Summary. Administration of 5-fluorouracil (5-FU) caused enlargement of the adrenal glands in Wistar and A X C rats. Results of simultaneous administration of cortisone and/or ACTH suggest that this enlargement is due to stimulation of secretion of ACTH rather than to enhancement of its activity. The enlarged adrenals of animals bearing the Walker tumor, Murphy-Sturm lymphosarcoma or Morris hepatoma 3924C did not enlarge further under treatment with 5-FU. They were reduced in size by administration of cortisone, more so in the case of the Walker tumor. The suppressive effect of cortisone was not overcome by simultaneous administration of 5-FU in rats bearing the lymphosarcoma or hepatoma but was in those with the Walker tumor. The ACTH-secreting mechanism in the latter is apparently more sensitive both to suppression by cortisone and to stimulation by 5-FU. The Walker tumor was sensitive to the tumoristatic action of cortisone and relatively resistant to that of 5-FU, whereas the converse was the case for the lymphosarcoma and hepatoma. These 3 tumors therefore exhibit a reciprocal relationship between sensitivity to cortisone and to 5-FU similar to that reported for corticosteroid-sensitive and resistant strains of mouse lymphoma P-1798.

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Influence of Gonads and Adrenals on Growth of Solid Ehrlich Tumor. (29475)

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The induction and further development of neoplasms are often affected by the hormonal state of the host(1,2). The growth of solid Ehrlich tumor, a mammary cancer by origin, is much greater in male mice than in female mice, and the sex difference is reversed by castration(3). The present study attempts to elucidate the relationship of the gonads and adrenals to these observations.

Materials and methods. All animals were Ha/ICR Swiss mice, propagated at Roswell Park Memorial Inst. by cousin mating. Hy-

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TABLE I. Comparison of Growth of Solid Ehrlich Tumor in Male and Female Swiss Mice.

Day	Host	Mean body wt* $\pm \hat{s}$ (g), based on (n) mice	P	Mean tumor wt $\pm \hat{s}$ (mg), based on (n) mice	P
4	male	33.4 ± 1.8 (10)	P<.001	75 ± 39 (10)	.05<P<.1
	female	27.8 ± 2.6 (10)		105 ± 31 (10)	
8	male	32.7 ± 3.4 (10)	.001<P<.01	409 ± 243 (10)	.001<P<.01
	female	27.6 ± 2.5 (10)		150 ± 138 (10)	
12	male	35.1 ± 1.9 (10)	P<.001	1223 ±1231 (10)	.02<P<.05
	female	30.1 ± 1.7 (10)		328 ± 250 (10)	
16	male	37.0 ± 3.3 (10)	P<.001	3259 ±2521 (10)	.001<P<.01
	female	30.9 ± 2.9 (10)		301 ± 212 (10)	
20	male	37.0 ± 3.0 (10)	P<.001	2293 ±1268 (10)	.001<P<.01
	female	30.4 ± 2.2 (10)		822 ± 552 (10)	

* Body weight was calculated by subtracting weight of tumor from total weight of the mouse bearing it.

potetraploid Ehrlich tumor stock, supplied in 1959 through the courtesy of Dr. T. S. Hauschka, was employed throughout the study, and was maintained in female mice by intraperitoneal inoculation of 1×10^7 cells/mouse at 8- to 9-day intervals. Solid Ehrlich tumors were produced in mice by subcutaneous injection of ascitic Ehrlich tumor cells into the right shoulder region. The animals received a standard pellet diet (Rockland Farms, Inc.), with drinking water *ad libitum*.

The following hormones were used: Testosterone Propionate, Schering; Estrone, Squibb; and Hydrocortisone Acetate, Merck Sharp & Dohme. Testosterone propionate (T) or estrone (E), dissolved in corn oil, was injected intramuscularly into the hind legs. Hydrocortisone acetate (HCA), suspended in isotonic saline, was injected subcutaneously into the left flank. Control mice received the corresponding vehicles. Castration and adrenalectomy were performed one month before tumor inoculation. Control animals underwent sham surgery. Adrenalectomized mice were given 0.9% sodium chloride solution *ad libitum* after surgery. Data obtained were analyzed by Student's t test.

