

resistant C3H/JAX strain were quite comparable in size to those of the untreated C3H/CMC mice during the period 7 to 12 days following implantation. On the 11th day, for example, subcutaneous tumors measuring 13 to 21 mm across were present at each implantation site in the four hosts, and these growths were approximately the same size on the 12th day. On the 14th and 15th days, however, the growths were all much smaller, and by the 18th day they had disappeared entirely from all four mice. The guinea pig serum in general proved equally inhibitory in mice of the two sorts. Thus growths failed to appear in 3 of the 4 C3H/CMC mice given 2 cc of guinea pig serum, while small bilateral growths made a much delayed appearance in the fourth animal of this group; the same was true of the group of C3H/JAX mice given 2 cc of guinea pig serum, there being complete inhibition of the growths in 3 of the 4 animals, with delayed appearance of bilateral growths in the remaining one. 1 cc of guinea pig serum gave complete inhibition of the growths in 1 of the 4 C3H/CMC mice, with delayed appearance of growths in the other 3, while this amount of serum completely inhibited growth in two of the C3H/JAX mice and delayed the appearance of growths in the remaining two. The 0.5 cc of guinea pig serum likewise inhibited the growths markedly but incompletely in the relatively susceptible and resistant mice respectively, the results being quite comparable in the 2 groups. The findings as a whole made it plain that the inhibitory effects of the guinea pig serum was not notably enhanced in the resistant mice.

Summary. In the light of a previous observation—that normal guinea pig serum is highly effective against 6C3HED lymphoma cells *in vivo* but not *in vitro*, the host animal obviously participating in the reaction *in vivo*—a number of experiments were done to learn whether isoantibodies, capable of acting in conjunction with guinea pig serum against the lymphoma cells, might be present in the serum of normal mice of susceptible and resistant strains, in that of susceptible hosts carrying 6C3HED lymphomas, or in that of mice that had overcome implanted 6C3HED lymphoma cells and proved immune to re-implantation with them. The findings are given in some detail. They provide strong but not conclusive evidence that such isoantibodies are absent from the blood of mice in all the categories mentioned above. It follows that host factors or conditions of some other sort might profitably be sought to account for the participation by the host in the reaction whereby normal guinea pig serum, injected into animals carrying 6C3HED lymphomas, brings about death of the lymphoma cells.

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Relationship of Vitamin E to Embryonic Development of Avian Eye. (21259)

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The relationship of eye disorders to vit. E has been reported in a study of retrolental fibroplasia in human infants(1), and in an

eye disorder suggestive of retrolental fibroplasia in rats(2). Jensen(3) stressed the importance of vit. E in practical turkey rations in relation to embryonic mortality and hatchability. The effect of adding alpha-tocopheryl acetate to a practical type diet, with combina-

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tions of dried whey and fish solubles, on hatchability and embryonic mortality in Beltsville Small White turkeys will be reported herein.

Procedure. Seventy-two turkey hens were randomized into 8 groups of 9 hens each. These birds had been reared in electrically heated batteries with raised screen floors for 6 weeks, in standard growing batteries with raised wire floors from the 6th through the 12th week, and in pens with raised wire floors for the remainder of a 30-week period prior to the initiation of this experiment, as described in (4). The diet fed during the first 10 weeks consisted of 55% soybean oil meal, 20% ground yellow corn, 19% ground milo, 3% dicalcium phosphate, 2.5% ground oyster shell and .5% salt. This diet was supplemented with the following vitamins as indicated on a per lb basis: 2 mg riboflavin, 12.5 mg calcium pantothenate, 20 mg niacin, 400 mg choline chloride, 6 μ g vitamin B₁₂, 2 mg menadione, 160 mg manganese sulfate, 4500 I. U. vit. A and 1200 I. C. U. vit. D₃. From the 10th through the 30th week, soybean oil meal was decreased to 40% and ground yellow corn and ground milo were increased to 25 and 29% respectively. At the beginning of the present experiment soybean oil meal was decreased to 25% and ground yellow corn and ground milo increased to 35 and 34% respectively, and vit. A was increased to 9000 I. U./pound. The source of vit. A was a stabilized product which had been sealed in through the use of hydrogenated vegetable fat. Each group of 9 birds was placed in a 10' by 12' pen with a raised wire floor. Four nests were also placed in each pen. Feed and water were supplied *ad libitum*. Each pen of females was mated with a male of the same breed reared on the range of the Poultry Husbandry Department of the Texas Agricultural and Mechanical College System. Toms were rotated weekly to obtain maximum fertility. Groups 1, 3, 5 and 7 were fed the basal diet unsupplemented, supplemented with 5% fish solubles, with 3% dried whey and with 5% condensed fish solubles and 3% dried whey respectively. Groups 2, 4, 6 and 8 were fed the same diets except that the latter 4 mentioned groups also received a supplement of alpha-tocopheryl acetate at a level of

