Effect of Estrogen and Progesterone on Leukocyte Glycogen and Alkaline Phosphatase in Dogs (34178)

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In the mature neutrophil leukocyte, the major functional organelles are limited to the specific neutrophil granules and glycogen particles (1, 2). Changes in these cytoplasmic constituents can be determined by measuring glycogen and leukocyte alkaline phosphatase (LAP) activity, the latter being a marker for the specific granules (1).

Leukocyte glycogen and LAP are significantly higher in females than in males (3), suggesting hormone effects. During the later months of pregnancy when blood estrogen and progesterone levels are elevated (4, 5), there is an elevation of leukocyte glycogen and LAP (3). The synthetic estrogen $\alpha\alpha'$-diethyl-4,4-stilbendial, (stilbesterol), also causes significant elevations of both leukocyte glycogen and LAP in males treated for carcinoma of the prostate (3). In contrast, another synthetic estrogen, 17-ethynylestradiol 3-methyl ether (mestranol), given to humans as an anovulatory agent, caused an increase in LAP but not glycogen (3). Failure to cause elevation of both glycogen and LAP may have been due to the relatively small dose of mestranol used. The present study was performed to test this possibility and also to determine whether progesterone could cause similar changes in leukocytes.

In this study the response of male dogs to mestranol or progesterone was studied. Both were given in high doses relative to human therapeutic regimens and the effect on leukocyte glycogen and LAP values was studied. The results show that mestranol can produce an increase in leukocyte glycogen and LAP values. Progesterone caused an increase in LAP only.

Methods. Leukocyte suspensions were derived from blood which had been treated with dextran to remove red cells as described previously, (3, 6) with the following modifications: 2 ml of dextran were mixed with 10 ml of blood, and the leukocytes were sedimented and washed at 65*g*. This speed lowered platelet contamination to less than 8000 platelets/mm^3 in the final suspension. Since platelets contain only 1/100 as much glycogen as neutrophils (7), their contribution was negligible.

Leukocyte alkaline phosphatase scores were determined on fresh blood smears by the histochemical method of Kaplow (8).

Two groups of nine mongrel male dogs were used as subjects. Two or more blood samples were taken from each dog on consecutive days during a control period. These values were averaged for each group. One group was then given mestranol 0.24 mg (3 tablets) daily in a small amount of food; and blood samples were taken on the sixth through the eighth day and averaged for the group. The second group of dogs received 5 mg/kg of progesterone in sesame oil by daily intramuscular injection (five or more times the human dose).

Results. The first group of dogs (Fig. 1) had leukocyte glycogen levels of 7.12 $\pm$ 0.45 SE mg/10^9 neutrophils and LAP scores of 7.5 $\pm$ 1.3 SE per 100 neutrophils. After 5 days of mestranol treatment the average leukocyte glycogen content rose to 9.03 $\pm$ 0.43 SE mg/10^9 neutrophils and the LAP scores rose to 19.2 $\pm$ 2.3 SE per 100 neutrophils. These increases were statistically significant ($p<0.005$ and $p<0.025$, respectively).

In the second group of dogs (Fig. 1) the control values were 7.46 $\pm$ 0.45 SE mg of glycogen/10^9 neutrophils and 8.2 $\pm$ 1.4 SE

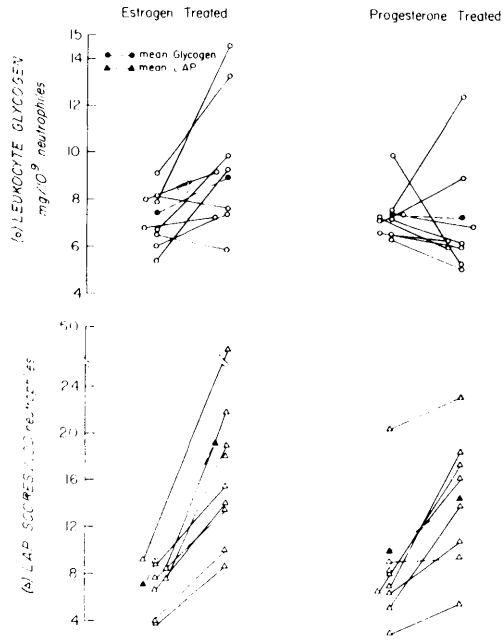

FIG. 1. Leukocyte glycogen and LAP before and after drug treatment: (upper and lower left), leukocyte glycogen (○) and LAP (△) values before and after treatment with mestranol. Posttreatment values for glycogen and LAP are significantly higher than the base line values ($p<0.005$ and $p<0.025$, respectively). (upper and lower right), Glycogen and LAP values before and after progesterone treatment. Posttreatment values for LAP alone are significantly higher than the base line values ($p<0.001$).

LAP score per 100 neutrophils. After 5 days of progesterone treatment the average leukocyte glycogen was 7.48 ± 0.54 SE mg/10^9 neutrophils, which was not significantly different from the control values. The average LAP score, however, rose to 14.6 ± 0.98 SE per 100 neutrophils which was significantly elevated ($p<.001$).

Discussion. These studies show that mestranol, when given in high doses, can result in elevation of *both* glycogen and LAP in leukocytes. In the doses ordinarily used in humans (about one-fifth of the dose used here) the stimulus was insufficient to produce readily detectable changes in glycogen content (3). Perhaps changes in LAP are more readily detectable due to technical factors. Species differences cannot be absolutely ruled out in the response, but animal testing is a reasonable alternative to testing humans with unusually high doses of the hormone.

Both leukocyte glycogen and LAP (as a specific granule marker) seem to vary in parallel instances in which they have both been measured. This led to a hypothesis that, since these two organelles represented the major functional machinery of the mature cell (9), their synthesis might be controlled by a single genetic mechanism. If an unequivocal instance could be found in which one varied independently of the other, this hypothesis would be untenable. Mestranol as a stimulus proved to be capable of stimulating both in appropriate dose, but progesterone therapy (in high doses) does result in elevation of LAP *without* change in glycogen. This, then, implies that these two organelles are controlled separately during leukocyte development.

Summary. Mestranol, a synthetic estrogen, causes significant increases in both leukocyte glycogen content and leukocyte alkaline phosphatase activity in dogs. Progesterone, by daily intramuscular injection, causes significant elevation of leukocyte alkaline phosphatase activity, but leukocyte glycogen content is unchanged.

This work was supported by the Blood Research Fund of the Mecical College of Virginia and Grant CA 08482-04 from the U. S. Public Health Service. Y. Abul-Fadl, M.D. was supported by a fellowship of the Leukemia Society of America, Inc. We thank LaVerne W. Cooper, Mary Vaughan, and Ronald Hamon for technical assistance, and Dorothy Barnes and Lucille Gouldin for expert secretarial assistance. We are indebted also to G. Watson James, III, M.D., for his support. Requests for reprints should be addressed to R. B. Scott, M.D.

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Received April 29, 1969. P.S.E.B.M., 1969, Vol. 132.