Growth of Ehrlich tumor in both sexes. Ascitic tumor cells, 5×10^6 suspended in 0.2 ml of saline, were inoculated subcutaneously into each mouse on day 0; 10 males and 10 females were killed on each of days 4, 8, 12, 16, and 20, and well demarcated solid tumors were excised and weighed. Tumor growth (Table I) was much greater in males than in females for the first 16 days. Thereafter, tumor weight in males began to decline because of necrosis, whereas tumors in females continued to grow. Body weight showed a parallel sex dependency.

Effects of castration, adrenalectomy, and HCA administration on tumor growth. Mice were distributed among 6 treatment groups for each sex: 1, sham surgery; 2, castration; 3, castration and HCA; 4, sham surgery and HCA; 5, adrenalectomy; 6, castration and adrenalectomy. Each mouse received 5×10^6 cells subcutaneously on day 0. HCA, 1 mg/day/mouse, was started on day 5 and ended on day 14, a total of 10 injections. All animals were sacrificed on day 15, and the solid tumors were excised and weighed.

Ovariectomy produced a remarkable increase in body weight as well as tumor weight (Tables II and III). This stimulative effect of ovariectomy was observed whether the mice were adrenalectomized or not.

Orchiectomy caused decreases in body weight and tumor weight, but the differences in tumor weight were not statistically significant after either adrenalectomy or sham surgery. HCA generally had a suppressive effect on body weight and tumor weight. In females with sham surgery, however, HCA treatment resulted in increased tumor weight but decreased body weight.

Adrenalectomy significantly decreased body weight in both males and females with sham surgery and in castrated females, but not in castrated males. Tumor weight in these mice

TABLE II. Growth of Solid Ehrlich Tumor in Swiss Mice, as Related to Hormonal Status of Host.

Group No.	Host	Hormonal manipulation*	Mean body wt $\pm \hat{s}$ (g), based on (n) mice	Mean tumor wt $\pm \hat{s}$ (mg), based on (n) mice
1	male	S	37.2 ± 3.0 (8)	1460 ±1680 (8)
1′	female	"	29.3 ± 2.6 (9)	130 ± 55 (9)
2	male	C	31.3 ± 2.9 (9)	1360 ±1010 (9)
2′	female	"	35.6 ± 4.3 (8)	596 ± 186 (8)
3	male	C + HCA	26.5 ± 2.9 (8)	439 ± 182 (8)
3′	female	"	25.6 ± 2.4 (10)	295 ± 189 (10)
4	male	S + HCA	27.8 ± 3.1 (8)	405 ± 93 (8)
4′	female	"	23.9 ± 2.0 (10)	401 ± 237 (10)
5	male	A	33.3 ± 2.2 (10)	1581 ±1024 (10)
5′	female	"	25.1 ± 2.8 (10)	200 ± 205 (10)
6	male	A + C	30.8 ± 3.4 (10)	954 ± 612 (10)
6′	female	"	29.3 ± 1.9 (7)	520 ± 331 (7)

* S, sham surgery; C, castration; HCA, hydrocortisone treatment; A, adrenalectomy.

was not much affected by adrenalectomy. Apparently the effect of adrenalectomy on host and tumor is subject to modification under the influence of other endocrine organs. The response of the tumor was not completely identical with that of body weight in all treatment groups, but parallelism was found in several instances, suggesting that the same principle governs both body weight and tumor weight.

Effects of gonadal hormones on tumor growth. Mice with or without gonads or adrenals were given E (50 γ/day/mouse) or T (1 mg/day/mouse) on days −5, −3, −1, 2, 4, 6, 8, 10, 12, and 14, tumor inoculation being done on day 0. Control mice were treated with the vehicle, corn oil. The solid tumors were excised and weighed on day 15.

TABLE III. Statistical Analysis of Data in Table II.

Difference between groups	Body wt	Tumor wt	Parallelism between body wt & tumor wt
1 − 1′	P<.001	.02 <P<.05	Yes
2 − 2′	.02 <P<.05	.05 <P<.1	
3 − 3′	.3 <P<.4	.1 <P<.2	
4 − 4′	.001<P<.01	.9 <P	
5 − 5′	P<.001	P<.001	"
6 − 6′	.3 <P<.4	.1 <P<.2	
1 − 2	P<.001	.8 <P<.9	
1′ − 2′	P<.001	P<.001	"
2 − 3	.001<P<.01	.02 <P<.05	"
2′ − 3′	P<.001	.001<P<.01	"
1 − 4	P<.001	.05 <P<.1	
1′ − 4′	P<.001	.001<P<.01	No
1 − 5	.001<P<.01	.8 <P<.9	
1′ − 5′	.001<P<.01	P=.3	
5 − 6	.02 <P<.05	.1 <P<.2	
5′ − 6′	.001<P<.01	.02 <P<.05	Yes
2 − 6	.7 <P<.8	P=.3	
2′ − 6′	.001<P<.01	.5 <P<.6	