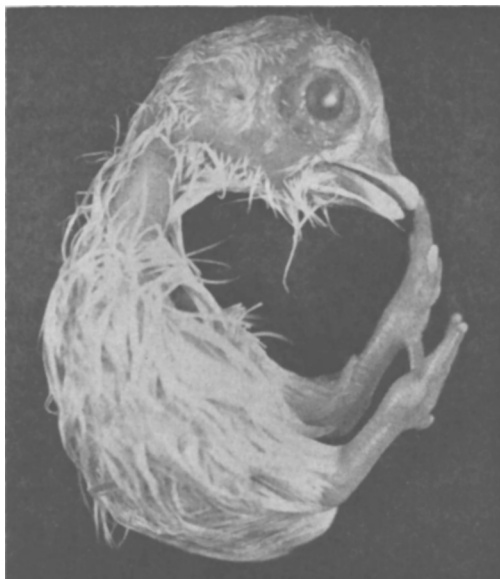


FIG. 1. Abnormal eye of a 21-day turkey embryo, from hen not supplied with supplementary alpha-tocopheryl acetate.

20 mg/lb. Eggs were collected at 4 stated intervals each day, were kept in a cooler at 55°F and were set once each week. Eggs were candled at 7, 14, 21 and 24 days. Infertile eggs and those containing dead embryos were broken to determine the approximate time of death. Eggs removed during incubation and those which failed to hatch on the 29th day of the incubation period were broken and a gross examination was made of the embryos. This report is concerned with the embryos that were at least 21 days of age or over. Live embryos were preserved in formalin or Zenker's fixative for later histopathological studies. Sections were stained with hematoxylin and eosin. Body measurements and skeletal measurements were also made of some of the embryos.

Results. Supplementation of the all-vegetable protein diet with fish solubles, whey, or a combination of these ingredients had no effect on the occurrence of the anomalies described herein.

A cloudiness in the central portion of the lens was found among the embryos from eggs laid by hens fed an all-vegetable protein diet without added vit. E. This condition was observed in both of the eyes in 13 of 30 live embryos examined on the 29th day. These

embryos pipped the shell but did not hatch. Seven of this number had hemorrhages in the vitreous humor. One or both of the eyes frequently had a bulging of the cornea, that gave the embryo a protruding eye appearance in extreme cases. The cornea was found to be slightly irregular in some embryos when examined with a binocular microscope. A yellowish-white spot of material was seen in the eyes between the lens and the cornea in a few specimens, as shown in Fig. 1, picturing a 21-day embryo that was alive when removed from the egg. This condition also occurred at later stages of embryonic development. An irregular-shaped iris was often associated with the bulging cornea, which made the pupil likewise irregular. Upon dissection of the lens, pressure applied firmly to the outside resulted in its rupture with a splitting out of a smaller miniature lens and the loss of a watery fluid. In some instances, a still smaller lens-like structure could further be forced out. The conjunctival blood vessels were dilated in many embryos and there were some with edema of the eyelids. The pecten was present but extensive observations on the pecten were not made. The eyes of the embryos obtained from dams supplemented with vit. E were apparently normal. The pupil was rounded, the iris evenly pigmented and the lens was clear. Dissection of the lens showed it to be a typical solid crystalline lens. The vitreous humor was clear.

The results of a preliminary histological examination of the lens from the 2 groups of embryos is shown by camera lucida drawings in Fig. 2. Fig. 2A is a section near the center of the lens of a 29-day embryo from a dam that was fed added vit. E. A portion of the iris and ciliary body are included. Fig. 2B is a similar section from near the center of the lens of an embryo of the same age where the dam did not receive supplemental vit. E. The lens is apparently the same diameter as 2A, but is more flattened and has a small lens body inside the outer lens layer. Degenerative changes have occurred throughout the lens, especially in the central and posterior portions. These observations are based on histological sections of both eyes from 5 of the 29-day embryos of the non-supplemented groups and the same number of embryos pro-

