the introduction of iron, either by accident or by contaminated chemicals.

Discussion of results. The analyses of "cell rich" and "cell poor" sweat from 25 different normal subjects are given in Table I.

"Cell rich" indicates sweat just as it came from the glove. "Cell poor" refers to the supernatant obtained after centrifugation. The former material was opalescent, the latter clear. By far the larger amount of iron was present in the "cell rich" sweat. With one exception all determinations of "cell poor" sweat showed less than 0.64 μg of iron per cc, which is below the limits of accuracy of this method.

In order to check the results further a rubber glove was applied to a subject's arm after the usual preparation. Distilled water was allowed to remain in contact with the arm for one hour and the contents of the glove were then analyzed for iron. The material obtained was opalescent and rich in epithelial cells; it yielded an iron content of 8.5 μg/cc, corresponding to "cell rich" sweat. The supernatant after centrifugation gave a blank reading for iron. This has been repeated several times with similar results.

From the data obtained it is concluded that the loss of iron in human sweat is negligible. Loss of iron from desquamation of the epithelium may be considerable, although no quantitative estimates were made. Further iron balance work with reference to the loss of iron by means of desquamation of epithelial cells is indicated.

Summary. "Cell poor" and "cell rich" sweat samples from 25 normal subjects have been analyzed (total of 118 determinations). The iron content of "cell poor" sweat was negligible. On the other hand "cell rich" sweat contained a high concentration of iron. This iron loss is not an essential function of the process of sweating, but is associated primarily with desquamation.

Received March 13, 1950. P.S.E.B.M., 1950, v74.

Effect of DDT on Testes and Secondary Sex Characters of White Leghorn Cockerels. (17805)

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With the introduction of the insecticide DDT (2,2-Bis(p-chlorophenyl) 1,1,1-Trichloroethane) and its subsequent widespread application, there came a need for a complete understanding of its toxicity, and metabolic effects in animals other than insects. Studies on several species of mammals have resulted in a rather wide variety of findings regarding the action of this compound. In general, mammals are extremely sensitive to relatively small amounts of DDT. On the other hand, birds have been shown to possess a high degree of resistance to this substance(1). Since experiments dealing with the effects of DDT on birds are notably less numerous than similar studies on mammals, it appeared that additional information regarding this important compound might be obtained from a study designed to reveal the effects of long-term poisoning in chickens. In the course of this study on cockerels, some interesting effects were noted on the development of the testes and secondary sex characters.

Method. White Leghorn cockerel chicks were selected at random and divided into 2 groups, one to serve as controls and the other as experimental animals. They were placed in a commercial brooder and allowed free access to food and water. Two series of experiments were carried out involving a total of 30 controls and 40 treated birds. Starting on the 8th day after hatching, the experimental chicks were given daily subcutaneous injections of purified DDT (M.P. 109°). To

