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Influences of dehydroepiandrosterone acetate on ovarian oocytes in mature cycling rats

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Summary. Degeneration of ovarian oocytes occurred to a remarkable extent in rats with polycystic ovaries induced by dehydroepiandrosterone acetate (DHA-Ac) administration. The ratio of degeneration oocytes, compared with the total oocytes examined, finally exceeded 70%.

Experimental induction of polycystic ovaries (P.C.O.) in the rat, in which anovulatory cycles followed by cystic change in the ovarian follicles were observed, was reported by Mahesh et al.[2] and by Ward et al.[3]. The characterization of the cystic follicles was attempted[4]; however, further details of the changes are required, as an animal model for P.C.O. The present study was performed to elucidate the influence of the pathologic environment on the ovarian oocytes.

Wistar rats, 9 weeks of age, were used. The animals were kept in air conditioned rooms at 24 °C with lights switched on at 09.00 h and off at 21.00 h. Solid laboratory chow and water were supplied ad libitum. DHA-Ac was administered s.c. 1 mg/100 g b.wt/day (1-mg group) or 10 mg/100 g b.wt/day (10-mg group) for 4, 7, 11 and 14 consecutive days respectively. A non-treated (control) group received injections of the vehicle only. Vaginal smears were obtained by lavage every day to eliminate non-cycling rats before the treatment and to determine estrus cycles during the treatment. In the 10-mg group 96% of the animals showed constant diestrus smears until day 5 of administration whereas the smears in the 1-mg group were less uniformly constant. Animals showing diestrus smears were preferred for oocyte collection in both the control and DHA-Ac-treated groups.

The ovaries were dissected free of adipose and connective tissue and were transferred to a Falcon dish (3.5 cm in diameter) containing 5.0 ml of physiological saline solution. The oocytes were liberated by random puncturing under a stereoscopic microscope, using 10-fold magnification[5]. The oocyte-containing solution was transferred using a glass micro-pipette to a test tube with several drops of 1% aceto-orcein solution.

The test tube was left for about 30 min. The supernatant was discarded, and the whole sediment was mounted on a glass slide with a cover slip[5]. The oocytes were examined

Degeneration ratio is defined as percent ratio of degenerating oocytes to the total oocytes examined. Note the remarkable increments in the degeneration ratio for the 1-mg group on day 14 and for the 10-mg group on day 11, and that the ratios in both groups exceeded 70% ultimately

Group	Number of animals	Oocyte observed	Physiological stage			Abnormal findings of oocyte				Degeneration ratio (mean ± SD) %
			Dictyate	M-1 to 1-P.B.	Total	Deformation	Fragmentation	Others	Total	
Control	8	357	172	17	189	98	13	57	168	47.1 ± 6.8
1-mg/100 g b.wt/day										
4-d	4	201	80	13	93	77	10	21	108	53.7 ± 5.9
7-d	4	213	75	13	88	58	31	36	125	58.7 ± 3.8
11-d	4	238	82	15	97	69	41	31	141	59.2 ± 3.3
14-d	4	236	52	18	70	81	52	33	166	70.3 ± 4.5
10-mg/100 g b.wt/day										
4-d	4	179	80	10	90	59	4	26	89	49.7 ± 7.0
7-d	4	213	91	14	105	57	17	34	108	50.7 ± 5.3
11-d	4	205	43	13	56	93	28	28	149	72.7 ± 5.6
14-d	4	211	49	15	64	68	30	49	147	70.0 ± 13.0

under a phase contrast microscope with 100–400-fold magnification. The physiological stage of meiotic maturation was classified as intact germinal vesicle (dictyate) and metaphase I (M-1) to metaphase II with extrusion of the first polar body (1-P.B.). Oocyte abnormalities suggesting degeneration consisted of a) obvious deformation of oocytes described as a helmet, a kidney, an amoeba or an ovoid shape, b) fragmentation of the oocyte and c) others such as oocytes with cytoplasmic granulation or pigmentation[6].

Degeneration ratio is defined as percent ratio of degenerating oocytes to the total oocytes exmained. As was shown in the table, the degeneration ratio of the 10-mg group increased significantly in comparison with that of the control group; the ratio $72 \pm 5.6\%$ (mean $\pm$SD) was reached on day 11 ($p < 0.01$), and this ratio was maintained on day 14. A similar remarkable increase in the degeneration ratio of the 1-mg group was observed on day 14, when it reached $70.3 \pm 4.5\%$.

