Development of Electrical Activity in Cerebral Hemispheres of the Chick Embryo.* (21095)

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Several authors have studied the EEG during both pre- and post-natal growth in rabbits (1-4), cats(5), guinea pigs(6,7) albino rats (8) and human foetus(9). The chick embryo is excellent material for recording and analyzing development of the electroencephalogram (EEG) as well as for comparison with morphologic, histochemical and other data obtained from study of cerebral hemispheres. The fairly simple structure of its nervous system, the uniformity in development, the possibility of determining exact age and facilities for using this animal for research have greatly helped in conducting this work.

Material and method. Seventy-three Rhode Island Red chick embryos varying in age from 9 to 20 days of incubation were used. Egg shell and membranes were pierced over the air chamber and the head made to protrude through the opening. Until the 14th day proper fixation of head is enough to obtain the EEG. Thereafter the embryo was immobilized with curare (d-tubo-curarine chloride, 0.3-0.6 mg) in order to avoid recording artifacts due to muscular activity. The dorsal aspect of one or both hemispheres was uncovered by removing the parietal cartilage, the dura mater, and 2 to 6 electrodes consisting of Ag-AgCl-cotton wool-Ringer's solution were placed thereon. In some of our experiments an Ag-AgCl wire was placed in the beak as a reference electrode. Bipolar leads from the cerebral electrodes and/or "unipolar" leads with the common reference electrode were used. The *cerebral electrodes* cover the dorsal part of the hemispheres.† This wide zone includes, towards the rostral region and dorsomedially, the nucleus diffusus dorsalis and dorsolateralis, and dorsolaterally the thin lateral corticoid area L2. Caudally it includes the cortical areas, field d and field a + b + c. Two silver electrodes were introduced into the egg on either side of the body in order to record the electrocardiogram (EKG) and the artifacts due to movements of the embryo. A 2 beam cathode oscillograph with 4 stages for direct coupled amplification, a Grass 8 channel electroencephalograph and Offner 4 channel electroencephalograph were used. In the younger embryos high amplification ratios were used (up to 1 mm per microvolt). Embryos were placed at an oven at 38°C because room temperature exposure reduces EEG amplitude and frequency. Environment was generally kept saturated with moisture, as any drying of the brain considerably alters electrical activity, while electrode drying causes artifact. Under these conditions perfect vitality of the embryo can be kept for more than 24 hours as the EKG and EEG show. Strychnine activity was induced with strychnine sulfate crystals or with 1 mm square filter paper soaked in a saturated solution of this salt. In every case the strychnine was placed upon the dorsal aspect of the hemispheres near one of the electrodes. Metrazol was used with the same technic.

The following *controls* were made: a) electrodes fidelity was checked by joining them with a filter paper soaked in Ringer's solution and/or by making a record using the same amplification and position after the embryo death; b) movements causing serious artifacts were checked by means of the electrodes within the egg; and c) the vitality of the embryo by the EKG.

Results. 1. *"Spontaneous" electrical activity.* As the study of 64 embryos shows, "spontaneous" electrical activity appears on the 13th day of incubation. This activity is irregular with a mean frequency of 2.5 to 3 per sec., of low voltage (5-10 μv), sporadic, in short trains of waves, alternating with

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† Topographical references are according to Kuhlenbeck(10).