1. Woodard, G., Nelson, A. A., Calvery, H. O., *J. Pharm. and Exp. Ther.*, 1944, v82, 152.

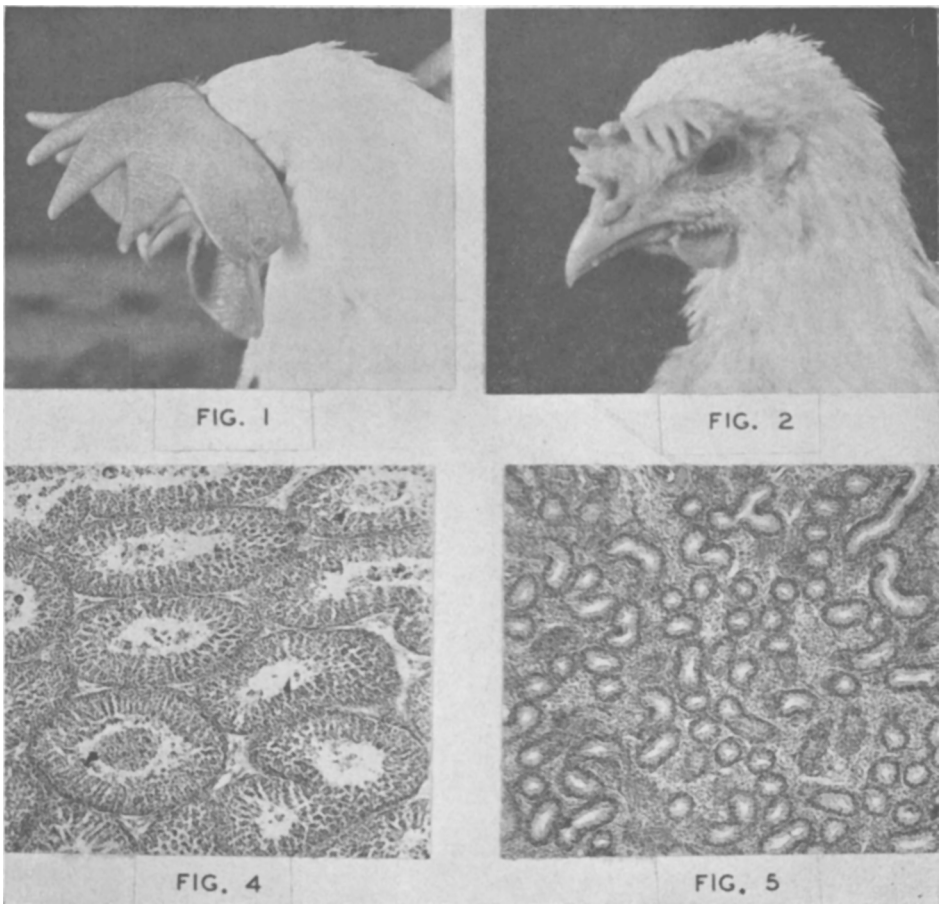


FIG. 1. Control bird, age 60 days.
FIG. 2. Treated bird, same age.
FIG. 4. Section of testis from control bird (× 60), age 86 days.
FIG. 5. Section of testis from treated bird (× 60), same age.

minimize the possibility of lesions, the site of injection was varied. The amount of the compound administered was gradually increased during the course of the experiment from an initial dose of 15 mg per kilo of body weight in the young birds to a maximum of 300 mg per kilo near the terminal stages of the study. The solvent used was rendered chicken fat. Control birds received an equivalent amount of the fat each time the treated birds were injected. All birds were treated for at least 60 days and some were carried for as long as 89 days. Careful weight records were kept throughout the course of the study. At the termination of the experiment the animals were sacrificed and the testes removed, weighed and prepared for sectioning.

Results and discussion. As treatment proceeded, it became increasingly evident that there were significant differences in the development of the secondary sex characters between the two groups. After about 25 days of treatment, the experimental animals began to display marked inhibition of combs and wattles both in regard to size and color. As the experiment progressed, these differences became more striking. Fig. 1 and 2 serve to illustrate the effects after 52 days of treatment. At this stage comb measurements were made which further demonstrated the difference between the two groups. The aver-

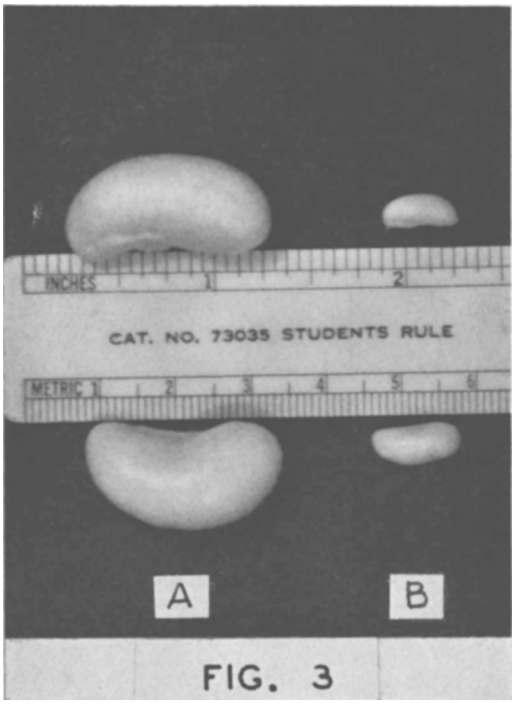


FIG. 3

Showing the comparative size of testes from normal bird (A) and those from DDT-treated animal (B) of the same age. (85 days).

age comb size (taken as length plus height) for the control group was 11.7 cm as compared to an average of 7.56 cm for the experimental birds. A few instances of comb measurements made after 80 days of treatment were even more striking, the control combs measuring 21.3 cm and treated, 8.9 cm. These observations led us to suspect an involvement of the testes in the production of this phenomenon, since it is well known that atrophy of head furnishings results from castration of Leghorn cockerels.

Therefore, when the birds were sacrificed, first attention was given to the stage of development of the testes. In a group which was killed at an age of between 80 and 90 days, the average testes weight of the treated animals was 1.05 g, while the average for the normals was 5.63 g. Fig. 3 serves to illustrate the relative size of the gonads from treated and untreated birds of the same age. Tissue sections were prepared of the testes of both treated and normal animals and these further emphasize the effect which was produced. Fig. 4 and 5 provide a means of comparison. Careful examination of the sections reveals that the amount of intertubular tissue in the organ from the treated bird is far in excess of that found in the control animal. In contrast to this observation it can be seen further that the tubular development has been markedly retarded in the treated testis.

