stance that might have been split off by the action of thrombin. Iodinated fibrinogen differs so much from fibrinogen, and its preparation involves so many additional steps, that it is more likely to be free of possible adsorbed impurities than the original fibrinogen. Working with iodinated fibrinogen has the further advantage that it is soluble in water, clots in water, and its solution can be deproteinized by adding salt to it. It was found that the supernatant of clotted iodinated fibrinogen gave a spot detectable by ninhydrin on paper chromatograms and contained a substance that stimulated isolated frog heart.

The experimental procedure is as follows. Iodinated fibrinogen is clotted with a small amount of thrombin. In a few hours a firm gel is formed. By warming the gel it reversibly liquefies. To the liquefied gel a concentrated Ringer solution is added (final salt concentration corresponds to a Ringer solution) to remove the protein. The supernatant when tested on frog heart in contrast to similar unclotted supernatant stimulates the heart. The nature of this substance found in the supernatant of clotted fibrinogen is under investigation. Lorand (personal communication) believes that it is a peptide. Work is also in progress to find further evidence that this substance is really split from fibrinogen by thrombin and whether it is a specific heart stimulant.

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The Radiation Dose-Response Curve and Bacterial Mutations

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The shapes of the curves obtained when plotting biological response against radiation dose have yielded information on the nature of the reaction. Demerec and Latarjet (*1*), Hollaender *et al.* (*2*), and Mefferd and Wyss (*3*) have shown that some induced mutations increase linearly with dose, some increase exponentially, and still others exhibit breaks in the dose-response curves. The causes of these breaks that have been observed with ultraviolet irradiation are subject to conjecture. We have observed a different type of break which is superimposed on the ultraviolet dose-response curve and also results with x-ray and nitrogen mustard treatment. This is observed when the survivors of the mutagenic action are placed in broth and permitted to grow for several generations before the assay for mutants is made. This is often done with bacteria because with a number of bacterial mutations there is considerable delay between the action of the mutagen and the appearance of the mutations in the population; with the *Escherichia coli* mutation to phage resistance the maximum number is not attained until each organism has made about 10–13 divisions following the application of the mutagenic agent. Consequently, in order to observe the maximum of induced mutants the bacteria surviving mutagenic action are usually placed in a condition favorable to growth before an assay of the mutants is made. With some mutations additional induced mutations appear up to 13 generations after the application of the mutagen; with others no more appear after a very few divisions. It is when these so-called end-point mutants are plotted against the dose of the mutagenic agent that a break in the curve is superimposed on the less drastic break that has often been observed with the "zero-point" mutants. This first came to our attention when doses of ultraviolet light sufficiently large to kill all but a small fraction of *Bacillus anthracis* spores failed to increase the incidence of mutants in a culture grown from the survivors, although lower doses gave readily measurable increases. This was especially evident when rare mutations were sought but also applies to biochemical mutants and streptomycin resistance induced by ultraviolet light as well as when x-rays or nitrogen mustard are the inducing agents.

TABLE 1

EFFECT OF THE NUMBER OF SURVIVORS ON THE INCIDENCE OF END-POINT MUTANTS TO STREPTOMYCIN RESISTANCE IN *B. anthracis*

Inoculum size	Organisms inoculated	Mutants per million
	No irradiation	
.1 cc	1.4×10^6	13.8
.001	1.4×10^4	15.0
.00001	1.4×10^2	14.8
	Irradiated 40 sec	
1.0	2.8×10^5	49.5
.1	2.8×10^4	50.5
.01	2.8×10^3	17.3
.001	2.8×10^2	17.6
	Irradiated 60 sec	
1 cc	4.6×10^4	63
.1 cc	4.6×10^3	20
.01 cc	4.6×10^2	14

In the experiment reported in Table 1 a population of *B. anthracis* was subjected to ultraviolet light as indicated. Then inocula of various sizes were removed from each treatment and planted into broth and permitted to grow for 6 hr. The incidence of streptomycin-resistant mutants in the resulting population in unirradiated cultures was not affected by inoculum

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.

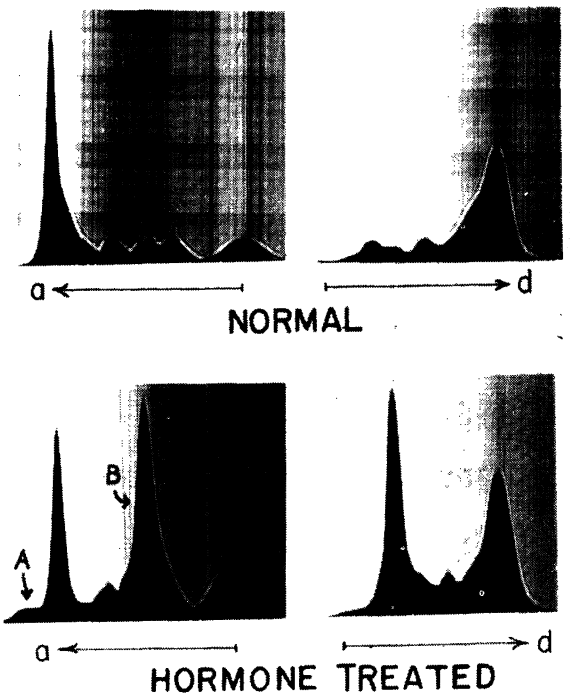

FIG. 1. Electrophoretic comparison of the serum proteins from normal and estrogen-treated cockerels. Schlieren diagrams in borate buffer (pH 8.6) after 7,200-sec electrophoresis.

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-

[1] Contribution No. 451 of the Department of Chemistry and Contribution No. 194 of the Department of Poultry Husbandry, Kansas State College, Manhattan.