal survival being 143 hours, the average 180 hours, the maximal 239 hours. Thirty-one percent of the survivals exceeded 200 hours. The maximal survival as indicated by the spontaneous activity of