11-Dehydrocorticosterone Acetate (Compound A) in Normal and Tumor Bearing Mice. (17878)

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It has been shown that 11-dehydro-17-hydroxycorticosterone (Compound E) as well as adrenal cortical extracts have a restraining effect on the growth of leukemias and lymphoid tumors of mice and rats(1,2,3,4). We have found no reference in the literature to such an action of 11-dehydrocorticosterone (Compound A). The study here reported provides evidence that Compound A has pronounced biological effects. Among these is the ability to extend the life of mice bearing a transplantable leukemia and to modify the growth of a lymphosarcoma.

Materials and methods. Normal male and female strain *AK (RIL)* mice when 55 to 69 days of age, young strain *DBA* mice bearing transplantable leukemia P1534, and strain *C3H* mice bearing lymphosarcoma 6C3HED were employed. The 11-dehydrocorticosterone acetate was suspended in saline, 50 mg/cc, and unless otherwise noted, subcutaneous injections of 4 mg/day were used in 2 divided doses. Control mice received similar physiological saline injections. The experiments were restricted by the fact that only a few milligrams of Compound A were available and no more could be obtained. The transplantable leukemia and lymphosarcoma were transferred by subcutaneous injection of the local tumor mass with a 12 gauge trochar. Mice bearing leukemia P1534 typically die suddenly about 10 days after tumor transfer. The local mass of the lymphosarcoma is well enlarged at 6 days and very large at 12 days. Mice bearing this tumor die about 3 weeks after tumor transfer. All mice were maintained at 22°C, fed Purina Fox Checkers *ad libitum* with a supplement of sunflower seeds and rolled oats.

Results. Three criteria have been employed in determining the response of the normal mice to Compound A: (1) body and organ weight, (2) hematologic studies, and (3) histologic studies. The first two are shown in Table I; the latter is reserved for a later report. Although the findings must be considered preliminary, a few points stand out: (1) body weight was well maintained, (2) organs related to the lymphatic system; spleen, thymus, and mesenteric lymph nodes were reduced in size. Females were started in diestrus and autopsied in diestrus. The treated females were acyclic and the non-treated cyclic, and there appears to be some evidence of reduction in accessory reproductive organ weight. Microscopic observation showed the adrenal cortex of males and females to be greatly reduced; in the females the X zone was large and vacuolated.

Four criteria were employed in studying the response of the transplanted leukemia to Compound A: (1) duration of life, (2) hematologic studies, (3) growth of leukemic cells at the site of inoculation, and (4) the infiltration of leukemic cells as measured by weights of infiltrated organs. The results are shown in Tables II, III and IV. The tumor mass at the site of inoculation was well controlled, (Tables II and III) the duration of life was extended following a 6-day period of treatment with Compound A both

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TABLE I.
Comparative Organ Weights and Hematologic Findings of Male and Female AK (RIL) Compound A Treated and Control Mice; Treated for 7 Days with Autopsy on the 8th Day. Body wt in g.

	Treated		Non-treated	
No. of mice	2	2	4	4
Mean age (days)	60	60	62	62
Mean body wt, at start	25.2	22.8	24.6	22.0
Mean body wt, at autopsy	25.8	24.5	25.6	22.9
Mean organ wt (% of autopsy body wt)				
Thymus	.020	.028	.248	.420
Spleen	.117	.114	.338	.363
Mesenteric lymph nodes	.032	.034	.155	.205
Liver	7.15	6.45	6.48	6.38
Brain	1.75	1.86	1.71	2.02
Heart	.911	.738	.797	.801
Kidney	.924	.698	.876	.723
Vesicular glands and prostates	.597		.790	
Testes and epidydimi	.520		.547	
Preputial	.366		.410	
Bulbo-urethral	.052		.092	
Uterus, ovaries, vagina		.391		.679
Submaxillary	.530	.426	.590	.424
Adrenal	.005	.010	.008	.015
Pituitary	.004	.007	.006	.009
Hematologic findings				
Mean total R.B.C. $\times 10^6$	8.2	9.4	10.7	11.1
" " W.B.C. $\times 10^3$	12.5	24.5	9.4	5.8
" % lymphocytes	8.0	3	57.6	59.2
" % monocytes	1.5	1	3.8	3.6
" % polymorphonuclear leukocytes	90.5	96	38.5	36.9
" % eosinophils	.0	.0	.1	.3

TABLE II.
Effect of Compound A on Lymphatic Leukemia. DBA Mice Treated for 6 Days Starting 2 Days After Tumor Transfer. Body wt in g.

	Treated tumor	Non-treated tumor	Non-treated non-tumor
No. of animals	3	3	5
Mean body wt at start	14.7	16.4	—
" body wt at 9 days	15.5	20.4	17.9
" tumor mass at 9 days in sq mm (L x W)	0	180.0	0
" days survival	15.4	10.7	—
Hematologic findings 7 days after tumor transfer			
Mean total R.B.C. $\times 10^6$	7.1	8.5	9.7
" total W.B.C. $\times 10^3$	9.1	13.5	7.1
" % lymphocytes	8.5	49.0	62.0
" % monocytes	0.2	5.5	5.5
" % polymorphonuclear leukocytes	91.3	45.2	32.4
" % eosinophils	.0	.3	.1

when given early (Table II) and late (Table IV) in the course of the disease, the mean total W.B.C. including the mean percent lymphocytes was lower (Tables II, III and IV) and spleen and thymus size was lower (Table III) in the Compound A treated groups.

The criterion for determining the response of the lymphosarcoma to Compound A was the mean tumor size. This tumor growth was well controlled for the short time of the present experiment (Table V).

TABLE III.