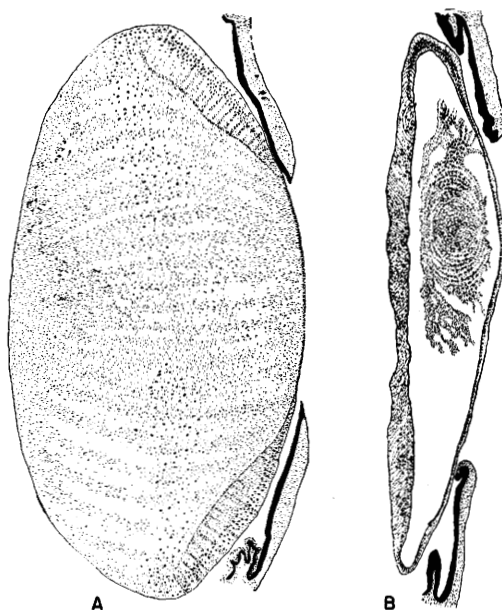


FIG. 2. Lens of turkey embryo (29 days); (A) from hen fed a supplementary source of alpha-tocopheryl acetate; (B) not supplemented.

vided supplemental alpha-tocopheryl acetate. Microscopic section of the eyes of a 24-day embryo from the unsupplemented group showed it to be as in Fig. 2B. Degenerative changes have also been observed in the retina. Further studies will be necessary to determine the extent of the degeneration and when such changes are discernible.

Measurements of the principal skeletal parts of live embryos from the supplemented and unsupplemented groups were made. These embryos were 29 days of age, and had pipped the shell but did not hatch. The legs, wings, and body length of the embryos from the groups not supplied additional alpha-tocopheryl acetate were less than the supplemented groups. The tarsometatarsus and toes of the unsupplemented groups were edematous, which made this part of the leg appear extremely shortened. Ten of the 30 live embryos examined from the unsupplemented groups on the 29th day of the incubation period also had an edematous area on the neck at the back of the head. This condition did not occur in the alpha-tocopheryl supplemented groups.

Caution should be observed in interpreting abnormalities in dead embryos. However, 71

dead embryos from dams fed the diet not supplemented with alpha-tocopheryl acetate were examined. The number of dead embryos for each day between the 21st and the 28th day were 1, 2, 1, 11, 6, 19, 15, and 16 respectively. Fifty-seven of these embryos possessed a cloudy lens, 31 showed a hemorrhaged vitreous humor, and 44 had an edematous area on the neck at the back of the head. Sixty of these were smaller than normal embryos of the same age. The anomalies described did not occur in the groups supplemented with alpha-tocopheryl acetate.

Two hundred eggs were obtained from a Broad Breasted Bronze commercial turkey flock near the end of the hatching season. The turkey hens from which these eggs were obtained had been fed a good turkey breeder mash throughout the laying season. Sixty fertile eggs produced 25 poults. A high early embryonic mortality occurred. Eight of the embryos which died between the 24th and 28th day of the incubation period had a cloudy lens, were smaller in size, and were identical with embryos from dams that did not receive supplemental vit. E in the present report.

The alpha-tocopheryl acetate content of a representative sample of egg yolks from each group was determined in the laboratory of Dr. Philip L. Harris, Distillation Products Industries, Rochester, N. Y. Egg yolk from groups fed the alpha-tocopheryl acetate contained 3.6 times as much tocopheryl as those where the diet was not supplemented with this compound.

The results relative to embryonic mortality and hatchability and the influence of vit. E are similar to those obtained by Jensen(3), who stated that niacin and grass juice were not effective in reducing embryonic mortality or enhancing hatchability, but that alpha-tocopheryl acetate fed at the level used in the present experiment (20 mg/lb) was effective in increasing hatchability. No description of the embryos was given in the Jensen report (3). In the present instance supplementa-

tion of the all-vegetable protein diet with fish solubles, whey or combinations of these did not improve hatchability nor decrease embryonic mortality. The results agree also with the work of Jensen(3) that vit. E may be a more critical nutrient in turkey laying rations than heretofore considered. Further work will be necessary on the earlier stages of embryonic development in order to determine the onset and course of the anomalies described.

Summary. A cloudiness in the central portion of the lens was found in embryos from eggs laid by turkey hens fed an all-vegetable protein diet without added vit. E. Associated with the lens disorder was a hemorrhaged vitreous humor, a smaller embryo with edematous areas on the neck and feet, and a high embryonic mortality between the twenty-fourth and the twenty-eighth day of the incubation period. Supplementation of the all-vegetable protein diet with alpha-tocopheryl acetate was effective in preventing the anomalous conditions described, while fish solubles, dried whey, or combinations of these substances had no effect.

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