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EEG Studies of Normal and Encephalomalacia Chicks.* (27807)

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Although review of the literature fails to show the use of EEG in the study of function or abnormalities of the chicken brain, we have used this technic for studying nutritional encephalomalacia, one of the vitamin E deficiency syndromes. This condition, as described by Pappenheimer, involves primarily the cerebellum and to some extent the medulla and cerebrum, sparing the optic lobes (1-3). We have compared EEG records of standard tracings, photostimulation and pentylenetetrazol-induced convulsions, using normal birds, and chicks with various stages of nutritional encephalomalacia.

Administration of diets deficient in Vit. E induces in chicks certain well known syndromes, *i.e.,* encephalomalacia, exudative diathesis and muscular degeneration. In former studies of vitamin deficiencies, each bird usually showed more than one of these syndromes. Recent experiments have made it possible to differentiate nutritional factors pertinent to each syndrome by modifying the diets and producing single syndromes in individual experimental chickens. For example, selenium protects against exudative diathesis(4). Linoleic or arachidonic acid is necessary for development of encephalomalacia(5). Selenium and antioxidant protect chicks from muscle degeneration when they are fed a low sulfur containing diet(6). Diets deficient in arginine protect chickens from myopathy when they are on myopathy-producing diets(7).

Chickens fed for 2 or 3 weeks on diets which produce encephalomalacia exhibit various clinical signs which include ataxia, uncontrollable bicycling movements, coma and death. At the time our tracings were made a variety of these signs were evident.

Material and methods. Nichol 108 cockerels were placed on encephalomalacia producing diets, with controls kept on basal diets. EEG tracings were made from birds of each group selected at random from ages 4 to 20 days. Suitable restraining containers eliminated the use of any sedating drugs during the procedure. Med-Art Subdermal electrodes, shaped like a hook were passed through a pinch of skin over either side of the head, electrode #1 on the left, #2 on the right. An indifferent electrode, #3 was inserted over the posterior head region. A Grass 8 channel EEG machine was utilized, recording 2 or 3 birds simultaneously. Paper speed and voltage are indicated in the figures. Grass model PS 1 photostimulator was mounted with light source 5 inches directly before the birds; light intensity was 16. Photostimulation with and without blindfolds, and at varying frequencies lasted for periods of 10 seconds.

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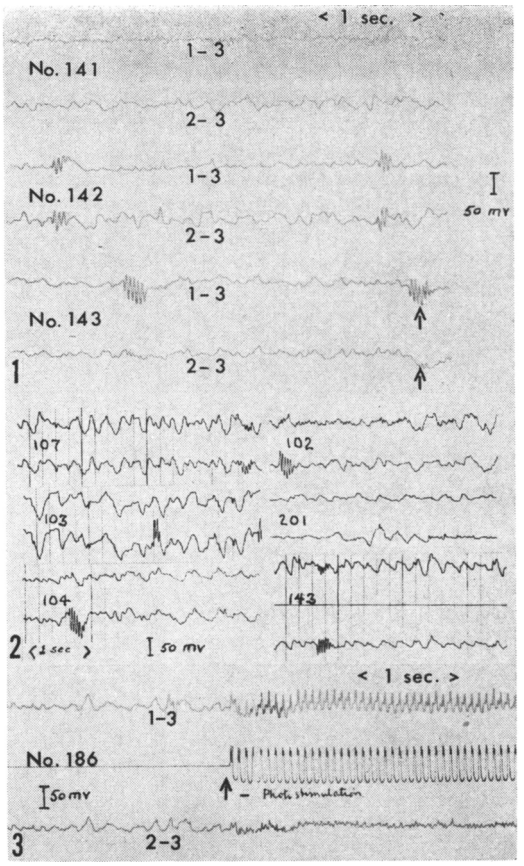

FIG. 1. EEG of 3 chickens age 14 days, on control diet. Arrows point to twitch trace produced by eye blink, swallowing or comb movement.

FIG. 2. EEG tracings of chickens with clinical signs of encephalomalacia. These tracings are from runs of abnormal slowing.

FIG. 3. Effect of photostimulation. Activation and driving of tracing when flicker rate is 14 cycles per sec. Control animal.

