of the terminal lesion, which is aortic rhexis. In fact, lowering of systolic blood pressure in 2 weeks by 50 mm of Hg in turkeys treated initially at 17 weeks of age might be the reason that older turkeys were essentially refractive to DES-induced aortic ruptures. However, this explanation is not completely convincing because in Exp. 2 more females died which were injected first with DES at 6 weeks than did females which were treated initially at 8 weeks (Tables I, II). Yet, the systolic blood pressures of untreated 8 weeks old females were 200 mm Hg as contrasted to 170 mm Hg for 6 weeks old control females.

Summary. Male and female turkeys were first injected with DES at 6, 8, 10 and 17 weeks of age. A high incidence of aortic ruptures occurred in all birds except those which were injected initially at 17 weeks of age, and these were essentially refractory to the disease. Total serum cholesterol was elevated and systolic blood pressure was lowered by all treatments.

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Convulsive Responses in Developing Chickens.* (30223)

ANTONIA VERNADAKIS AND ALAN BURKHALTER
(Introduced by Robert M. Featherstone)
Department of Pharmacology, University of California School of Medicine, San Francisco

Functional development of the nervous system of the chicken has been studied in a number of ways. For example, electroencephalographic activity has been recorded in chick embryos and was found to increase gradually in amplitude from the 15th day of incubation to hatching time(1,2). Castillo and Vizoso(3) reported that action potentials can be recorded on stimulation of the sciatic nerve in 9-day-old chick embryos. The conduction velocity was low on the 10th day of incubation and progressively increased to hatching time.

The present investigation was designed to study further the functional development of the chick embryo central nervous system (CNS) by observing maximal electroshock seizures (MES) and spinal cord convulsions.

Materials and methods. The chick embryos and chicks used in this study were from eggs of White Leghorn chickens. Spinal cord convulsions were produced by direct stimulation of the spinal cord. Square-wave, supramaximal (30V, 1 msec pulse duration) stimuli were delivered by a Grass stimulator. The technic of spinal cord stimulation was that described by Esplin and Freston(4) and modified by Vernadakis(5).

Maximal electroshock seizures were produced by a stimulator delivering a sine wave current of 60 cycles/sec for 0.2 second through corneal electrodes. The apparatus and techniques have been described in detail by Woodbury and Davenport(6). Stimulus intensities from 50 to 300 milliamperes (mA) were employed. Chick embryos younger than 14 days of incubation could not be stimulated since the embryos were too small to handle.

Results. Durations of leg flexion for 14-

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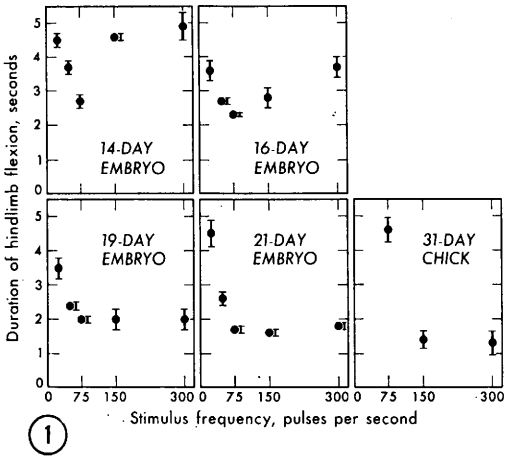

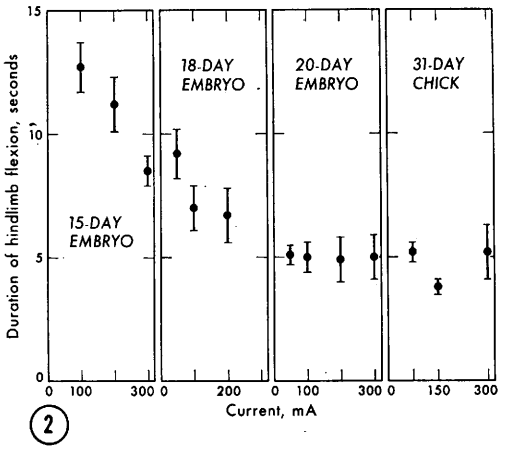

FIG. 1. Spinal cord convulsions. Duration of leg tonic flexion at various frequencies of spinal cord stimulation in developing chickens. Points with bracketed lines represent means and standard errors. Each mean is based on 4 to 8 animals.