Strangely enough, in spite of the uniform symptom of anovulation, little observation has been focussed on the oocytes of experimental animals or patients with the P.C.O. syndrome. Knudsen et al.[7] reported that in rats it was possible to induce ovulation using pregnant mare serum gonadotropin (PMSG) and/or human chorionic gonadotropin (HCG) after DHA administration, but the recovery of ovulated ova from the Fallopian tubes by flushing was as low as 2–4 ova per animal. The increase in the degeneration ratio of ovarian oocytes seen in the present study cannot be the full explanation for this poor recovery of ova from the Fallopian tubes, but may be an important factor, because a degenerated oocyte probably does not contribute to ovulation. Mrinal et al.[8] categorized human ovarian oocytes into 4 basic types, and reported that large numbers of oocytes from P.C.O. (77%) were degenerating and frequently contained massive clumps of chromatin material associated with the nucleolus. Our results show that oocyte degeneration occurs in the animal model as well, and the degeneration ratio finally exceeded 70%. In this respect, the experimentally induced P.C.O. in rats, and human P.C.O., seem to have a common feature. As for the mechanism which gives rise to degeneration of ovarian oocyte and the relationship with cystic change of ovarian follicles, future work is awaited.

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Hypercalcitonemia in pernicious anemia

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Summary. Gastrin has been suggested as a natural secretogogue of the hormone calcitonin. We have found hypercalcitonemia in 55% of patients with pernicious anemia, and the gastrin levels, although usually increased, did not correlate.

Several investigators have demonstrated that gastrin may be a secretogogue for the hormone calcitonin. In vitro, pentagastrin releases calcitonin from slices of normal human thyroid[1]. In vivo, the injection of pentagastrin increases both serum and urinary calcitonin[2,3]. In the pig, an increase of endogenous gastrin was thought to mediate secretion of calcitonin from the thyroid gland[4]. Patients with pernicious anemia are known to have high levels of endogenous gastrin, presumably as a result of their achlorhydria[5–7]. Accordingly, a study was undertaken of serum calcitonin levels of such patients.

Materials and methods. A morning fasting serum specimen was obtained from 11 patients with pernicious anemia on replacement with parenteral vitamin B_{12}. Calcitonin was determined by radioimmunoassay by the procedure we have described, using Ab-I, an antibody with recognition for several regions of the calcitonin molecule[8,9]. Serum gastrin was determined by a modification of the method of McGuigan[10]. Serum total calcium was determined by atomic absorption spectrometry, and serum ionized calcium was determined by the Orion ion flowthrough technique[11].

Results. The mean serum calcitonin was 412 ± 335 pg/ml (SD), and the range was 50–1,100 pg/ml (95% upper confidence limits for normal: 260 pg/ml). 6 patients, or 55%, had increased serum calcitonin. The mean serum gastrin was 1490 ± 1160 pg/ml, and the range was 126–3206 pg/ml (95% upper confidence limits for normal: 200 pg/ml). 81% of patients had increased serum gastrin. Serum total and ionic calciums were all within the normal range. There was no significant correlation between the level of serum calcitonin and gastrin, nor between either of these hormones and the serum total or ionized calcium.

Discussion. It has been demonstrated that the gastrointestinal hormone, gastrin, may influence calcitonin secretion. Hypercalcitonemia has been found in patients with increased serum gastrin due to Zollinger-Ellsion syndrome, and this was interpreted as suggesting a possible interhormonal relation[12]. In the present study, while hypercalcitonemia occurred in over half of the pernicious anemia patients, it did not correlate with serum gastrin levels. Fahrenkrug et al.[6] have measured serum calcitonin levels in pernicious anemia, and found no difference from controls, although all of their patients had increased serum gastrin. In contrast, Franchimont and Heynen[7] found increased serum calcitonin in 25% of their patients, and also noted no correlation with levels of serum gastrin. Serum calcitonin exists in multiple heterogeneous forms, and different antisera detect these various forms with different avidity[9]. This phenomenon may explain why all investigators are not in accord that hypercalcitonemia may occur in pernicious