Following a 20-minute EEG recording under standard conditions, photostimulation reactions and responses to injections of pentylenetetrazol were recorded. Subcutaneous injections of the latter in the posterior cervical region, in doses diluted to 0.25-0.5 ml, required for optimal response, doses of at least 70 mg/kg. Above this dosage convulsions invariably occurred, and doses of more than 80 mg/kg were always fatal. Indices for the beginning of EEG activation were carefully determined by the presence of changes not produced by artifacts, or present in standard runs, and by eventual development of convulsions following the first changes in EEG patterns. Latent periods from time of injection to activation patterns were measured.

Results. 1) Character of tracings. A. *Normal chicks.* These manifested a low voltage record with predominance of 5-7 cycles per second (Fig. 1). Unlike the response in lambs(8), auditory stimuli did not activate the record. Eye blinking or comb movement periodically caused brief bursts of high voltage 30 cycle per second discharges. No other body movements were reflected in the tracings. Tracings from the 2 hemispheres varied in amplitude in some birds. B. *Encephalomalacia chicks.* Bursts of high voltage, excessive slowing to 3-4 cycles per second for periods of approximately one to 3 seconds, appeared erratically in the experimental chicks (Fig. 2); intervening record resembled the normal.

2) Photostimulation. All chicks were sensitive to flicker stimulation; the optimal rate was 14 per second (Fig. 3). Blindfolded chickens did not show this pattern, which immediately returned when the blindfold was removed.

3) Pentylenetetrazol convulsions. Tracings of both normal and encephalomalacia chicks were activated by a similar threshold dose of the drug, 70-80 mg/kg. The latent period of activation in experimental birds was twice that of the normal chicks; encephalomalacia 63-102 seconds; normal 30-49 seconds.

Discussion. While histological examination of the brains of chickens from which tracings were recorded have not been made, such studies from cage mates showed lesions most prominently in the white fiber tracts of the cerebellum, and to a lesser extent in the midbrain. Apparently they represent infarcts. Since the chicks are less than one month old, the possibility that tracings represent those of immature birds was resolved by examination of tracings in mature chicks, which are similar. Adding ethoxyquin to the disease producing diet prevents the development of lesions until the antioxidant is withheld for several days as shown by Machlin (9).

Activation by photostimulation could have been anticipated in both sets of chickens,

since the thin cortex and the optic lobes showed no gross lesions. Activation in this study served to demonstrate to us, that the tracings were in fact electroencephalograms and not artifacts.

The prolonged latent period for activation by pentylenetetrazol in the encephalomalacia chicks suggests the importance of midbrain structures and the reticular activating system in induction of convulsions. Further investigation is required.

The reported technics are useful in the study of certain vitamin deficiency states affecting the central nervous system, particularly since such states are readily produced in pigeons and chickens.

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Dietary Fatty Acids and Plasma Alkaline Phosphatase After Bile Duct Ligation.* (27808)

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It is known that the activity of the nonspecific alkaline phosphomonoesterase in plasma of experimental animals increases after bile duct ligation. Large increases have been described in dogs(1,2); smaller increases were observed in the cat(3) and rat (4). It will be shown here that the rise in alkaline phosphatase in the plasma of rat following bile duct ligation is contingent on the presence of unsaturated fatty acids in the diet.

In normal rats the level of the nonspecific alkaline phosphatase in the plasma depends to a considerable extent on the diet: after starvation or after feeding a fat-free diet the activity of the enzyme is low(5). Adding fat to the diet increases the activity of the alkaline phosphatase(6). Weil and Russell(5) found that among the lipid components of the food only unsaturated fatty acids are able to increase the enzyme activity.

Since it would appear that the increased level of the nonspecific alkaline phosphatase in plasma after bile duct ligation is of hepatic origin, the question arises if there is also some relation of the level of plasma alkaline phosphatase to the dietary fatty acids after ligation of the common bile duct.

Methods. The experimental animals were albino rats of Sprague-Dawley strain and dogs. Blood was drawn in rats by cardiac puncture, in dogs from the femoral vein. Ligation of the common bile duct proximal to the duodenum was done under ether anesthesia.

Alkaline phosphatase in plasma: Blood (0.1 ml) obtained by cardiac puncture was diluted with 0.9 ml of 0.15M sodium chloride, centrifuged and the supernatant removed for analysis. The method for determination of the enzyme and the reaction mixture have been described earlier by Huggins and Morii

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