nancies was not the same in the mice on the diet supplemented with ether extract of whole egg as in those receiving alcohol extract of egg yolk or powdered egg from which the ether extractable part was removed. In the latter 2 groups the incidence of mammary cancer was considerably higher and that of the other types of malignancies (lung adenocarcinoma, lymphosarcomas, etc.) lower than in Group 2. This difference in incidence of the above two groups of malignancies is probably due to a difference in composition of the extracts used, since it is known that some lipids are more soluble in alcohol and others more in ether. In the case of Group 4, it is believed that the carcinogenicity is not due to the egg proteins, but to the unextracted lipids. These and other results indicate that there is more than one carcinogenic lipid in eggs.

Some slides were examined by Dr. Stephen S. Sternberg, Sloan-Kettering Inst., and some by Drs. Paul Kotin and Thelma B. Dunn, National Cancer Inst., to whom I am greatly indebted.

1. Szepsenwol, J., PROC. SOC. EXP. BIOL. AND MED., 1963, v112, 1073.

Received May 15, 1964. P.S.E.B.M., 1964, v116.

Effects of 5-Fluorouracil and Adrenocortical Hormones on Tumors and Adrenals in Rats Bearing Transplanted Tumors.* (29474)

A. CANTAROW, J. J. RUPP AND J. W. GODDARD
Departments of Biochemistry and Medicine, Jefferson Medical College, Philadelphia, Pa.

We have found that 5-fluorouracil (5-FU) inhibits fetal growth, liver regeneration, testosterone-induced growth of the seminal vesicles, and somatotropin-induced growth of the epiphyseal cartilage(1). The high toxicity of 5-FU for hypophysectomized rats interfered with similar studies of its effect on ACTH-stimulation of adrenal growth. However, the results suggested that this process might not be inhibited by 5-FU. The present study was undertaken for the purpose of investigating this matter further. Observations were extended to tumor-bearing rats because of the possibility that the hypophyseal-adrenocortical mechanism may respond differently in the presence of a tumor than in the normal animal.

Materials and methods. Male rats (Barkbridge Farms, N. J.), weighing about 150 g, were fed laboratory chow and had free access to water. Tumors were implanted subcutaneously in the groin by trocar technic, the Walker 256 tumor and the Murphy-Sturm lymphosarcoma in Wistar and the Morris hepatoma 3924C in A X C rats.

(1) In the studies of effects on adrenal weight, treatment with hormones and 5-FU was begun when tumor growth was well established. Cortisone acetate was injected subcutaneously, 5 mg daily for 10 days, ACTH gel subcutaneously, 5 units twice daily for 5 days, and 5-FU intraperitoneally, 25 mg/kg/day for 5 days. In the combination treatment schedules, ACTH or/and 5-FU was/were given on the last 5 days of cortisone administration.

(2) In the studies of effects on tumors, these were allowed to grow for 10 days. The adrenocortical steroids were administered daily from day of implantation, and 5-FU was given daily for the last 5 days. The several dosage schedules are indicated in Table IV.

All animals were sacrificed by exsanguination under light ether anesthesia 24 hours after the last injection.

Results and discussion. Effect of 5-FU on adrenals of normal rats (Table I). Administration of 5-FU resulted in significant increase (49%) of adrenal weight in normal Wistar rats. Body weight was maintained and there was no other evidence of drug toxicity in these animals. Increased adrenal

* Supported by Contract SA-43-ph-2387 from Cancer Chemotherapy National Service Center, Nat. Cancer Inst., N.I.H., U.S.P.H.S.

TABLE I. Influence of 5-Fluorouracil on Adrenal Weight in Normal Wistar Rats.