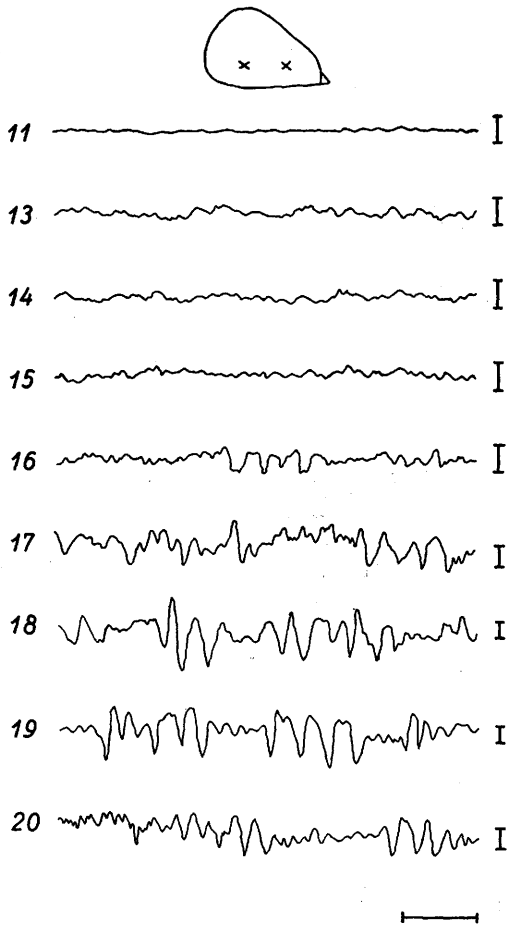

FIG. 1. *Beginning and development of the EEG in the chick embryo.* 11-20 days of incubation. Standardization: 50 microvolts. Time: one sec. In the upper part is shown the distribution of the electrodes upon the surface of the cerebral hemisphere; bipolar leads. 11: the small oscillations seen are produced by the apparatus. 13-15: "early" rhythm. 16: start of "late" rhythm. 17-20: further development of the "late" rhythm. For details, see text.

"silent" periods lasting several seconds. In no case was electrical activity observed before the 13th day (Fig. 1—11 and 13). EEG thus appears in relatively early stage of embryonic development the same as in guinea pigs(6,7), but unlike rabbits(2) and albino rats(8) where it appears later. On the 14th and 15th days there is little increase in frequency and voltage. The "silent" periods become continually shorter until, on the 15th day, they practically disappear (Fig. 1—14, 15). Low voltage and silent periods are also characteristic of EEG in early development phases in other animals(2,6-8) and in human foetus (11).

On the 16th day of incubation the pattern of the EEG changes considerably. High voltage wave trains, ranging from 20 to 100 μv, appear upon the preceding fundamental activity, showing a frequency of 3-4 per second. On the 16th day they are brief and sporadic. On the next 3 days (17th to 19th) they become progressively longer and more constant (Fig. 1—16-19). The appearance of other rhythm on the 16th day of incubation agrees with what is seen in the guinea pig and the rabbit in which at the end of fetal development more than one rhythm appears(2,6,7). In order to distinguish these 2 types of waves, we have given the name "early" rhythm to the low voltage waves appearing on the 13th day, and that of "late" rhythm to the slower high voltage waves observed from the 16th day. The main difference between these rhythms is the voltage. After the 16th day the "early" rhythm gradually increases in voltage and frequency until the 19th day. Meanwhile there is an increase in the number and duration of the "late" rhythm wave trains. The periods in which "early" rhythm is still visible becomes shorter. Voltage and frequency of "late" rhythm shows no significant changes. On the 20th day the EEG becomes dysrhythmic and the voltage decreases (Fig. 1—20). At this time there is considerable pulmonary respiration, and anoxia provoking embryo death in 1-2 hours is caused when curare stops respiratory movement.

At all ages electrical activity varies from place to place. The rhythms are not synchronous in the 2 hemispheres and voltages are different. In the dorsal aspect of the hemisphere the waves are more constant and of higher voltage rostrally.

Exposure of the brain of the embryo to a dry atmosphere for 15 to 30 minutes alters the EEG considerably inducing an epileptoid activity similar to that observed in adult mammals through drying of the brain(11). Random high voltage spikes appear at first, becoming more frequent with time. Subsequently regular discharges of spikes are seen, reaching a maximum and decreasing gradu-

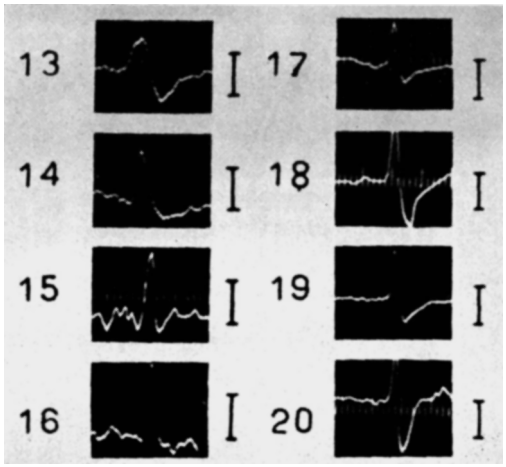

FIG. 2. *Development of strychnine activity in the chick embryo.* Incubation of 13-20 days. Standardization: 20 microvolts for 13-16 days, 50 for 17 days and 200 for 18-20 days. Time indicated in 15, 18 and 20, 50 millisec. (the same in all records). For explanations see text.

ally, while random spikes reappear. These discharges are repeated at intervals. This epileptoid activity may last as long as 24 hours without appreciable change. The 14th day is the earliest time at which this phenomenon has been observed.

