THE ADVERSE EFFECT OF OESTROGEN ON THE RESISTANCE OF MICE TO STRESS

By

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Stressing agents such as formalin, cold, etc., produce adrenal hypertrophy accompanied by splenic and thymic atrophy in experimental animals (Selye, 1950). Oestrogen administration generally results in similar changes, and because of the similarity of response, oestrogens occasionally have been referred to as stressors (Selye, 1940).

In current studies of mouse mammary cancer, we have been administering oestradiol by the subcutaneous implantation of 3–4 mg. pellets. Young C3H/He mice show little effect of such treatment, other than a slight decrease in body weight. In view of the possible stressor role of oestrogen mentioned above, it is of interest to determine whether or not this routine oestrogen treatment has effects, other than upon the mammary gland and other target organs, which might complicate interpretation of data. In particular, we have been interested in the possibility of an increased susceptibility to subsequent stress, resulting from hormonal treatment. In the present study, we have compared the resistance of normal and oestrogen-pretreated BALB/c («low mammary-tumour») mice of both sexes to formalin injections and to cold. Because of the involvement of adrenals, spleen, and thymus in the response both to stress and to oestrogen, special attention was paid to changes in these organs.

MATERIALS AND METHODS

A total of 196 BALB/c mice of both sexes, 65 ± 2 days of age, was used in these studies. 85 mice received β-oestradiol pellets weighing 3–4 mg. implanted subcutaneously by trocar either on the medial surface of the right thigh or on the dorsum. The other 111 animals received cholesterol pellets similarly implanted (with the exception
of 29 untreated males in the formalin experiment). Each animal was kept in a separate cage during the course of the experiments.

**Formalin experiments.** – 84 male mice were separated into 3 groups: 29 unimplanted, 25 cholesterol-implanted, and 30 oestrogen-implanted. 21 days after implantation of the steroid pellets, formalin treatment was commenced on 10 unimplanted, 15 cholesterol-implanted, and 20 oestrogen-implanted animals. The formalin treatment consisted of twice daily subcutaneous injections of 0.06 ml. 10% formalin (4% formaldehyde) into the dorsal region, beginning at the level of the scapulae and giving each subsequent injection 2 mm. caudal from the preceding site. On the 8th day the quantity of injected formalin was doubled (to 0.12 ml. per injection). At the time of death or of termination, a complete autopsy was performed, and the existence of the implanted pellet was verified. Paired adrenals, thymus, and spleen were weighed on a Roller-Smith balance, and a ratio was computed representing mg. organ weight/gm. final body weight. Adrenals were fixed in Bouin’s fluid and/or formol-saline for histologic study. Paraffin sections were stained with alum-hematoxylin-eosin or modified Masson’s trichrome.

A similar experiment was performed employing 25 cholesterol-implanted and 24 oestrogen-implanted female mice. Formalin was injected as above into 15 and 14 mice respectively from each of these two groups beginning on the 21st day of the experiment.

**Cold experiments.** – 27 male mice were separated into two groups: 14 cholesterol-implanted and 13 oestrogen-implanted. 11 days after pellet implantation each animal was placed in an individual cage in a cold room at +5°C. until all but one of the oestrogen-implanted mice had died. For the first 20 days at this temperature the animals protected themselves from the cold by nesting in the shavings. On the 21st day the shavings were removed, and the empty pans were changed every few days. Autopsies were performed at time of death or sacrifice, and the adrenal glands were weighed and preserved.

Similarly, 36 female mice were separated into two groups: 18 cholesterol-implanted and 18 oestrogen-implanted, and were treated as described above. However, the shavings were removed from all cages at the time of subjection to cold (11th day); hence, the effect of the cold stress was more quickly evident.

**RESULTS**

It is clear from examination of Figs. 1–3 that prior oestrogen treatment of the mice greatly reduced resistance to formalin and to cold stress. After 10 days of formalin treatment, all oestrogen-treated mice had died, whereas about 2/3 of the male and 1/3 of the female controls remained alive. Among the cold-stressed male mice, all but one of those implanted with oestrogen had died by the 24th day in the cold room after shavings had been removed, whereas 42% of the cholesterol-implanted mice were still alive. No oestrogen pellet could be found at autopsy in the single surviving, presumably oestrogen-implanted, male mouse. No female mouse subjected to cold after 10 days of oestrogen treatment and kept in shavings-free cages from the beginning of the cold stress survived more than 11 days, whereas about 1/3 of the stressed cholesterol-implanted
controls survived until the 18th day of cold treatment at which time the experiment was terminated.