No. of rats	Treatment	Adrenal wt, mg/100 g* ± S.E.	
25	None	17.0 ± .17	
20	Cortisone, 5 mg/day × 10	9.0 ± 1.28	
19	ACTH, 5 U b.i.d. × 5	24.7 ± 1.41	
22	5-FU, 25 mg/kg/day × 5	25.3 ± 1.94	
17	Cortisone + 5-FU	13.0 ± .98	(*vs* cortisone P <.01) (*vs* 5-FU P <.01)
25	Cortisone + ACTH	20.1 ± 1.72	(*vs* cortisone P <.01) (*vs* ACTH P <.05)
21	5-FU + ACTH	29.3 ± 2.85	(*vs* ACTH P <.05)
16	Cortisone + ACTH + 5-FU	20.5 ± 1.43	

* Carcass weight (minus tumor).

hypertrophy after unilateral adrenalectomy has been observed in rats treated with 5-FU, 5-fluorodeoxyuridine, thioguanine, thioguanosine, and 6-mercaptopurine riboside(2). Gass *et al*(3) reported enlargement of the adrenals and thymic involution after administration of Myleran, actinomycin D, L-sarcolysin, and 4-aminopyrazolo (3,4-*d*)pyrimidine. This was interpreted as probably due to a toxic effect of these agents. Perhaps also relevant is the report(4) that an amount of 5-FU that killed 20% of intact dogs killed 80% of adrenalectomized dogs receiving a maintenance dose of cortisol. Administration of larger amounts of hormone afforted complete protection to the adrenalectomized animals.

The size of the adrenals was reduced by cortisone (about 47%) and increased by ACTH (about 45%). The increase with 5-FU was comparable to that induced by ACTH. 5-FU induced significant increase (44%) of adrenal weight in animals with simultaneous adrenal suppression by cortisone, and in those with stimulation by ACTH (19%). However, it was without effect in animals receiving both ACTH and cortisone. These observations suggest that 5-FU produces enlargement of the adrenals through the medium of increased secretion of ACTH rather than by enhancing the effect of ACTH.

TABLE II. Influence of Transplanted Tumors on Adrenal Weight.

	No. of rats	Adrenal wt, mg/100 g* ± S.E.
Wistar control (no tumor)	24	17.0 ± .17
Walker 256	32	25.2 ± 2.64 (P <.01)
Murphy lymphosarcoma	30	26.7 ± 2.28 (P <.01)
A × C control (no tumor)	24	16.3 ± .26
Morris hepatoma 3924C	20	25.7 ± 2.14 (P <.01)

* Carcass weight (minus tumor).

Effects of cortisone and 5-FU on adrenals of tumor-bearing rats. The adrenal glands were consistently larger than normal in the presence of growing tumors (Table II). Under the experimental conditions employed, there was no significant difference between the Walker tumor, the Murphy-Sturm lymphosarcoma and the Morris hepatoma 3924C with respect to their effect on adrenal size. Similar observations have been reported previously for mice bearing Sarcoma 180(5, 6,7) and rats bearing the Jensen sarcoma(8), Walker tumor(8,9,10), and Murphy lymphosarcoma(11,12). Association of this enlargement with thymic involution and decreased adrenal lipid and ascorbic acid content(6,7, 12, 13), and reports that it does not occur in hypophysectomized animals(7,9) suggest that it is due to ACTH stimulation. There are reports of increase in urinary and plasma corticosterone(5,11,14), and of increased corticosterone secretion rate(11) which did not differ significantly from that of normal rats subjected to acute operative stress. It has been reported, however, that the patterns of change in nucleic acids and certain enzymes in the adrenal differ from those incident to states of nonspecific stress(15).

Cortisone reduced the adrenal weight in

tumor-bearing animals (Table III), somewhat more in those with the Walker tumor (63%) than with the lymphosarcoma (46%) or hepatoma (40%). In the case of the Walker tumor the values were lowered to levels approximating those obtained in cortisone-treated rats without tumors. This response to cortisone supports the view that adrenal enlargement in these animals is due to increased hypophyseal secretion of ACTH rather than to production of a similar substance by the tumor, as has been demonstrated for certain neoplasms in man(16).

TABLE III. Influence of Cortisone and 5-Fluorouracil on Adrenal Weight in Tumor-Bearing Rats.

Treatment	Adrenal wt (% of control*)					
	Walker		Lymphosarcoma		Hepatoma	
	No. of rats		No. of rats		No. of rats	
Cortisone, 5 mg/day × 10	19	37 ($P < .01$)	24	54 ($P < .01$) (*vs* Walker $P < .01$)	18	60 ($P < .01$) (*vs* Walker $P < .01$)
5-FU, 25 mg/kg/day × 5	11	114 (N.S.)†	16	103 (N.S.)	16	95 (N.S.)
Cortisone + 5-FU	12	82 (N.S.)	14	51 ($P < .01$)	14	56 ($P < .01$)

* A control group of 12 untreated tumor-bearing rats was run simultaneously with each treated group.
† N.S. = Not significant.

