size. When .1 cc of the organisms that were irradiated for 40 sec was planted, the population resulting after 6 hr contained 50.5 streptomycin-resistant mutants per million cells; but when the equivalent inoculum was planted after 60 sec exposure, the incidence of mutants in the resulting population dropped back to that of the control. This is not the true incidence of mutants in the population, since a culture grown from an inoculum of 1 cc of the organisms irradiated for 60 sec had a mutation incidence of 63.0/million. Since 63 mutants/million is 1 mutant/16,000 cells, it is evident that in .1 cc of the cultures irradiated for 60 sec there were only 4,600 cells, and consequently no mutants to be transferred in the subculture.

The same situation holds in the organisms irradiated for 40 sec when .01-cc transfer was made to subculture. The mutation incidence then drops to that determined by the spontaneous mutation rate.

With bacterial mutations that occur at a low rate a study of the progeny of the survivors of a large dose of mutagen may fail tc reveal the mutagenic action.

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Electrophoretic Comparison of the Serum Proteins of Normal and Diethylstilbestrol-treated Cockerels[1]

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Variations in the calcium, phosphorus, and total protein of chicken and pigeon serums during the normal reproductive cycle have been repeatedly reported (*1–3*). Riddle and McDonald (*4*) indicated that similar fluctuations occurred in pigeons injected with diethylstilbestrol, and McDonald and Riddle (*5*) showed that the injected estrogen had the same effect on the calcium and phosphorous partition in the serum of the nonlaying pigeons as was found during normal egg production. Brandt, Clegg, and Andrews (*6*) demonstrated a marked difference in the electrophoretic pattern of laying and nonlaying chickens. The electrophoretic pattern of the laying hen contained an extra component and, in addition, had a much higher percentage of the slower moving globulin components. Since Riddle and McDonald were able to demonstrate a parallel in the calcium and phosphorous partition in laying and estrogen-treated pigeons, the possibility that a similar parallel exists in the serum proteins of laying hens and diethylstilbestrol-treated cockerels bears investigation.

In the preliminary investigation diethylstilbestrol pellets were implanted in both male and female chicks 8–10 weeks of age. At the end of 7 days blood was obtained from the wing vein, and the serum was prepared by mild centrifugation of the blood clot. A determination of the total calcium of this serum and the ultrafiltrate prepared from it demonstrated a partition similar to that of laying hens. Therefore the action of the estrogen was fairly rapid, and 1 week was sufficient time for the effect to be noticeable.

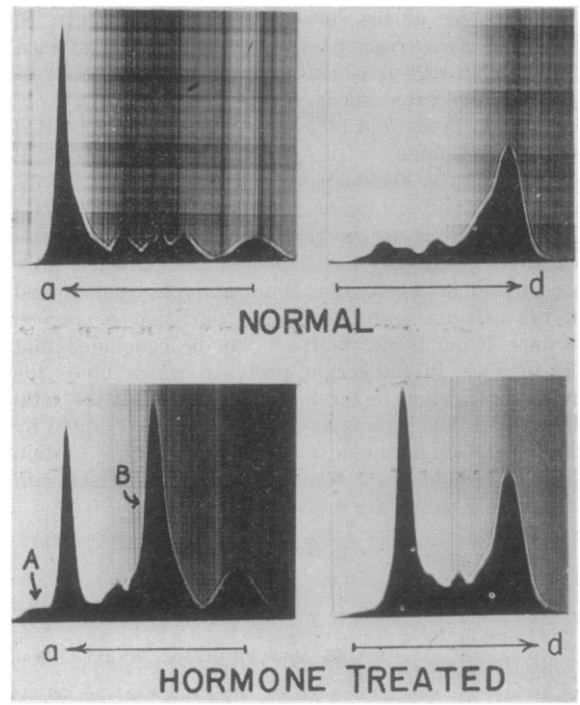

FIG. 1. Electrophoretic comparison of the serum proteins from normal and estrogen-treated cockerels. Schlieren diagrams in borate buffer (pH 8.6) after 7,200-sec electrophoresis.

For the electrophoretic analyses of the serum proteins of normal and diethylstilbestrol-treated birds, two groups of 8-week-old cockerels were employed. Diethylstilbestrol pellets were implanted in the necks of 5 birds. The other birds were used as controls. One week after the implantation the birds were sacrificed, and the serum prepared from the blood was subjected to electrophoretic analysis conducted in the same manner as described in a previous publication (*6*). All analyses represent individual chickens; serum samples were not pooled.