The oestrogen-implanted males and females both lost weight initially, a loss which was less evident in the females. By the end of the formalin experiment, the unstressed males showed a 10% loss in body weight, whereas the initial loss was regained by the females. The untreated controls gained about 4-5%. The characteristic reactions to formalin stress were observed, and progressive paralysis and necrosis of the hind limbs developed several days before death.

Fig. 4 summarizes the changes in adrenal weight seen in both the formalin and cold stress experiments. The organ weight (mg.)/body weight (gm.) ratios

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**Fig. 1.**

Daily % survival of male and female mice subjected to formalin stress 21 days after implantation of oestrogen pellet, as compared with their controls.

E = oestrogen-implanted; C = cholesterol-implanted or unimplanted.

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**Fig. 2.**

Daily % survival of male mice subjected to cold (see text) 11 days after implantation of oestrogen pellet (E), as compared with their cholesterol-implanted controls (C).

* indicates mouse that failed to show oestrogen part in autopsy.
Daily % survival of female mice subjected to cold 11 days after implantation of oestrogen pellet (E), as compared with their controls (C).

Response of adrenals from male and female BALB/c mice to oestrogen and/or stressors. Ratios of organ weight (both adrenals in mg.)/final body weight (in gm.) (mean ± SEM) are given, as well as the mean absolute weights of both adrenals (in parentheses).

C = cholesterol-implanted or unimplanted; E = oestrogen-implanted;
F = formalin-stressed; T = cold-stressed; n = number of mice.

and the absolute organ weights (the latter in parentheses) are given. In the BALB/c strain, the normal female adrenal is about twice as large as that of the male. Oestrogen treatment alone results in a doubling of the weight of the
male adrenal and in a significant but smaller increase in the female adrenal. Formalin or cold alone result in the expected stress-associated adrenal hypertrophy, and the addition of a stressor to oestrogen treatment results in adrenal weights significantly greater (at the 1% level of confidence) than those seen after oestrogen treatment alone, except in the formalin-stressed, oestrogen-

Fig. 5.
Sections of adrenals from male (above) and female (below) BALB/c mice to show effects of oestrogen treatment and/or formalin stress. Note juxtamedullary zone of «brown degeneration» after one month of oestrogen treatment. Masson's trichrome. × 115.
treated males, where the significance is at the 5% level. In the male, combination of oestrogen and stress result in significantly larger adrenals than did the stressor alone; in the female, however, no significant difference was seen.

Histologic examination of the adrenals reveals at least two processes consequent to oestrogen treatment: (1) adrenocortical hypertrophy and (2) alteration of the inner cortical zones. These changes are illustrated in Figs. 5 and 6. An X-zone is seen in the control 2–3 month old virgin female BALB/c mouse adrenal; however, this zone was not always distinguishable in the oestrogen-treated and/or stressed mice. The inner cortical alteration is identical with that previously described in several mouse strains as »brown degeneration«. A simple razor-blade section of the formalin-fixed gland reveals a distinct brown zone in the juxtamedullary region, which is not seen without oestrogen treatment. This zone consists of masses of highly vacuolated cells with indistinct cell boundaries. This picture is present in oestrogen-treated mice whether stressed or not.

Fig. 7 summarizes the splenic response to oestrogen and/or formalin stress. As can be seen, treatment with combined oestrogen and stressor causes significantly greater atrophy than either alone. Thymic atrophy was reflected in a decrease in the mean ratio of thymus weight (mg.)/body weight (gm.) from about 1.5 in the normal male and 2.0 in the normal female to about 0.6 in the stressed and/or oestrogen-treated male and about 0.4 in the female, with no
Fig. 7.
Response of spleen from male and female BALB/c mice to oestrogen treatment and/or formalin stress. Ratios of spleen weight (in mg.)/final body weight (in gm.) (mean ± SE_m) are given. Symbols as in Fig. 4.

significant differences in the degree of atrophy resulting from the several treatments.