The enlarged adrenals of the tumor-bearing rats did not increase further in size after administration of 5-FU. Although it is possible that ACTH secretion was already stimulated maximally as a result of the presence of the tumor, the fact that adrenal weights as high as 45 mg/100 g were found in animals with double tumor implants indicates that the ACTH-secreting mechanism was capable of considerably greater response to stimulation, at least under these circumstances.

5-FU overcame cortisone-induced suppression of the adrenals in rats bearing the Walker tumor, as in those without tumors, but not in animals bearing the lymphosarcoma or hepatoma. All 3 tumors induce essentially the same degree of enlargement of the adrenals (Table II), but the ACTH-secreting mechanism is apparently more sensitive both to suppression by cortisone and to stimulation by 5-FU in rats bearing the Walker tumor than in those with the lymphosarcoma or hepatoma.

Effects of corticosteroids and 5-FU on tumor growth (Table IV). Although the carcass weights of treated animals were slightly lower than those of controls, there was no consistent difference in this regard between animals bearing the 3 types of tumors. Growth of all 3 tumors was depressed by Decadron (9α-fluoro-11β, 17α, 21-trihydroxy-16α-methyl-1, 4-pregnadiene-3,20-dione) and prednisone (17α, 21-dihydroxy-1, 4-pregnadiene-3, 11, 20-trione) at dosage levels regarded as metabolically equivalent to 5 mg cortisone and 10 mg corticosterone. In these amounts, the latter 2 hormones inhibited growth of the Walker tumor but not that of the lymphosarcoma or hepatoma. With double this dose of cortisone (10 mg), growth of the lymphosarcoma was inhibited but that of the hepatoma was not affected. The difference in effectiveness of Decadron and prednisone as compared to that of cortisone and corticoserone suggests either (a) that the

TABLE IV. Effect of Corticosteroids and 5-Fluorouracil on Tumor Growth.

Treatment*	% change tumor wt†				
				Double tumor	
	Walker	Lympho-sarcoma	Hepatoma	Walker	Lympho-sarcoma
Cortisone, 5 mg/day × 10	−60	±	±	−55	±
Cortisone, 10 mg/day × 10	−55	−40	±	−45	−45
Corticosterone, 10 mg/day × 10	−55	±	±	—	—
Decadron, .16 mg/day × 10	−50	−70	−45	−45	−60
Prednisone, 1.2 mg/day × 10	−45	−45	−40	—	—
5-FU, 12.5 mg/kg/day × 5	±	−40	−65	±	−50
5-FU, 25 mg/kg/day × 5	±	−75	−45	±	−70
5-FU, 25 mg + cortisone, 5 mg	−60	−60	−55	−55	−65
5-FU, 25 mg + cortisone, 10 mg	−50	−65	−60	—	—

* 16-24 animals in each group. A control group of 12 untreated tumor-bearing rats was run simultaneously with each treated group.

† All stated numerical values (calculated to nearest 5%) are statistically significant ($P < 0.01$).

lymphosarcoma and hepatoma may differ from the Walker tumor in their ability to metabolize the natural but not the synthetic types of hormone or (b) that the tumoristatic action of these substances is not related quantitatively to other recognized metabolic activities of adrenocortical steroids.

Growth of the hepatoma and lymphosarcoma was inhibited by 5-FU, but not that of the Walker tumor. Simultaneous administration of cortisone did not alter the tumoristatic effect of 5-FU on the lymphosarcoma and hepatoma, nor did 5-FU alter that of cortisone on the Walker tumor. In the case of these 3 tumors, therefore, sensitivity to 5-FU is associated with relative resistance to cortisone, and *vice versa*.

To explore this apparent relationship further, the effects of 5-FU and steroids were investigated on growth of the Walker tumor and lymphosarcoma implanted simultaneously in the same animal, one in each groin. In untreated animals both tumors grew essentially as in single implantations. Each exhibited the same type of response to administration of 5-FU, cortisone and Decadron as when present alone (Table IV). A similar reciprocal relationship between tumor sensitivity to adrenocortical steroids and to 5-FU has been reported for corticoid-sensitive and corticoid-resistant strains of the P-1798 mouse lymphoma(17). These differed from the tumors in the present study in that the cortisone-resistant subline was resistant also to Decadron and prednisone (18). However, the experimental design also differed in that the steroids were administered only after the tumors had become well established.

