regular 4–5 day estral cycle. The animals were kept on a 14-hour light and 10-hour dark schedule and water and food were available ad libitum.

Atropine sulphate (150–250 μg) was tamped into capillary tubes (O.D. 300 micra-ID 150 micra), the tips of the tubes being sealed with a thin layer of sucrose. The animals were anesthetized with sodium penthobarbital (30 mg/kg) and the tubes were implanted stereotaxically, according to the DE GROOT atlas, in the lateral anterior hypothalamus. The tubes were fastened to the skull surface with dental acrylic. In a control group, tubes containing paraffin were implanted in the same zone.

Daily vaginal smears were taken during the post-operative period.

Hemicastration was accomplished by the lumbar route simultaneously with the implantation of atropine or paraffin. The excized ovaries were used as a control of the weight of the remaining ovary in each animal.

In a group of animals regarded as absolute controls, only hemicastration was carried out.

At the end of the experiment the animals were killed under ether and the remaining ovary, the uterus, adrenals, thyroids and pituitary were carefully dissected and weighed on a torsion balance. Histological sections of both ovaries and uteri were performed. The brains were fixed in 10% formalin and serial sections were made and examined to determine the precise location of the implant.

In the hemicastrated atropine-implant animals there was no change in the remaining ovary (Table). In those animals hemicastrated and implanted with tubes containing paraffin in the same area and with the same evolution in time, compensatory hypertrophy developed (Table).

In the controls, the increase in ovarian weight was greater than in the paraffin-implanted animals, although the difference was not significant. Some other hemicastrated atropine-implanted animals, allowed a longer evolution (28 days) and exhibited the type of ovarian compensatory response seen in the controls.

The vaginal smear showed no difference in length or in number of days of cornification in any of the groups, neither was there a significant change in the weight of the other endocrine organs. In the hemicastrated atropine-implanted animals, the prolonged dioestrus seen in whole animals was not observed.

It is known that the appearance of an ovarian compensatory hypertrophy is permanently inhibited by lesions of various hypothalamic areas. Animals allowed to live longer demonstrated that the atropine-induced inhibition of gonadotrophin release was a transient occurence.

The same dose of atropine found to arrest ovulation, temporarily inhibits the appearance of ovarian hypertrophy in the hemicastrated animals. Since the cholinergic inhibition is different in whole and hemicastrated animals, we assume this to be related to the type and amount of folliculotrophic hormones released[4].

Group	No. of animals	Evolution (days)	Increment of ovary weight mg/100 g body weight	
			Mean	Range
Atropine	10	12–14	0.80	—2.6–3.5
Paraffin	10	12–14	6.55	2.9–11.3
Control	10	12–14	10.37	4.0–23.1

Résumé. Des injections d'atropine dans l'hypothalamus antérieur et latéral de la rate font disparaître l'hypertrophie compensatrice de l'ovaire qui suit l'hémicastration. On conclut que dans ces conditions, l'atropine agit par des voies cholinergiques en relation avec la sécrétion des gonadotrophines.

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Peritoneal Fluid Cytodifferential Changes Associated with the Administration of Cortisone

Cytodifferential changes in the cellular composition of abdominal fluid offers a unique opportunity for studying endocrine influences on the peritoneal cavity[1–7]. We observed that the relative proportion of cells in aspirated cytologic specimens were characteristically altered during the estrous and menstrual cycles[1,2,5,7]. Furthermore, estrogen administration increased polymorphonuclear leukocyte counts and reduced mesothelial cell distributions whereas male hormone had an opposite effect. Since cortisone administration produced no increase in mononuclear cells in peritoneal fluid[7–9] we find it difficult to explain the decrease in blood lymphocytes by a movement of cells from blood into peritoneal fluid following glucocorticoid injection. Possibly, cells originate locally and cortisone inhibits mitosis as well as the release of these cells into body fluids[9]. However, physiologic saline administration markedly changed the normal cellular content of peritoneal fluid[10]. In the present study, we investigated the influence of cortisone in saline administered s.c. on the cellular distribution of peritoneal fluid in adult female mice.

Method. We injected micronized cortisone in 0.5 ml physiologic saline (0.9%) s.c. for 21 days at daily doses of 0.025, 0.1, 0.5, 1.5 or 5.0 mg to adult female mice (CF-1 strain; 20–25 g; 6–38 animals/group). One group of mice received no injections (untreated control) while another group (saline control) was given 0.5 ml physiologic saline daily without steroid. We weighed the animals on day 1 and day 22, immediately before autopsy, we aspirated each animal for abdominal serous fluid and then removed and weighed the adrenals and thymuses. The average weight of these organs was expressed as mg/100 g body weight.