Ischemia of the brain leads to the disappearance of electrical activity in 30 to 60 seconds, in some cases preceded by a transient increase in frequency and voltage, very marked at times and leading to a discharge of spikes.

2. *Electrical activity induced by strychnine.* In 45 embryos a study of the EEG changes due to local application of strychnine was made. The strychnine spikes appear on the 13th day, *i.e.*, at the same age that the "spontaneous" electrical activity(7). The same occurs in foetus of guinea pigs and in the newly born albino rat(8). The spikes are generally biphasic with a negative surface wave followed by a positive surface wave (Fig. 2). The negative wave is shorter than the positive, and is characteristic of these spikes, since it is always present and has approximately the same duration throughout a given experiment. In some cases the spikes are negative and single phased, polyphasic in others. The shape of the spikes is the same throughout a given experiment. The spikes are nearly always isolated, appearing at intervals, but occasionally may appear grouped together forming trains in which the frequency increases to a maximum and then decreases, constituting a spike discharge similar to that obtained by drying or ischemia. The spikes spread rapidly to the whole of the dorsal aspect of the homolateral hemisphere and to the opposite hemisphere. When the strychnine spike activity reaches a certain intensity, violent movements of the whole embryo ensue.

The strychnine activity varies considerably with embryo development. The duration of the spikes decreases as the embryo grows (Fig. 2) as in the case of the rabbit(3) and albino rat(8) in post-natal growth. The duration—age ratio curve of the daily averages is approximately exponential (Fig. 3). The duration of the negative wave is measured at the moment it crosses the isoelectric line, and the mean value is determined for at least 10 spikes. The extreme values found were 278 milliseconds on the 13th day and 51 milliseconds on the 20th. The amplitude of the spikes increases with age, although not so uniformly as the decrease in duration. The older the embryo, the greater the frequency of the spikes. On the 13th day there is usually an interval of over 5 minutes between spikes; on the 20th day there are 20-25 spikes per minute. This increase with age is not regular. The younger the embryo the greater the time that elapses between the local appli-

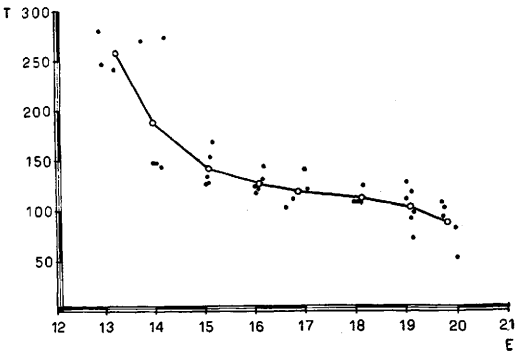

FIG. 3. *Strychnine activity in the chick embryo: duration-age ratio curve.* T: duration of negative phase of spike in millisec. E: days of incubation. Black dots: duration of the strychnine spikes in each experiment. White dots: general average duration on each day of incubation. For explanation see text.

cation of the strychnine and the appearance of the first spike, ranging from 50 minutes on the 13th day to 5 minutes on the 19th and 20th days.

The background electrical activity increased as a result of local application of strychnine in most of the experiments. Metrazol applied locally has the same qualitative effect as strychnine sulfate, but its action is weaker and not as constant.

Discussion. The "spontaneous" activity probably originates in the cell groups of the surface of the cerebral hemisphere. Bipolar leads from electrodes only 1-2 mm apart obviate the possibility of picking up the activity of deep structures. Besides, this "spontaneous" activity appears at the same time as the strychnine spikes, the origin of which has been shown in mammals to be in the superficial layers of the cortex(12). This fact is important, inasmuch as the rate of development of the deep nuclei is faster than that of the surface areas. The "spontaneous" electrical activity and that produced by drying are greatest in the dorso-rostral region. The diffuse dorsal and dorso-lateral nuclei are well developed at this level, consisting of 2 compact unstratified masses of cells, the forerunners of the neocortex of mammals(10). On the other hand, in the dorso-caudal region wherein the cortical areas a + b + c and d are already well developed, the electrical activity is not as great.

