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On the Nature of the First Forelimb Movements of Mammalian Embryos.

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Somatic movements occurring in response to faradic stimulation were studied in cat embryos delivered by experimental Caesarian section. The cats were decerebrated by ligating carotid and basilar arteries, after which one to 3 hours were allowed for recovery from ether anesthesia. Embryos were delivered as promptly as possible and examined while their placental circulation was intact and before uterine contractions had caused placental separation. All experimentation was carried out in a bath of physiologic saline solution maintained at the temperature of the cat's body. The combined diameter of the nichrome-wire stimulating electrodes was less than one millimeter and they were insulated except at their tips. Strength of current was not measured but could be borne upon the tongue. At the conclusion of physiologic studies, the embryos were prepared for histologic examination by the pyridine-silver technique.

It was seldom possible to obtain reflex movements in embryos less than 14 mm. long. But when the electrodes were placed directly over developing muscle masses of the shoulder region contractions were elicited with perfect regularity. Motor and sensory spinal nerves are well formed at this stage; free nerve endings appear beneath the epithelium of the forelimb and a few primitive endings are present upon the myoblasts of the shoulder muscles. Within the spinal cord, motor and associational mechanisms are well represented but morphologic reflex arcs are incomplete because the sensory dorsal funiculus collaterals are just forming and have made no connections with other neurons.

In embryos of about 23 days' insemination age (14 mm.), the sensory nerves have grown down the dorsal and volar aspects of the forelimb to within half a millimeter of its most distal point. There they end in free nerve endings immediately beneath the epithelium. There is little muscle formation distal to the shoulder but in the shoulder muscles many free and grapelike endings were observed upon the myoblasts. In the spinal cord, unisegmental structural reflex arcs have been completed by the growth of sensory collaterals into the groups of associational neurons of the dorsal gray matter and

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even to the motor neurons which supply the forelimbs. When, in these embryos, the points upon the hand reached by the new sensory nerve fibers were stimulated with the wire-electrodes a quick outward movement at the shoulder sometimes followed. This movement was not accompanied by any others, although a local extension of the head separately could sometimes be obtained by stimulating the nose region in a similar manner. Stimulation of points upon the body not yet reached by sensory nerves or at levels whose spinal cord segments contained less mature structural reflex arcs never resulted in movements of this type.

The responses in question were distinctly different from those obtained by direct stimulation of muscle masses. They exhibited fatigue to a degree not seen with direct stimulation, a just perceptible latent period following the stimulus was often seen, and they were definitely stereotyped and invariable reactions. Furthermore and of primary importance, they could be obtained only in the first few minutes of the experiment before the uterus had contracted upon the placenta. Contractions due to direct muscle stimulation occurred in dead embryos; but even in mildly depressed living specimens or in those injured slightly during delivery no movements occurred in response to localized stimulation of receptor endings. If spread of current were the cause, all should have shown the phenomenon. There can be no doubt that the movement in question is a true reflex of the simplest type involving afferent conductor, center, efferent conductor and effector.

By 26 days (18-20 mm.), a variety of reflex responses to faradic stimulation could be obtained. Stimulation of the tip of one paw often produced local abduction of the ipsilateral forelimb at the shoulder with or without flexion at the elbow, it sometimes produced contralateral forelimb reflexes, and frequently movements of the head. Stimulation of the dorsal aspect of one hand was followed by an ipsilateral forelimb reflex and ipsilateral head flexion; stimulation of the volar aspect caused contralateral head flexion. Reflexes always involved muscles at the same level or at levels rostral to that stimulated, but not caudally. These physiologic observations are in harmony with what is known concerning the histologic structure of embryos at this time. It has been shown[1] that the early fiber tracts of the embryonic spinal cord pass rostrad, and only later is there a rostral to caudal integration of structural reflex patterns. It has further been shown that the first structural reflex arcs are unisegmental, involving sensory collaterals from the lateral part of the

[1] Windle, W. F., *J. Comp. Neurol.*, 1933, **58**, 643; 1934, **59**, 487.

dorsal funiculus which terminate at approximately the level at which their dorsal roots enter the cord. In the 18 mm. embryos, the ascending branches of the dorsal root fibers give off collaterals farther rostrad which make contact with commissural neurons whose discharge would excite motor cells supplying the neck muscles.

Barcroft and Barron[2] have reported some experiments in sheep fetuses in which they dissected the median nerve and the lateral popliteal nerve, severed them, and stimulated central and peripheral ends. They found that no movements followed stimulation of the central end of the median nerve in sheep younger than 44 days old and none followed similar stimulation of the lateral popliteal before 47 days. They concluded, therefore, that the primary afferent neurons distributed to the limbs have not made functional connections with the anterior horn cells prior to these times and that any movements seen earlier are not true reflexes. It would seem that negative evidence obtained after mutilating operations upon such delicate forms as sheep embryos younger than 44 days old cannot serve as sound basis for the conclusions they have drawn. The tissues of sheep embryos 38 days (31-32 mm.) to 45 days old (57 mm.) are very susceptible to injury. Even if it were possible to keep the uterus from contracting upon the placental cotyledons (and setting up a state of partial anoxemia) for the time necessary to complete a dissection of the nerves in question, and even if such dissections could be made without loss of a significant percentage of the small total blood volume of the fetuses, the trauma involved in the procedures would certainly be such as to render the nervous system of the specimens no longer irritable to faradic stimulation.

It may be concluded from the experiments in cat embryos that early forelimb movements in response to faradic stimulation of the epithelium over newly formed sensory nerve endings of the hand are true spinal type reflexes. These reflexes are not part of a total mass reaction pattern when they first appear. But with progressive development of central reflex mechanisms more complex and integrated reflexes become elicitable.

[2] Barcroft, J., and Barron, D. H., PROC. SOC. EXP. BIOL. AND MED., 1937, 36, 86.