

NEW EVIDENCE AGAINST A PROGESTERONE-LIKE ACTION OF ASCORBIC ACID.

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INTRODUCTION.

It is well known that ascorbic acid is present in the corpus luteum and that its concentration increases in the uterus during progestational changes due to the action of progesterone (Pincus and Berman, 1937). Kramer *et al.* (1933) reported that lowered concentration of ascorbic acid in the guinea-pig can prevent proper progestational proliferation. These authors further observed that the removal of this vitamin from the diet of a pregnant guinea-pig can cause abortion. These evidences led Israel and Meranze (1941) to suspect that ascorbic acid is associated with progesterone in bringing about progestational proliferation and that even alone it may be able to cause similar changes. In their experiments on infantile rabbits these authors actually demonstrated that vitamin C has progesterone-like effect on the uterus. However, Pratt (1943) carefully repeated Israel and Meranze's experiments but could not obtain any evidence whatsoever of a luteoid-like action of ascorbic acid.

From a review of the above works it becomes evident that ascorbic acid has some interesting rôle to play in uterine physiology. We, therefore, decided to open up the problem once again and to determine whether this vitamin has any progesterone-like action. We used female pigeon as the material in our studies because we felt that it would be worth while to provide data on this rather disputed point from an altogether different species of animal. Moreover, the physiological action of progesterone on the genital system of the female pigeon is well known (Kar, 1949*a*) and, therefore, it occurred to us that it would be interesting to compare the actions of this vitamin and the luteoid on the female genital system of this avian species.

EXPERIMENTAL.

Adult female pigeons were used in this study. A total of 15 birds were experimented on, of which a group of 5 were left uninjected to serve as controls. A second group of 5 birds were injected intramuscularly with ascorbic acid ('Cevalin', Eli Lilly Co., Indianapolis, U.S.A.). The daily injections (50 mgm.) were made into the breast muscles and continued for a period of 7 days. The remaining lot of 5 birds were first injected with diethylstilbestrol (5 mgm. daily for 4 days) and subsequently treated with ascorbic acid at the rate of 50 mgm. daily for 7 days. All the birds were kept in cages and maintained under uniform husbandry conditions throughout the duration of the experimental period.

Autopsy followed 24 hours after the final injections. The ovary and the oviduct were carefully dissected out, weighed to the nearest mgm., and finally fixed in alcoholic Bouin's fluid for histological studies. Serial sections, 6 microns in thickness were prepared by the paraffin method and stained with Ehrlich's hematoxylin followed by eosin.

RESULTS AND CONCLUSION.

Controls.—Gross examinations at autopsy revealed a condition which is typical of the ovary of an adult bird. The presence of a number of large follicles and innumerable smaller ones are the notable macroscopic features of the organ. The larger follicles are full of yolk and are extremely hyperemic. The oviduct is a very conspicuous structure which occupies the major part of the peritoneal cavity. The convolutions of the duct are an index to its remarkable length. The entire organ is also richly vascularized.

Histological examination confirms the macroscopic findings that the ovary is in a state of full activity. The larger follicles are full of yolk and the follicular epithelium shows a high degree of stratification. The smaller follicles exhibit various stages of cytosomal differentiation. The interfollicular connective tissue is extremely well developed and a marked vascularity is also evident. Microscopical examinations of the oviduct show that the organ is at its height of secretory activity. The serosa, muscularis, and the sub-mucosa are extremely well developed. The endometrium shows pronounced glandular activity and a bordering epithelium of the stratified variety. The oviducal lumen exhibits a tortuous condition owing to the inward projection of the villus-like folds of the endometrium. The sub-mucosal connective tissue extends throughout the axial portion of these folds and in between the endometrial glands. A pronounced hyperemic condition of the duct is also visible.

Ascorbic acid treatment.—Progesterone has marked inhibitory effects on the genital system of the female pigeon (Kar, 1949a). The luteoid causes suppression of the follicular growth and pronounced infantilism of the oviduct. No such inhibitory effects are, however, visible in the genital system of the present material after ascorbic acid treatment. Gross appearance of the ovary is similar to that of the control birds. The organ is full of large and small follicles. There are no indications, whatsoever, of any hypoplastic change which, however, is to be expected if the vitamin acted like the luteoid on the ovary. Further, no signs of infantilism could be detected in the oviduct. The macroscopic features of the latter are comparable in every way with those of the oviduct of an adult bird. Histological examinations of the ovary and the oviduct also confirm our macroscopic findings. The microscopic appearance of these organs is similar to those of the ovary and the oviduct of the control pigeons and no inhibitory effects of the vitamin are discernible.

The above findings demonstrate clearly that unlike progesterone, ascorbic acid does not produce any inhibitory changes in the genital system of the female pigeon. The ovary and the oviduct of the vitamin-treated birds continue to remain unaltered histo-physiologically, and this is undoubtedly a very strong evidence against any luteoid-like action of vitamin C.

Ascorbic acid treatment in pigeons primed with estrogen.—We have already mentioned in a previous section that a batch of experimental birds were first treated with estrogen and subsequently with ascorbic acid. The object of this procedure was to expose only the oviduct to any possible luteoid-like action of the vitamin. It is well known that the ovarian function is suppressed by the action of estrogen but the secretory activity of the oviduct is stimulated to a very marked extent (*vide* Kar, 1949b). This procedure, therefore, served our purpose very well in targeting the effect of ascorbic acid exclusively on an experimentally-stimulated oviduct. To ensure accuracy, we examined the genital system of the estrogen-treated animals by laparotomy before the commencement of vitamin injections. The condition of the ovary, as we expected, was definitely infantile, but the oviduct was very much swollen and unusually hyperemic. Gross examinations at autopsy revealed similar conditions of the genital system. Histological examinations of the ovary showed that estrogen treatment caused inhibition of the follicular develop-

ment and atrophy of the stromal elements. Indications of estrogen-induced stimulation, however, were evident in the histological preparations of the oviduct. The serosa, muscularis and the sub-mucosa appeared greatly thickened. The endometrial glands were much enlarged in size and the oviducal lumen appeared to be clogged with the secretions of these glands.

The above findings are, undoubtedly, not indicative of any inhibitory action of ascorbic acid on the oviduct. It may be recalled here that progesterone has pronounced inhibitory action on the oviduct of this species (Kar, 1949a). The new evidence which we present in this report, therefore, appears to be against any progesterone-like action of ascorbic acid as described by Israel and Meranze (1941).

SUMMARY.

Ascorbic acid was injected intramuscularly into normal female pigeons and also into female pigeons previously primed with estrogen. Contrary to a previous report no effects similar to those of progesterone were obtained.

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