On the 12th day of incubation the nuclear differentiation of the telencephalon of the chick embryo can be regarded as completed. Later on, with the development of fibers, the centers acquire their ultimate structure and synaptic connections are established(10). It is precisely at this stage that the EEG appears and that the cells are enabled to discharge synchronously with strychnine stimulation. As the cells and their intercellular connections develop, "spontaneous" electrical activity becomes more evident and the capacity to synchronize increases. These facts agree with what has been found in other animals(3,7,8).

Various authors have established certain characteristics of the biochemical differentiations of the brain in the developing chick. The adenylpyrophosphatase (apyrase) activity of the brain in the chick embryo rises steadily from the 12th day of incubation on (13). The *ribonucleic acid* increases suddenly after the 13th day of incubation in the chick(14). Synthesis of phosphatides is especially marked from the 13th day of incubation on. The galactolipids increase suddenly between the 10th and the 13th day and the sphingomyelin appears between the 13th and the 16th day(15).

All these facts, ascertained by various investigators, have established that the electrical activity of the brain appears in the chick embryo at a critical time in the morphological and biochemical development of the cells. In opposition Nachmansohn(16) found that colinsterase increases suddenly just before hatching when, according to our experiments, electrical activity is already well developed.

Kuo(17) has analyzed various aspects of the physiological development of the embryo. From the 10th to the 14th day of incubation a considerable reduction in the massive movements of myogenic origin is observed and localized wriggling and jerking appear. This change in the patterns of the movements is very interesting as the EEG appears at this same stage, indicating a basic function of the central nervous system. From the 15th to the 18th day, rudimentary motor responses appear in answer to various sensorial stimuli (light, sound, rotation, and vibration). At this stage the EEG develops greatly and the "late" rhythm appears.

Summary and conclusions. 1. A study of the beginning and development of the EEG in 73 chick embryos is made. 2. "Spontaneous" electrical activity and that produced by strychnine appear simultaneously on the 13th day. 3. "Spontaneous" activity undergoes changes during the course of embryonic development. The "early" rhythm appearing on the 13th day gradually increases in frequency and voltage and becomes more constant. On the 16th day the high voltage "late" rhythm appears, becoming more constant on subsequent days. 4. Strychnine causes the appearance of spikes, which are generally biphasic, and increases the amplitude, frequency and constancy of the "spontaneous" activity in most experiments. 5.

With development of the embryo, the strychnine spikes become of shorter duration and higher voltage, pointing to a better synchronization of the neurones. Frequency increases and spikes begin to appear earlier as the embryo grows. 6. From the 14th day spike discharges are to be seen in the embryonic brain, similar to the epileptic discharges produced in man and animals by certain agents like anoxia, strychnine, metrazol, and drying. 7. The bioelectric development is compared with the histological, cytological, and biochemical development of the brain, and with the physiological activity of the embryo.

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Interaction of Heparin with Auto-Agglutinins in Idiopathic Acquired Hemolytic Anemia.* (21096)

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Heparin was long considered the physiological regulator of the mechanism of blood coagulation(1,2). Later findings demonstrated its influence on plasma components belonging to the immune system, namely complement(3,4), and iso-agglutinins(5,6). It was also found that the antibody titer of rabbits sensitized against bacterial antigens increased manyfold upon previous or simultaneous administration of heparin(6). Astrup presented evidence which seemed to disprove the theory of heparin as a physiological anticoagulant. He showed that in no instance where "the tendency of blood to coagulate was greatly increased," could a rise in the blood heparin level be observed. This fact, with the other biological relations of heparin, has led him to advance the hypothesis that the "primary function of heparin in the organism should be looked for in connection with immunological processes"(7,8). Owren observed that the *in vivo* destruction of red blood cells in a patient suffering from acquired hemolytic anemia was retarded by large doses of heparin(9). Because of an increased bleeding tendency, the experiment had to be stopped after a few days, and this withholding of heparin resulted in a severe rise in the rate of cell destruction. No further comment as to the reason for using heparin and its possible mechanism of action was given, appar-

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