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Introduction

Most of the literature about the *in vivo* effects of estrogens seems to be primarily concerned with vertebrate reproduction. This emphasis is a natural extension of the earliest recognition of induced estrus. However, it has obscured some reports of the effects of estrogens on organs and tissues which have no known reproductive or post-reproductive functions. The main purpose of this report is to draw attention to one such case.

The presence of large, spherical and acidophilic bodies in the cytoplasm of cells of the spleen of the guinea pig was first reported in 1889 (12). Since then, one hundred or more papers have been published about the presence of these structures in blood cells. At least three of these papers are useful reviews (2, 6, 12), but the most authoritative (2) is only available in Italian, as is most of the literature. In general, the published data refer to differential cell counts of blood smears and no quantitative information which would establish the origins or roles or fates of these bodies has been found.

These bodies, known as Kurloff or Foà-Kurloff Bodies and referred to here as FKB, are abundant in the blood of old guinea pigs, females, and particularly, pregnant females. They are especially numerous a few days after injection of estrogens, even in microgram quantities, whereas progesterone and testosterone are very much less effective. They are rare in very young and gonadectomized adult guinea pigs. There are several reports that gonadectomized guinea pigs also lose the capacity to produce FKB in response to treatment with estrogens (2, 6, 8).

At present we are terminating an investigation of the induction of FKB by estrogens, their origins, and their cytochemical morphology. This report deals primarily with the cytochemical morphology since the data on origins and induction have not been completed. However, there is no question of the greater number of FKB in the spleen of old and female guinea pigs, their relative rarity in the bone marrow, and their absolute rarity in the lymph nodes. Also, a splenic or subcutaneous pellet of diethylstilboestrol induces, within ten days, an increase of FKB/spleen nuclei from less than 2 percent to

about 15 percent in young guinea pigs of both sexes. This induction occurs even when the animal (tested only in males) is dying from 2,4-dinitrophenol incorporated into the diet. This powerful inductive effect is being used to study the origins of the FKB.

We have not been able to induce a clear quantitative increase in the FKB of the spleen by small amounts of estradiol (10 μgms. 100 gm. body weight) which have been effective in inducing increases in circulating FKB (6). Individual variation in the numbers of FKB even in control males makes it difficult to determine threshold or minimal requirements for estrogens. It is our opinion that this kind of information practically requires inbred strains of animals. Our other interests do not permit us to take on the maintenance of such a colony.

We have attempted to maintain guinea pig spleen on the chorioallantoic membrane so that possible direct effects of estrogens in the induction of FKB might be observed, but no maintenance or induction of FKB was observed.

Materials and Methods

The cytological observations reported here are from the spleens of untreated adult female guinea pigs. All material was sectioned at 6 or 8 micra and fixed in 4 percent neutral formaldehyde or cold acetone as noted. Carbohydrate was recognized by the periodic acid-Schiff method with nonoxidized, acetone-extracted, pyridine-extracted, and diastase controls (4), sulfated colloids by toluidine blue (4), protein by brom phenol blue (9), nucleic acids by a toluidine blue modification of the azure B method (3), and desoxyribosenucleic acid, carbohydrate, and protein simultaneously by a combination of techniques.

Observations

The Foà-Kurloff Bodies typically occur as large, regular, clear spheres "capped" on one side by a cup-shaped nucleus, which appears in cross-section as a crescent. The FKB takes up most of the cytoplasm. It is surrounded by a layer of nonbasophilic cytoplasm. The nucleus is condensed and may be pycnotic.

The results of the cytochemical methods are each presented in the following manner: the previous use of the technique, or a comparable technique, with FKB, the chemical significance of the technique, our observations, and the relation of our observations to treatment with estrogens.

Foà-Kurloff Bodies have been reported to color consistently with the periodic acid-Schiff method, as have the Russell Bodies which they resemble in morphology and location (10). A positive reaction

is indicative of glycol and/or mono-amino glycol groups and is characteristic of many carbohydrates (4). However, a negative reaction is not proof that glycol groups or carbohydrates are absent since not all the glycol groups of some carbohydrates react (5). The controls employed here are sometimes said to make the method specific for polysaccharides (4), but this seems questionable (10). In this study the FKB colored strongly and the Russell Bodies colored intensely. The intense reaction of Russell Bodies also occurred after fixation in cold acetone, but the color reaction of FKB did not, although extraction with acetone or hot pyridine did not affect the color reaction of FKB fixed in formaldehyde. This seems to be the first report of a chemical means of distinguishing between FKB and Russell Bodies and its implications for selective extraction of the FKB carbohydrate from guinea pig spleen are obvious. It should be emphasized that the term "carbohydrate" is used loosely and that all we know is that aldehydes are produced by periodic acid oxidation of material which appears to be lipoidal, but not typically so.

The toluidine blue modification of the azure B method for nucleic acids colored the FKB purple, which suggested the presence of sulfated colloids. At neutral pH (4), toluidine blue colored the FKB a clear red. Similar metachromatic staining of FKB has been reported (1). The homogeneous red color of FKB is a striking contrast to the deep blue color of plasma cells. Thus, despite the similarities of FKB and Russell Bodies, the cells which appear to produce them are quite different. The great increase in FKB which can be induced by estrogens is not accompanied by any obvious increase in plasma cells. We do not know if an increase in plasma cells induced by antigen would be accompanied by any change in the numbers of FKB.

The staining of FKB with the Brom phenol blue method for protein has not been reported previously. The method is reported to be a specific and sensitive method for protein (9). Both Russell Bodies (11) and the FKB seem to contain small amounts of protein.

The coloring of FKB with a combination of periodic acid-Schiff, Feulgen-Azure A, and acid dye methods has not been reported previously. The colors signify high concentrations of carbohydrate, desoxyribosenucleic acid, and protein, but the combined technique is primarily designed for convenient observation rather than cytochemical exactness. It is the best general method for FKB. Details of the technique and references will be provided upon request and will be included in a later publication.

Discussion

The observations cited here strongly suggest that the FKB are a morphologically and chemically distinct form of leukocytic differen-

tiation. However, the fact that estrogens induce an increase in similar, but smaller, bodies in the monocytes of other species, including the human (2), suggests that the FKB is a quantitative exaggeration of a process common to many species, rather than the result of a qualitatively unique process.

It should be noted that it is easy to induce estrogen-dependent tumors in the guinea pig by prolonged doses of estrogens. This does not seem to have received much attention even during the recent enthusiasm for estrogen-dependent tumors of hamsters and mice. The induction of such tumors is a separate and well-documented, but apparently little known, subject with a thirty year history. Such tumors may be accompanied by or contain many FKB. (References on request.)

It is likely that the FKB have a bearing on the interpretation of two other types of publications. One type reports that the blood of female guinea pigs contains more eosinophils than does the blood of the male. These reports make no mention of FKB which are several times more numerous in the blood of the female. Since the FKB can be stained with acidic, as well as basic dyes, it is possible that they are being counted in some laboratories as eosinophils. Another type of publication reports that estrogens stimulate phagocytosis of soluble dyes by cells of the spleen of guinea pigs. The "phagocytized particles of dye" are obviously identical with FKB which are known to stain with vital stains. Unfortunately, these observations have been taken to prove that estrogens stimulate phagocytosis in general. No mention is made of FKB, and the authors appear unaware that the bodies they observed are produced in response to estrogen alone. (References on request.)

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