DISCUSSION

It is evident that pretreatment of BALB/c mice with moderate doses of oestrogen as conducted herein results in a considerably reduced resistance to the stressors employed (formalin, cold). This is a facet of the organismal response of mice receiving oestrogen as a tumour-inciting agent, which cannot be ignored. With the pellet method of oestrogen administration the daily dosage can be estimated, based upon Bishop & Folley's (1952) calculations that the absorption rate in man and in the rat of oestradiol from pellets (50 and 100 mg.) was around 0.24% per day. With the 3–4 mg. pellets used herein, this would presumably mean a daily absorption of about 7.5–10 μg., a dose of oestradiol higher than the physiologic level. Daane & Lyons (1954) used a daily dose of 2.5 μg. oestrone to induce mammary development in male mice, which is presumably near the physiologic level. Whereas the body weight changes observed may indicate a toxic effect of the oestrogen, uterine hypertrophy was not excessive (no pyometria) in the female, and keratinizing metaplastic changes were not seen in the anterior prostates of the male.

As early as 1936, Selye, Harlow & Collip presented data which were inter-
interpreted to indicate that oestrogen acts as a stressor in Selye’s conventional sense. In later experiments wherein enormous daily doses of oestrogen were employed (as high as 2 mg. per 100 gm. rat), Selye (1940) interpreted his observations in terms of the general adaptation syndrome (see also Selye, 1950, p. 46). In these terms as applied to our experiments, one could refer to the oestrogen itself as a stressor, with formalin or cold serving as a second stressor. The »doubly stressed« mice succumb more readily owing to the decrease in »crossed resistance«. Lurie et al. (1949) state that »mice undergo the alarm-reaction and respond with the general adaptation syndrome as described by Selye«. The increased susceptibility to stress after oestrogen administration can be variously interpreted. Adrenocortical hypertrophy is generally observed following oestrogen administration (Allen & Bern, 1942; Tepperman et al., 1943), and some evidence from the rat indicates increased corticotrophin and (gluco)corticoid secretion resultant from oestrogen treatment (Gemzell, 1948, 1952; Selye, 1950, p. 333). Gemzell’s data (1948) point to adrenocortical activation even in the physiologic dose range. Such stimulation could result from the specific hormonal action of oestrogen upon the hypothalamus-hypophysis system and/or the non-specific action of a toxic agent which increases the utilization of corticoids by the tissues. Despite the possession of hypertrophic and possibly hyperfunctional adrenals, the oestrogen-treated mouse is evidently less able to withstand a stressful situation. Selye (1955) states that the toxic effects of oestrogen are masked by the activated pituitary-adrenal cortex axis.

Recently Vogt (1955) concluded that hexestrol inhibited normal adrenal function in the rat. However, her description of the resultant hypertrophied adrenals as being engorged with blood and depleted of lipid differs from what we observe in our oestrogen-treated mice, where the adrenals are pinkish-white in colour and lipid-laden. In addition, hexestrol did not inhibit adrenocortical secretion in the rabbit, so that the rat response to this synthetic oestrogen may be species-specific.

The oestrogen-induced weight loss of about 10 % in the male and the failure to gain in the female point to a possible restriction of food intake and interference with normal growth. This may be engendered in part by increased glucocorticoid secretion (and/or by »conditioning« the action of glucocorticoids – see Selye, 1955), since cortisone and related compounds are known to have such effects (e.g., Meites, 1952). Caloric restriction – resulting from decreased food intake and decreased efficiency of absorption of food from the gut and subsequent utilization (cf. Meites, 1952) – may be a major factor in the observed decreased resistance to stress. However, Meites (1949) reported no decrease in food consumption of rats treated with 0.1 mg. of oestrone or oestradiol daily. Furthermore, Cataldi (1953) has claimed that the administration of oestradiol benzoate protects female rats to some extent from the effects of simultaneous inanition. In the formalin experiment (Fig. 1), the untreated female mice showed
greater susceptibility to stress than the males. Oestrogen-treated females also were less resistant than oestrogen-treated males, despite the fact that the loss of body weight was less severe in the female than in the male.

In addition to caloric restriction, vitamin deficiency may play an important role in the decrease in resistance. Meites (1952) has shown that administration of vitamin B₁₂ mitigates in part the inhibiting effects of stilbestrol administration on growth in rats. Thus, there is a variety of ways in which oestrogen may effectuate the decreased resistance to subsequent stress; however, involvement of the adrenal cortex cannot be overlooked.