A typical electrophoretic pattern of the serum protein components of the normal group (Fig. 1) was similar to the pattern obtained previously when the serums of cockerels, nonlaying hens, and young chicks were analyzed (*6*). On the other hand, the electrophoretic pattern of the serum proteins of the group treated with diethylstilbestrol was remarkably similar to the pattern obtained when the serum of laying hens was employed. The fast-moving component, A, previously found in the pattern of the serum of laying hens was clearly evident in the patterns of the diethylstilbestrol-treated cockerels, and, in addition, the in-

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crease in size of the slower moving components, B, paralleled the increase previously shown to occur when pullets begin egg production (*6*). In the one case attempted, the same serum protein changes were observed in a 2-year-old male bird that had been treated with the hormone.

These results demonstrate that the injection of diethylstilbestrol will cause changes associated with egg formation to occur in the serum proteins of male birds. As previously mentioned, similar changes have been noted in the total calcium and phosphorus and in the calcium and phosphorus partition of pigeon serums. From these results it can be concluded that the increase in the serum proteins, which binds the increased calcium in the laying hens so that the total diffusible calcium remains constant, may be caused by the female sex hormone. The properties of the protein fractions found in the serums of normal and estrogen-treated male birds are under investigation.

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Carbon Dioxide and Root Hair Development in *Anacharis* (*Elodea*)[1]

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Considerable interest has been shown in the fact that the normally hairless roots of *Anacharis* become coated with root hairs when they penetrate the mud. In a recent paper (*1*) two attempts at explanation are reviewed. In the first, soil particles are regarded as a stimulus and, in the second, light is considered the deciding factor. Cormack (*2*) and King (*3*), by completely excluding all light, reported abundant production of root hairs in water alone.

In an effort to understand the effect of light on the root, Cormack made a microscopic study. Unlike the roots grown in light, those grown in darkness had no cuticle. He assumed that the toughness of the cuticle prevented the extension of the epidermal cells as root hairs. Roots grown in the light had chloroplastids in the epidermal cells, whereas these were absent in roots grown in the dark. Lee and Priestley (*4*) had pointed out that the saturation of fats by oxygen is one stage in the formation of cuticle. Unsaturated fatty materials migrating to the surface in the presence of oxygen would be toughened into cuticle. Cormack considered the oxygen produced in the epidermal cells of the root during photosynthesis to be involved in this reaction. In the absence of light no oxygen would be produced, and the unsaturated fats would wash away without being changed. He tested this by administering ethylene gas to inhibit chlorophyll formation. After this treatment the usual stains gave no evidence of a fatty layer, and root hairs were produced in the light.

While investigating the development at the stem apex, the writer has frequently grown the cultivated and native *Anacharis* in nutrient solutions of high carbon dioxide tension. A continuous stream of minute bubbles of this gas flowed through the solution in covered 4-liter Pyrex jars. Under this treatment the green roots of *Anacharis* grown in light were invariably covered with root hairs. There was an abundant supply of oxygen, for as soon as the carbon dioxide tension built up the sprigs were buoyed up by the increased oxygen in the air spaces, and it was necessary to weight the plant down by pierced sections of glass slide to keep it immersed. Presumably carbon dioxide concentration is often the limiting factor in photosynthesis. On staining with Sudan IV, a fatty layer on the outside of the roots was observed, the root hairs breaking through this layer. This would seem to contradict Cormack's evidence; nevertheless, a study of the nature of this fatty layer was undertaken. On warming sections of the roots in 10% potassium hydroxide, the hairless root lost only a small part of its staining film, whereas the root grown in high carbon dioxide tension completely lost its coating of fatty material, which therefore could not be considered as cuticle. To discover if the fats were oxidized, a 1% aqueous solution of osmic acid was used. It is reduced and blackened if the fats are unsaturated. Soaking sections of hairless roots in the solution overnight produced a slight darkening on the outside of the fatty layer. Only a very thin outer layer was still unsaturated. The hairy roots produced in abundant carbon dioxide showed a definite blackening in the whole thickness of the layer. After saponification with the alkali no darkening of the epidermal walls occurred in either hairy or hairless roots, although the cuticle was still retained on the latter. The carbon dioxide in some way had prevented the oxidation of fats to form a cuticle, and the formation of root hairs was not prevented.

Another problem presented by the high carbon dioxide tension is that of pH. Cormack (*5*) in his investigations on *Brassica* has shown that the formation of root hairs is linked with the change of pectic acid to calcium pectate in the outer wall of the epidermis. He demonstrated that this took place in cells having a pH above 5.8, none developing on cells of a pH of 4.6–4.8. In the writer's experiments the pH of the solution in which the plants were grown was 3.8. In order to understand this discrepancy, a crude attempt

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