The effect of E on tumor growth was rather inhibitory (Tables IV and V). Body weight in female mice was increased by E. Tumor weight was decreased by T in adrenalectomized mice but not in castrated males. These discrepancies might be explained by the interfering action of other endocrine organs, on one hand; and by the difference in hormonal responsiveness between the host cells and the parasitic tumor cells, on the other hand.

Orchiectomy resulted in a significant decrease in tumor weight as well as body weight, and adrenalectomy in female mice caused a remarkable reduction in not only body weight but also tumor weight, in contrast to the preceding experiment, in which a suppressing effect of adrenalectomy was observed for body weight but not tumor weight. In male mice, adrenalectomy also decreased body weight but not tumor weight.

Discussion. Several investigators have tried to find a possible relationship between the growth of Ehrlich ascites tumor and the hormonal status of the host, but the data obtained thus far are inconclusive(4-6). In a study of the chemotherapeutic effects of hydrocortisone on Ehrlich ascites tumor, a striking difference in tumor growth was noticed between male and female mice bearing

TABLE IV. Growth of Solid Ehrlich Tumor in Swiss Mice, as Related to Hormonal Status of Host.

Group No.	Host	Hormonal manipulation*	Mean body wt $\pm \hat{s}$ (g), based on (n) mice	Mean tumor wt $\pm \hat{s}$ (mg), based on (n) mice
1	male	S	40.7 ± 1.5 (9)	4407 ±2274 (9)
1′	female	"	33.9 ± 3.7 (9)	1340 ± 993 (9)
2	male	S + T	41.3 ± 4.9 (10)	2707 ±1655 (10)
2′	female	S + E	34.3 ± 3.1 (11)	747 ± 424 (11)
3	male	C	33.5 ± 2.3 (10)	2569 ± 840 (10)
3′	female	"	36.2 ± 2.5 (9)	2906 ±1574 (9)
4	male	C + T	39.2 ± 2.7 (10)	2413 ±2543 (10)
4′	female	C + E	38.9 ± 5.3 (11)	1539 ± 782 (11)
5	male	A	34.9 ± 2.7 (9)	3961 ±1729 (9)
5′	female	"	30.7 ± 3.7 (10)	441 ± 375 (10)
6	male	A + T	37.7 ± 3.8 (10)	1954 ± 989 (10)
6′	female	A + E	35.2 ± 1.7 (8)	506 ± 378 (8)
7	male	A + C	36.5 ± 4.1 (10)	2911 ±1706 (10)
7′	female	"	30.7 ± 2.5 (9)	1829 ±1393 (9)
8	male	A + C + T	33.0 ± 2.7 (10)	1748 ±1001 (10)
8′	female	A + C + E	34.9 ± 3.0 (8)	929 ± 510 (8)

* T, testosterone treatment; E, estrone treatment; other abbreviations as in Table II.

solid Ehrlich tumor(3). Failure to obtain an appreciable sex difference with the ascitic form of the tumor is probably due to the fact that the ascitic Ehrlich tumor reaches the "saturation" cell number much earlier than the solid form, thus killing the host before any influence of the gonads on tumor growth is detected in terms of cell count. The present study confirmed the observation that the growth of solid Ehrlich tumor was stimulated by the presence of the testes and inhibited by the presence of the ovaries(3).

TABLE V. Statistical Analysis of Data in Table IV.