FIG. 2. Maximal electroshock seizures. Duration of leg tonic flexion at various current intensities of brain stimulation in developing chickens. Points with bracketed lines represent means and standard errors. Each mean is based on 4 to 8 animals.

to 21-day-old chick embryos and for 31-day-old chicks at various frequencies of spinal cord stimulation are shown in Fig. 1. After termination of leg flexion, extension occurred and continued for a variable period. The flexion-extension sequence was present at all frequencies employed. The curves obtained for the duration of flexion as a function of frequency were U-shaped in embryos 14 and 16 days old. The duration of flexion did not change significantly with higher stimulus frequencies in 19- and 21-day-old embryos, and 31-day-old chicks. The briefest flexion was produced with 75 pulses per second in 14- and 16-day-old chick embryos.

Durations of leg flexion for 15- to 20-day-old chick embryos and for 31-day-old chicks at various current intensities of brain stimulation are illustrated in Fig. 2. At each stimulus intensity the duration of flexion decreased with age. Flexion was almost always followed by extension. Termination of extension, however, was variable. Chick embryos and chicks that exhibited flexion but failed to exhibit extension were not included in the data.

Discussion. Spinal cord convulsions and maximal electroshock seizures were studied in chick embryos and chicks after hatching. The duration of flexion was used as a measure of seizure intensity. It has been shown that for both maximal electroshock seizures(7) and spinal cord convulsions(4), the shorter the duration of flexion the more severe is the convulsion. In both tests used, the degree of response was age dependent. In spinal cord convulsions, changes in duration of flexion as a function of frequency of stimulation (Fig. 1) were observed at all age periods. With high-frequency stimulation the duration of flexion decreased with age, indicating an increase in intensity of convulsions as the animals mature. At early embryonic development, U-shaped curves were obtained with increasing frequency. The U-shaped curve was most prominent in 14-day-old chick embryos. In studies(5) on spinal cord convulsions in developing rats it was found that the U-shaped curve for duration of flexion as a function of frequency of stimulation is most pominent in 1-day-old rats. This U-shaped curve may suggest that the efficiency of high-frequency stimulation is low during early development of the spinal cord when many nerve fibers are not completely myelinated.

Although electroshock flexor-extensor convulsions were elicited in chicks of all ages studied, the duration of flexion decreased with age indicating that the intensity of the seizure progressively increased with age (Fig. 2). Also it was observed that in the younger

embryos the duration of flexion decreased with increasing current intensity whereas in older embryos and chicks it did not change. This suggests that a higher intensity of current is required to activate immature neuronal pathways.

It can be concluded from the present data that the intensity of both spinal cord convulsions and maximal electroshock seizures increased with development of the CNS. The degree of myelination of the neuronal pathways may play an important role in the intensity of seizure. It is known that myelination confers upon a nerve fiber the properties of lowered threshold, increased conduction velocity, and greater ability to carry repetitive impulses. Studies of myelination in the spinal cord of the chick embryos have shown that myelin sheaths appear in small numbers in the anterior column the 12th day of incubation(8). From the 15th day onward, myelination proceeds actively, spreading from the anterior columns laterally and posteriorly. Studies of myelination in higher centers of the chick CNS have not been reported.

The question as to whether seizure intensity may also depend on the development of neuromuscular elements can be answered partly by pharmacological studies by Kuo(9) and cytochemical studies by Drachman(10). Kuo reported that spontaneous contractions of skeletal muscle were observed as early as the 4th day of incubation in chick embryos. Such movements could be blocked by curare or curare-like agents. These drugs are thought to act at the level of the receptor substance of the neuromuscular junction. In histological studies by Drachman the earliest cholinesterase-containing end-plates were seen in the paraspinal muscles of 10-day-old chick embryos and were well developed in this region by 12 days. In the distal muscles of the leg, end-plates were seen at 12-13 days, and were well developed by 14 days. Localized cholinesterase activity represents an early response of the embryonic muscle membrane to innervation(11).

Thus it appears that in the chicken as in the rat the increase in seizure intensity with age is predominantly due to the development of the chick CNS.

Summary. Spinal cord convulsions elicited by direct spinal cord stimulation and maximal electroshock seizures produced by brain stimulation were studied in 14- to 21-day-old chick embryos and in 31-day-old chicks. In spinal cord convulsions, with high frequency stimulation the duration of leg flexion decreased with age. The curves obtained for the duration of leg flexion as a function of frequency were U-shaped in 14- and 16-day-old embryos. The duration of flexion did not change with higher stimulus frequencies in 19- and 21-day-old embryos, and 31-day-old chicks. In maximal electroshock seizures the duration of flexion decreased with age at each stimulus intensity. The decrease in the duration of flexion with age indicates an increase in the severity of convulsion. It is concluded that the particular pattern and intensity of seizure is related to CNS development and may be influenced by the degree of myelination of the neuronal pathways involved in seizure responses.

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