sion of the symptoms of myasthenia gravis in a moderately ill woman after administration of the hormone was reported by Soffer and collaborators[20] without application of objective testing procedures. The transitory impairment observed during the period of administration of the hormone was also observed by Hellman.[21] The increased general disability during the administration of the hormone may be due to changes in electrolyte distribution,[25] and in various metabolic processes (increase of secretion of some steroid hormone,[22-26] adverse effect on carbohydrate metabolism[25] and decrease of glutathione content of blood.[22]) These processes apparently offset the gradually improving function of the neuromuscular apparatus *per se*.[19]

The above investigations indicate that administration of the adrenocorticotrophic hormone induces changes suggesting the beginnings of an incomplete remission in patients with myasthenia gravis. If the view suggested by this laboratory[1-3] be valid, *i.e.*, that the immediate cause of the symptoms of patients with myasthenia gravis is a decrease in the synthesis of acetylcholine, then the observation that administration of the hormone increases the synthesis of acetylcholine[4] becomes extremely pertinent to an understanding of the apparent remission of symptoms observed in patients with myasthenia gravis after administration of the adrenocorticotrophic hormone. Because of its long lasting effects, it is inferred that the adrenocorticotrophic hormone acts upon some basic regulatory mechanism.

Summary. 1. Five patients moderately to severely ill with myasthenia gravis received 400 mg adrenocorticotrophic hormone given in amounts of 20 mg every 6 hours.

2. Changes that may indicate the beginnings of an incomplete remission occurred after completion of the injections. These changes consisted of decrease of the symptoms and outward manifestations of muscle dysfunction, disappearance of the abnormalities noted in the electromyogram, increased work performance on the ergograph, and increase to normal of the ability of serum to support acetylcholine synthesis. The incomplete remission is long lasting.

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17214. Alkaline and Acid Phosphatase Activity of the Embryonic Chick Retina.

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Evidence has been accumulating which indicates that the phosphomonoesterases may be associated in some way with the differentiation of embryonic tissue. Moog[1] has demonstrated that the common alkaline and acid phosphomonoesterase activity increases

1 Moog, F., *J. Cell. and Comp. Physiol.*, 1946, **28**, 197.

considerably during early embryonic development and reaches its maximum in whole chick embryo homogenates by the 10th day of incubation. From these and previous observations (Moog[2]), it appears that these enzymes enter in some way into the chemistry of differentiation of tissue. Although the above findings are very convincing, nevertheless attention should be directed to the fact that the results were obtained from tissue which was not only differentiating, but also undergoing rapid growth. This is a complicating factor and does not entirely eliminate the possibility that these phosphatases may be concerned with growth rather than differentiation. It seemed to the author that this concept could be further tested by using a single tissue which could be followed through its embryonic development from the time it begins to differentiate until it becomes functional. One tissue in the chick embryo which lends itself well to such a study is the retina. According to Weysee and Burgess[3] differentiation of retinal cells begins about the 12th day of incubation and is complete shortly before hatching.

The present study was designed to determine whether any relationship exists between the stages of differentiation and the phosphatase activity of the embryonic chick retina.

Material and methods. New Hampshire Red chick embryos were used throughout this study. The eggs were obtained from a local hatchery and incubated in a commercial type incubator at a constant temperature of 102°F and a humidity of 90%. The retinas were removed from the eyes of 3 embryos of the desired age, blotted to remove excess moisture and weighed. The tissue was ground in a high speed micro grinder* to a uniform homogenate. The homogenate was then diluted with unbuffered saline solution (Krebs Ringer solution containing approximately .03M $MgSO_4$) so that 1 ml contained from 40-50 mg of fresh tissue. The substrate was combined with the buffer as follows: Alkaline mixture (pH 9.1) 1.09 g disodium phenylphosphate and 10.0 g of sodium barbital per liter. Acid mixture (pH 5.0) 1.09 g disodium phenylphosphate dissolved in 800 ml of a 0.2 N sodium acetate and 200 ml 0.2 N acetic acid. The enzyme activity was measured by a method modified from Moog[1] as follows: All experiments were carried out in test tubes (17 × 150 mm) containing 0.4 ml of substrate mixture. After pre-heating for 5 minutes in a bath kept constant at 37.5°C, 0.1 ml of homogenate was added to each tube. The preparations were then shaken for 20 minutes and the reaction stopped by adding 4.5 ml of 10% trichloroacetic acid. The precipitate was removed by filtration and 3 ml of the clear solution was analyzed for inorganic phosphorus by the method of Fiske and SubbaRow.[4] Ten minutes were permitted for the blue color to develop and all measurements were made with a Coleman spectrophotometer, calibrated with a series of standard K H_2PO_4 solutions. Experiments were always run in triplicate against 2 control tubes taken at zero time. Control values were subtracted from experimental values.