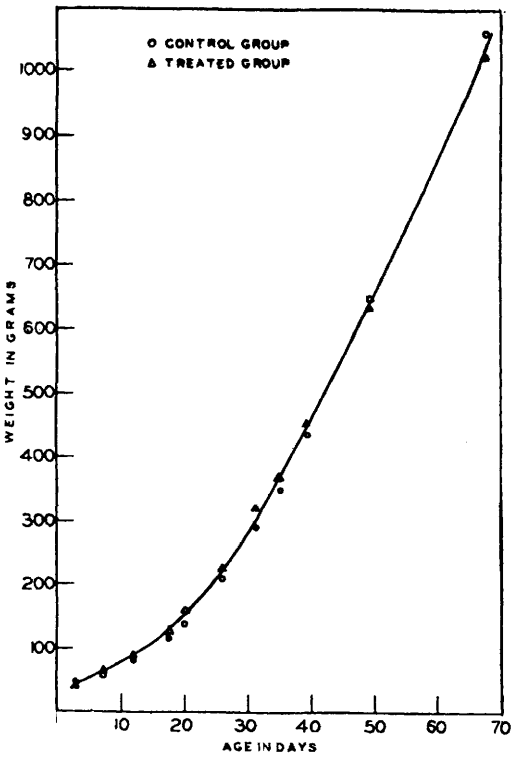
In noting the results of the treatment, it was necessary to consider the possibility that the over-all effects did not occur as a result of a direct action of the DDT itself, but instead were the consequences of a generalized condition of poor health brought about by the presence of a toxic agent. It is not without significance that workers have attributed to the factor of inanition many of the results obtained by administration of the compound(2,3). In this study, no difference in food or water intake between the two groups was discernible from casual observation and the weight increments of the treated and control animals present a strikingly similar picture. The weights, when plotted for the entire course of the experiment, can be served by a single curve for both groups (Fig. 6). To further ascertain whether the poison was affecting the general health, red blood cell counts and hemoglobin measurements were made. The experimental birds were shown to have an average of 17.8% fewer red cells than the controls. This difference was not considered great enough to influence the general health. There were no significant differences in the hemoglobin values.

Attempts to explain the results obtained in this work are difficult since the literature does not contain any evidence which shows similar effects in other animals. There is one report however, which lists occasional testicular atrophy in rats as a result of DDT feeding experiments(1). No significance was placed on the finding since it occurred in only rare instances.

The effects noted here are such that they

2. Nelson, A. A., *et al., Pub. Health Rep.*, 1944, v59, 1009.

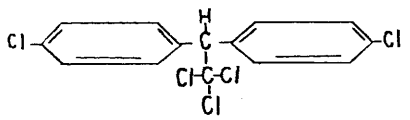
3. Sarett, H. P., and Jandorf, B. J., *J. Pharm. and Exp. Ther.*, 1947, v91, 340.



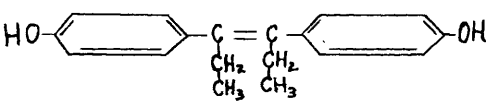
WEIGHT CURVE

FIG. 6

might easily be duplicated by the administration of an estrogen. It seems, therefore, that the possibility of an estrogenic action of DDT is at least worthy of consideration. In speculating along these lines, it is interesting to note the degree of similarity between the molecular configuration of DDT and certain synthetic estrogens, especially diethylstilbestrol.



DDT



Diethylstilbestrol

Similar chemical structures by no means insure a common biological action; however, it is interesting to note that introduction of chlorine into the stilbestrol molecule has been reported to result in a compound that still possesses some estrogenic activity(4). Assuming that DDT was acting in such a capacity, it is very possible that it might be doing so through an inhibition of pituitary gonadotrophins. Noble(5), among others, has reported a decreased gonadotropic content in rats treated with diethylstilbestrol. Experiments have been designed to determine whether or not DDT is acting in this fashion.

Another factor that must be considered is the manner in which chickens metabolize DDT. Perhaps there occurs a slight alteration in the structure of the compound which enables it to produce the effects noted. That chickens do handle the substance differently from mammals is suggested by the difference in tolerance levels exhibited. It is quite possible that doses of the same magnitude as those administered to chickens in this experiment might produce comparable effects upon mammalian testes. However, these levels are so high as to be fatal for most mammals.

Summary. 1. White Leghorn cockerels were subjected to injections of DDT beginning 8 days after hatching and continuing for as long as 89 days.

2. The development of the comb and wattles was greatly inhibited as a result of the treatment. This effect is attributed to the marked retardation of the testes growth which was noted.

3. These findings suggest that DDT may exert an estrogen-like action in white Leghorn cockerels.

We wish to express our appreciation to E. I. du Pont de Nemours and Co., for generously supplying the DDT used in this study.

4. Dodds, E. C., *et al., Proc. Roy. Soc. Lond.* (B), 1944, v132, 83.

5. Noble, R. L., *Lancet*, 1938, v235, 192.

Received March 31, 1950. P.S.E.B.M., 1950, v74.