The Walker tumor, Murphy lymphosarcoma and hepatoma 3924C exhibit equally high levels of incorporation of uracil and fluorouracil into RNA(19). The difference in their sensitivity to 5-FU is therefore attributable to difference at some point in the metabolic pathway from uridine to DNA. Acquired resistance to 5-FU has been reported to be accompanied in one line of Ehrlich ascites tumor by decreased uridine kinase activity(20), and in another by inability of 5-fluoro-2′-deoxyuridylic acid to inhibit thymidylate synthetase activity(21), presumably owing to alteration of the enzyme. There is no information as to the comparative sensitivity to adrenocortical steroids of these FU-sensitive and -resistant tumor lines.

No explanation is apparent for the peculiar association of sensitivity to one of these agents with resistance to the other. However, its existence in several types of tumors suggests that this relationship is not merely fortuitous. It is interesting, too, that the ACTH-secreting mechanism is apparently more responsive to FU-stimulation and to cortisone-depression in animals bearing the tumor most sensitive to cortisone (Walker) than in those bearing the tumors (lymphosarcoma and hepatoma) relatively resistant to cortisone.

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.

1. Paschkis, K. E., Bartuska, D., Zagerman, J., Goddard, J. W., Cantarow, A., *Cancer Research,* 1959, v19, 1196.

2. Palma, V., *Atti Acad. Med. Lombarda,* 1961, v16, 409.

3. Gass, G. H., Umberger, E. J., Davis, K. J., Davis, L. J., *Cancer Chemother. Rep.,* 1959, v3, 16.

4. Tipton, J., Regan, W. J., *Surgery,* 1963, v53, 495.

5. Hilf, R., Burnett, F. F., Borman, A., *Cancer Research,* 1960, v20, 1389.

6. Savard, K., *Science,* 1948, v108, 381.

7. Savard, K., Homburger, F., PROC. SOC. EXP. BIOL. AND MED., 1949, v70, 68.

8. Haven, F. L., Bloor, W. R., Randall, C., *Cancer Research,* 1949, v9, 511.

9. Ball, H. A., Samuels, L. T., PROC. SOC. EXP. BIOL. AND MED., 1938, v38, 441.

10. Begg, R. W., *Cancer Research,* 1951, v11, 341.

11. Millar, F. K., Toal, J. N., Brooks, R. H., Davis, J. O., White, J., *Am. J. Physiol.,* 1963, v205, 189.

12. Murphy, J. B., Sturm, E., *Cancer Research,* 1948, v8, 139.

13. Hilf, R., Breuer, C., Borman, A., *ibid.,* 1961, v21, 1439.

14. Peric-Golia, L., Jones, R. S., PROC. SOC. EXP. BIOL. AND MED., 1963, v113, 317.

15. Hilf, R., Cowett, V. W., Johnson, M. L., Borman, A., *Cancer Research,* 1962, v22, 449.

16. Meador, C., Liddle, G. W., Island, D. P., Nicholson, W. E., Lucas, C. P., Nuckton, J. G., Leutscher, J. A., *J. Clin. Endocrinol.,* 1962, v22, 693.

17. Lampkin-Hibbard, J. M., *J. Nat. Cancer Inst.,* 1962, v28, 569.

18. ———, *ibid.,* 1960, v24, 1353.

19. Cantarow, A., Williams, T. L., Paschkis, K. E., *Cancer Research,* 1962, v22, 1021.

20. Reichard, P., Sköld, O., Klein, G., Révész, L., Magnusson, P. H., *ibid.,* 1962, v22, 235.

21. Heidelberger, C., Kaldor, G., Mukherjee, K. L., Danneberg, P. B., *ibid.,* 1960, v20, 903.

Received May 15, 1964. P.S.E.B.M., 1964, v116.

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

MITSUO KODAMA* (Introduced by George E. Moore)

Department of Medicine, Roswell Park Memorial Institute, Buffalo, N. Y.

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-

* Present address: Nagoya University School of Med., Showa-ku, Nagoya, Japan.