Difference between groups	Body wt	Tumor wt	Parallelism between body wt & tumor wt
1 – 1′	$P<.001$	$.001<P<.01$	Yes
1 – 2	$.7<P<.8$	$.05<P<.1$	
1′ – 2′	$.4<P<.5$	$.05<P<.1$	
2 – 2	$P<.001$	$P<.001$	"
1 – 3	$P<.001$	$.02<P<.05$	"
1′ – 3′	$.1<P<.2$	$.02<P<.05$	
3 – 3′	$.02<P<.05$	$.5<P<.6$	
3 – 4	$P<.001$	$.8<P<.9$	
3′ – 4′	$.1<P<.2$	$.02<P<.05$	
5 – 5′	$.01<P<.02$	$P<.001$	"
5 – 6	$.05<P<.1$	$.001<P<.01$	
5′ – 6′	$.001<P<.01$	$.7<P<.8$	
6 – 6′	$.1<P<.2$	$.001<P<.01$	
1 – 5	$P<.001$	$.6<P<.7$	
1′ – 5′	$.05<P<.1$	$.01<P<.02$	
7 – 7′	$.001<P<.01$	$.1<P<.2$	
7 – 8	$.02<P<.05$	$.05<P<.1$	
7′ – 8′	$.001<P<.01$	$.1<P<.2$	
8 – 8′	$.1<P<.2$	$P=.05$	
1 – 7	$.001<P<.01$	$.1<P<.2$	
1′ – 7′	$.02<P<.05$	$.4<P<.5$	
5 – 7	$P=.3$	$P=.2$	
5′ – 7′	$.9<P$	$.001<P<.01$	
3 – 7	$.05<P<.1$	$.5<P<.6$	
3′ – 7′	$P<.001$	$P<.001$	"

Benton(7) observed that the growth of Sarcoma 180 in male mice was markedly inhibited by adrenalectomy, and that administration of deoxycorticosterone eliminated the depressive effect of adrenalectomy. The effect of adrenalectomy on Ehrlich tumor, on the basis of the present study, does not seem to be the same as that on Sarcoma 180. The growth of solid Ehrlich tumor in male mice is little affected by removal of the adrenals, and some inhibitive effect of adrenalectomy was observed in female mice, indicating that the adrenals stimulated the growth of Ehrlich tumor through the production of androgenic hormone.

Presumably the increased tumor growth in male mice is related to the anabolic action of androgen, although administration of testosterone failed to give any appreciable increase in tumor weight. Investigation is under way to find optimal conditions for detecting stimulatory action of the male hormone.

Ehrlich ascites tumor is known to be susceptible to respiratory inhibition by syn-

thetic estrogens(8). The inhibitory action of the ovaries on tumor growth may well be of a more complex nature. The tumor system employed in this study will serve as an instructive model in elucidating the regulatory mechanism of various hormones in cancer.

Summary. The growth of solid Ehrlich tumor, as compared with body weight, was studied in relation to gonadal and adrenal function in Ha/ICR Swiss mice. Both tumor weight and body weight were much greater in males than in females, and castration reversed the differences. Adrenalectomy was inhibitory for tumor growth in females, but not in males. Body weight was decreased by adrenalectomy in both sexes. Hydrocortisone was generally inhibitory for both tumor weight and body weight, but increased tumor weight in females with sham surgery. Estrogen treatment in castrated females significantly reduced tumor growth, but not body weight. Testosterone administration increased body weight in castrated male mice, but had little effect on tumor weight. These findings are discussed in the light of the hormonal responsiveness of the tumor.

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Exudative Diathesis and Muscular Dystrophy Induced in the Chick by Esters of Polyunsaturated Fatty Acids. (29476)

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The incidence and severity of vitamin E deficiency syndromes in chicks have been shown to be directly related to increasing levels of polyunsaturates when fed with diets low in vit. E(1,2). But, to our knowledge these syndromes have never been induced in chicks fed varying levels of polyunsaturates in diets containing supposedly adequate levels of vit. E and other related dietary protectants.

In a previous study(3) at our laboratory, however, it was observed that chicks fed the most unsaturated fractions of ethyl esters of fatty acids prepared from menhaden *(Brevoortia tyrannus)* fish oil in a diet containing supposedly adequate levels of vit. E and related dietary protectants developed exudative diathesis and muscular dystrophy as early as 9 days. The diets contained 44 ppm dl-α-tocopherol acetate, 167 ppm ethoxyquin, 0.3 ppm Se, and 0.9% sulfur amino acids. But, since this observation was incidental to another study, there was a question as to whether it might have been an artifact. Also, in the event that it was a correct observation, there was a question as to whether these disorders in some manner were due only to the ethyl ester form of the fractions or were related to the oxidation of the oil in the feed.

Experimental. Ethyl esters and triglycerides. Unfractionated ethyl esters were prepared from commercial, light, cold-pressed menhaden oil by a rapid alcoholysis method.

Four equal-volume fractions of ethyl esters were obtained by the "cyclic" molecular distillation technique of Cawley, Barnitz, and