Serous abdominal fluid was aspirated by a 27 guage needle from the animal's ventral surface. We spread the aspirated specimen on an albumin-coated slide, stained

Cytodifferential changes in female mice peritoneal fluid associated with the administration of cortisone

	Control	Saline	Cortisone (mg/day) × 21				
			0.025	0.1	0.5	1.5	5.0
No. of mice	38	30	15	17	33	17	6
Final body weight (g)	24.0 ± 0.3	24.1 ± 0.3	24.1 ± 0.2	22.9 ± 0.3	21.0 ± 0.2	20.7 ± 0.5	19.1 ± 1.4
Body weight change (g)	+2.4	+2.0	+2.6	+1.5	−0.4	−0.5	−4.7
Adrenals (mg/100 g)	36.3 ± 1.7	39.2 ± 2.1	40.5 ± 2.6	52.0 ± 2.8	42.0 ± 14.4	29.2 ± 2.6	21.4 ± 2.6
Thymus (mg/100 g)	221.2 ± 12.2	219.8 ± 12.3	239.1 ± 10.4	200.2 ± 16.3	99.6 ± 15.6	47.0 ± 7.9	41.2 ± 8.0
	Number of cells/200 cell count						
Mesothelial cells	104.9 ± 2.3[a]	91.3 ± 5.6	103.8 ± 3.8	96.2 ± 2.8	91.2 ± 4.5	48.4 ± 2.9	76.2 ± 12.4
Lymphocytes	55.1 ± 2.6	71.4 ± 6.2	44.5 ± 3.7	44.1 ± 3.4	47.5 ± 4.9	18.9 ± 3.1	10.2 ± 1.0
Polymorphonuclear leucocytes	10.1 ± 2.6	2.4 ± 0.8	12.8 ± 1.6	15.1 ± 1.2	4.4 ± 1.3	70.0 ± 4.7	84.3 ± 16.4
Histiocytes	9.2 ± 0.6	2.8 ± 0.7	15.3 ± 1.1	11.8 ± 0.9	4.2 ± 0.8	23.1 ± 1.8	16.3 ± 3.0
Mast cells	1.7 ± 0.2	0.2 ± 0.1	2.5 ± 0.5	1.8 ± 0.3	1.9 ± 0.4	2.1 ± 0.6	1.6 ± 0.4
Bare nuclei	18.5 ± 2.0	28.6 ± 3.9	19.5 ± 4.3	29.1 ± 4.0	49.8 ± 4.7	23.3 ± 4.7	9.7 ± 3.7
Monocytes	0.5 ± 0.1	0.1 ± 0.1	0.4 ± 0.2	0.2 ± 0.2	0.3 ± 0.2	0.2 ± 0.3	0.5 ± 0.2
Daisy cells	0.0 ± 0.0	0.2 ± 0.2	1.0 ± 0.3	1.2 ± 0.3	0.8 ± 0.3	13.9 ± 1.4	1.5 ± 1.0

[a] Mean ± S.E.

it by PAPANICOLAOU's procedure[11], and 200 consecutive cells were randomly counted and grouped as mesothelial cells, lymphocytes, polymorphonuclear leukocytes, histiocytes, mast cells, bare nuclei and daisy cells. Bare nuclei are cells without cytoplasm and daisy cells may represent a varient mesothelial cell with the nucleus bulging in the form of a daisy.

The standard error for adrenal, thymuses and body weights as well as for each mean cellular count was calculated using the formula, S.E. $= \sqrt{\Sigma d^2/N\,(N-1)}$. We used Student's t-test to obtain the necessary probability values (p) for significant differences between the various means[12].

Results. The distribution of cells listed in the Table indicates that s.c. injection of cortisone produced a marked alteration in the cellular distribution of female mouse peritoneal fluid. We observed that high doses (1.5 mg) of cortisone decreased mesothelial cell counts in peritoneal fluid when compared with saline control values ($p < 0.001$). Daisy cells, which are not generally seen in uninjected control smears of mice, represented about 7% of the total cellular counts of specimens aspirated from 1.5 mg cortisone-treated animals. Lymphocyte counts were gradually reduced by cortisone to a 5% cellular distribution and the rise in polymorphonuclear leukocytes represents a 35-fold increase. Small steroid doses appeared to normalize cellular changes in counts induced by saline alone. This was particularly evident with histiocytes, mast cells and monocytes. We recorded that mice given 5.0 mg steroid in saline s.c. each day for 3 weeks lost an average of 4.7 g body weight and only 6 of 22 animals survived. The decrease in adrenal and thymus weights clearly established the biological activity of cortisone.

Conclusion. Subcutaneous administration of cortisone in saline for 21 days over a daily dose range of 0.025 to 5.0 mg to adult female mice decreased lymphocyte and mesothelial cellular counts of peritoneal fluid. Possibly, cortisone inhibited the production of these cells. These data also suggest a conversion of mesothelial cells to the daisy cell variety and bare nuclei. An irritation produced by the higher cortisone doses could partly explain the rise in polymorphonuclear leukocytes. Nevertheless, smaller steroid doses tended to normalize histiocyte, mast cell and monocyte distributions in peritoneal fluid.

Résumé. L'administration par voie s.c. à des souris femelles adultes, d'une dose quotidienne (de l'ordre de 0,025 à 5 mg) de cortisone en solution saline pendant 21 jours, fait diminue le nombre des lymphocytes et des cellules mesothéliales du liquide péritonéal. Il est possible que la cortisone inhibe la production de ces cellules. Ces données suggèrent aussi une transformation des cellules mésothéliales en «daisy cells» à noyaux vides. L'irritation produite par les très fortes doses de cortisone pourrait, en partie, expliquer l'élèvation du nombre des leucocytes à noyaux polymorphes.

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