The situation in the BALB/c mouse is complicated by the process of «brown degeneration» of the inner cortical zones, the functional significance of which is unknown. It is possible that this process involves a decrease in corticoid production by the cells concerned; however, the so-called «degeneration» cannot be termed atrophic. In a sense, a new tissue appears between cortex and medulla, which accounts at least in part for the increase in the weight of the gland. «Brown degeneration» has been described in various strains of mice as occurring spontaneously with age (Cramer & Horning, 1937, 1939a; Blaisdell et al., 1941; Jones, 1948), with diets high in unsaturated fat (Tobin & Birnbaum, 1947), after oestrogen treatment (Burrows, 1936; Cramer & Horning, 1937; Lacassagne & Raynaud, 1937; Alpert, 1953), and after hypophysectomy (Jones, 1950; Ferguson & Visscher, 1953). Originally Cramer & Horning (1939a; Cramer, 1940) felt that its incidence was indicative of a susceptibility to mammary cancer, but this has not been borne out. Jones (1950) has described its occurrence in the «Bagg albino C» strain several weeks after hypophysectomy, and we have observed the phenomenon in our BALB/c strain one month after hypophysectomy of 2-month-old mice (Nandi, unpublished data). Oestrogen treatment of this strain for 3 weeks or more generally results in the appearance of a full-blown zone of «brown degeneration» (equivalent to Cramer’s stage 4; see Blaisdell et al., 1941). Whether or not there is a common etiology for this so called «degeneration» after oestrogen administration and after hypophysectomy has yet to be determined.

The oestrogen-adrenal relationship herein described does not seem to be true for all strains of mice. Experiments on the oestrogen-treated C₃H/He mouse (Bern, Westberg & Nyegaard, unpublished) similar to those reported herein indicate (1) a decreased resistance to cold stress, but not as notable as in the BALB/c, (2) no adrenocortical hypertrophy in the female, but a significant increase in adrenal size in the male, and (3) no evidence of «brown degeneration». Cold stress results in the usual cortical hypertrophy. An examination of strain differences in adrenocortical response to oestrogen has recently been completed (Westberg, Bern & Barnawell, 1956).

Observations on thymic and splenic atrophy after oestrogen treatment and in stress produced little new information. Thymic atrophy occurs after oestrogen
in the absence of the adrenal (e.g., Selye et al., 1936; Selye & Masson, 1939; Selye, 1955). An essentially similar response of the thymus and the spleen is seen herein after a stressor. The increase in splenic atrophy with combined oestrogen treatment and stress is similar to the aggravation by oestradiol of cortisol-induced thymicolymphatic involution reported by Selye (1955) in adrenalectomized rats. The normal female BALB/c mouse shows a considerably larger spleen than the male (Fig. 7); however, this sex difference disappears in stressful situations.

Oestrogen evidently does not always have a debilitating effect on the organism. Cramer & Horning (1939 b) reported that pretreatment with oestrogen protected mice from the adverse effects of adrenalectomy. (It is possible that oestrogen acting through the anterior lobe of the pituitary gland stimulates accessory adrenal tissue which is sufficiently active at the time of subsequent adrenalectomy to supply the needed corticoids). Pretreatment with oestrogen also protects mice for a limited period of time from the lethal effects of X-irradiation (Treadwell et al., 1943; Patt et al., 1949) and possibly anoxia (Desclin, 1952).

**SUMMARY**

This study establishes that treatment of BALB/c mice of both sexes with moderately high doses of oestrogen reduced their resistance to subsequent exposure to stress. Two-month-old mice were implanted with 3–4 mg. pellets of oestradiol for 11 or 21 days before subjection to stressful situations (formalin injections, cold). Adrenal changes induced by the oestrogen alone included cortical hypertrophy coupled with extensive «brown degeneration» of the inner cortical zones. Splenic and thymic atrophy resulted from the oestrogen and/or the stressor. Addition of a stressor to the oestrogen treatment significantly increased the extent of adrenocortical hypertrophy and of splenic atrophy over that resulting from oestrogen alone. The importance of considering the degree of adrenocortical involvement following oestrogen administration is emphasized.

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REFERENCES

Gemzell, C. A.: The Effect of Corticotrophic Hormone and Oestrogen on Liver Glyco-
Selye, H.: Am. J. Physiol. 130, 358, 1940.
Selye, H., Harlow, C. M. & Collip, J. B.: Endokrinologie 18, 81, 1936.