Results and discussion. The present study was confined to the retina from embryos beginning with the 12th day of incubation and ending approximately 3 days after hatching. This is the period during which the neural elements of the retina undergo maturation and differentiation into the various types of cells that characterize the functional organ. Although differentiation is very extensive during this time there is no appreciable change in the weight of the retina. The practice of using the weight of the tissue for computing data on chemical studies in embryonic tissues during different stages of development is sometimes considered a questionable procedure due to the changing concentration of the cytoplasm. However, since the chick retina did not increase more than 10% in weight over the period studied it seemed that there could be no serious objection in this case. The phosphatase activity is therefore expressed in terms of micrograms of inorganic

[2] Moog, F., *Biol. Bull.*, 1944, **86**, 51.

[3] Weysee, A. W., Burgess, W. S., *Am. Nat.*, 1906, **40**, 611.

* The homogenizer is one of our own design consisting of a stainless steel drill driven by a high speed motor.

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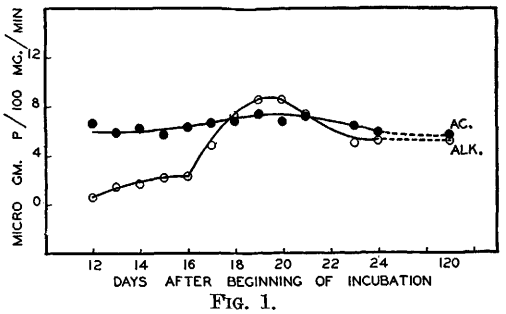

FIG. 1.

Alkaline (ALK) and acid phosphatase activity of the developing chick embryo retina.

phosphate liberated per 100 mg of fresh tissue per minute.

Fig. 1 depicts graphically the data obtained for both the alkaline (ALK) and acid (AC) phosphatase activity of whole homogenates of chick embryo retinas beginning with the 12th day of incubation and ending 3 days after hatching. The points on the curves represent means of data obtained from 3 separate series of embryos, incubated under identical conditions. It will be noted that the alkaline phosphatase activity at 12 days is almost negligible but rises gradually up to 16 days of incubation. Beginning with 16 days the activity increases rapidly up to 19 days where it reaches a maximum which is maintained through the 20th day after which it begins to fall at a rate comparable to the rise. However, about 3 days after hatching it levels off and maintains a rather constant rate.

The activity of the acid phosphatase, on the other hand, is relatively high at 12 days of incubation and does not appear to show any rise until about the 15th day. At this time there is a very small rise up to 19 days when it also decreases and eventually levels off at about 24 days. The broken lines on the graph designate the level of enzyme activity between the 24th and 120th day. Weekly analyses made between these ages showed no appreciable fluctuation in the enzyme activity and it is assumed that no further change took place.

It is interesting to note that the peak of both alkaline and acid phosphatase activity is reached on about the 19th-20th day of incubation. That the retina is differentiated at this time was demonstrated by the author,[5] when it was shown that 18 days after the beginning of incubation is the earliest stage in the development of the eye at which the pupillary constrictor reflex could be elicited by light stimuli.

Since these 2 events, namely completion of differentiation and development of maximum phosphatase activity correspond so closely it would appear that these enzymes may be concerned in some way with the morphogenesis of the retinal elements. These results are of interest therefore in the light of the findings of Moog, who demonstrated strikingly similar increases in both the alkaline and acid phosphatase activity of homogenates of whole chick embryos between the 2nd and 12th day of incubation during which time it was assumed that the tissues were undergoing differentiation. Thus the chick retina, which is known to differentiate between the 12th day of incubation and hatching time, lends further support to the concept that certain phosphomonoesterases may enter into the histochemistry of differentiation of embryonic tissues.

Summary and conclusion. 1. The alkaline and acid phosphatase activity was determined on whole homogenates of embryonic chick retina from the 12th day of incubation until 3 days after hatching.

2. The alkaline phosphatase activity increases slowly up to 16 days and then more rapidly to 19 days, when it reaches a maximum. After the 20th day it begins to decrease until 3 days after hatching, at which time it establishes a level of activity which is apparently constant throughout the life of the chicken.

3. The acid phosphatase activity is considerably higher than the alkaline at 12 days, but shows only a slight increase up to 19 days, after which it also falls to a new level.

4. Since the rise in activity of both acid and alkaline phosphatase correspond closely to the period of cellular maturation in the chick retina, this seems to give added support to the concept that these enzymes may be associated with the histochemistry of differentiation.

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