Effect of Compound A on Lymphatic Leukemia. DBA Mice Treated with 2 mg/day for 8 Days Starting the 1st Day After Tumor Transfer. Autopsy and Hematologic Readings on the 9th Day. Body wt in g.

	Treated tumor	Non-treated tumor	Non-treated non-tumor
No. of animals	3	3	3
Mean body wt at start	22	19	—
" body wt at autopsy	22	22	20.5
" tumor mass in sq mm (L x W)	0	144.0	0
Mean organ wt (% of body wt)			
Adrenal	.006	.009	.014
Spleen	.074	.164	.067
Thymus	.003	.014	.024
Hematologic findings			
Mean total R.B.C. $\times 10^6$	8.7	8.3	9.2
" total W.B.C. $\times 10^3$	5.2	17.7	5.6
" % lymphocytes	15.3	55.3	63.3
" % monocytes	3.2	5.0	4.8
" % polymorphonuclear leukocytes	81.5	38.7	31.9
" % eosinophils	.0	1.	.0

TABLE IV.

Effect of Compound A on Lymphatic Leukemia. DBA Mice Treated for 6 Days Starting the 7th Day After Tumor Transfer. Body wt in g.

	Treated tumor	Non-treated tumor
No. of animals	3	(6*) 3
Mean body wt, start	18.2	17.5
" body wt, 10 days	18.8	—
" days survival	14.8	10.7
Hematologic findings on 10th day after tumor transfer		
Mean R.B.C. $\times 10^6$	5.4	4.7
" W.B.C. $\times 10^3$	12.3	83.0
" % lymphocytes	12.8	57.3
" % monocytes	1.5	2.0
" % polymorphonuclear leukocytes	85.7	40.7
" % eosinophils	.0	.0

* 3 of 6 mice died of the leukemia before the 10th day and are not otherwise included.

Discussion. Lymphoid tumors were selected for study with the limited amount of Compound A available since there is a growing body of evidence showing that these tumors, as well as the normal lymphatic tissues of animals, undergo involution with increased adrenal cortical function. Reference has been made to the work of Heilman and Kendall, and others who have demonstrated tumor restraining activity of adrenal cortical extracts and Compound E. Recently evidence has been published showing adrenocorticotropic hormone (ACTH), presumably through stimulation of adrenal cortical function, also leads to the temporary regression of lymphoid tumors in man(5).

The quantitative problem is an important one. In the present experiments we have used at least four times the amount of Compound A that we would have been able to use of Compound E without causing serious disturbances or death of the animal. Speirs and Meyer have observed that a much larger dose, 25 γ, of Compound A is required to produce a reduction in eosinophil count similar to that obtained with 3 γ of Compound E(6).

Summary. The study provides evidence that Compound A (11-dehydrocorticosterone acetate) has pronounced biological activity. Among these is the ability to reduce the size of: (1) the adrenal glands, (2) tissues related to the lymphoid system and, (3) possibly the accessory reproductive organs. The life expectancy of mice bearing transplantable lymphatic leukemia P1534 was increased

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6. Speirs, R. S., and Meyer, R. K., *Endocrinol.*, 1949, v45, 403.

TABLE V.
Comparative Tumor and Organ Weight of Compound A Treated and Control C3H Lymphosarcoma Mice Treated for 6 Days Starting the 3rd Day; Autopsy the 9th Day. Body wt in g, tumor wt in mg.

	Treated tumor	Non-treated tumor	Non-treated non-tumor
No. of animals	3	3	3
Mean body wt at start	19.3	19.	—
" body wt at autopsy	20.0	23.7	19.3
" wt tumor mass	.0	1350	.0
Mean organ wt as % of body wt less tumor wt			
Spleen	.24	.74	.59
Mesenteric lymph nodes	.011	.030	.29
Thymus	.016	.093	.111
Adrenal	.007	.009	.011

and evidence of modification of the local lymphatic lesion was observed. Growth of the tumor mass of lymphosarcoma 6C3HED was prevented for a short experimental period.

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Role of the Pituitary in Growth of a Transplanted Rat Tumor.* (17879)

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The purpose of the present investigation is the study of the hypophyseal influence on the rate of growth of a transplanted rat tumor. This subject has received only a limited amount of attention in recent years, and as some of the earlier work is open to criticism regarding the completeness of hypophysectomy, we refer only to a more recent paper by Chamorro and Dobrovolskaia-Zavadskaia (1) for a bibliography on this subject.

Materials. Male rats of the Sprague-Dawley† strain were used throughout this investigation. The hypophysectomized animals were of the same weight range (80-90 g) as the controls at the time of the operation. The tumor used was the Walker Carcinosarcoma 256‡; Anterior Pituitary Powder, Parke-Davis & Co., No. H 1684E and whole dried Anterior Pituitary Lobes, Massone Institute, Buenos Aires.

Exp. I. Tumor implantation was made into the hypophysectomized rats 3 days after the hypophysectomy; the intact control animals were implanted at the same time. The animals were given a diet of Purina Laboratory Chow with tap water *ad libitum*. They were weighed twice weekly. The animals were sacrificed on the 14th day after tumor implantation, and the tumors removed and weighed immediately.

Exp. II. The conditions of this experiment were the same as for Exp. I with the exception that tumor implantation was delayed until 11 days after the surgical intervention, and that the animals were sacrificed on the 15th day following tumor implants.

Exp. III. Tumor implantation was made

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1. Chamorro, A., and Dobrovolskaia-Zavadskaia, N., *C. r. soc. biol.*, 1945, v139, 614.

† Supplied via air freight by Hormone Research Laboratories, Inc., Chicago, Ill.

‡ We wish to thank Dr. K. Sugiura of the Sloan-Kettering Institute for making this tumor available to us.