

one-half of the circulating eosinophiles were stored in these spleens the number of eosinophiles per oil immersion field would remain the same. Thus on the basis of these calculations, although approximate, it appears reasonable that the number of eosinophilic leucocytes seen in the spleen could account for the number disappearing from the circulating blood. Further investigations are being conducted in order to determine the fate of the eosinophilic leucocytes following adrenal cortical stimulation in splenectomized animals.

Summary. In a group of Swiss albino mice

injected with cortisone acetate it was found that 8 hours post injection the spleens contained a significant increase in the number of eosinophilic leucocytes. It is postulated that this may be the locale of the eosinophilic leucocytes during their disappearance from the circulating blood following adrenal cortical stimulation. The number of eosinophilic leucocytes found in the spleen could account for the number that disappeared from the circulating blood following a single injection of cortisone acetate.

Received January 8, 1951. P.S.E.B.M., 1951, v76.

Ascorbic Acid Concentration in the Adrenals of Lymphosarcoma-Bearing Mice.* (18478)

ELIJAH ADAMS[†] AND ABRAHAM WHITE[‡]

From the Department of Physiological Chemistry, School of Medicine, University of California Medical Center, Los Angeles.

Savard(1) has described recently a progressive reduction in ascorbic acid concentration in the adrenals of female Carworth Farm mice during the growth of a transplanted tumor (sarcoma 180). The lymphocyte is known to be an end cell of pituitary-adrenal cortical secretions, and the possibility that the products of lymphocyte destruction may exert a suppressive influence on this endocrine system has been noted in a recent publication(2). It seemed of interest, therefore, to investigate adrenal ascorbic acid levels in mice bearing a rapidly growing lymphosarcoma. The present report describes these observations as well as incidental observations concerned with a sex difference in the respon-

siveness of adrenal ascorbic acid to the administration of histamine and adrenocorticotrophic hormone (ACTH).

Methods. Male and female mice of the CBA strain (Strong) 8 to 10 weeks of age were used. Male mice were inoculated subcutaneously with a small fragment of lymphosarcoma(3) and were autopsied at varying intervals thereafter. Histamine acid phosphate (Abbott) was administered intraperitoneally as a solution in normal saline in doses ranging from 0.25 to 5.0 mg of histamine base per 100 g body weight. One mg of ACTH (Armour) was administered intraperitoneally as a fresh solution in distilled water. All intraperitoneal injections were given in a volume not exceeding 0.5 ml and all mice were sacrificed 1 hour following the administration of either substance. The determination of ascorbic acid in the adrenals of fasted mice was made at the end of a 48-hour period during which mice were maintained in individual metabolism cages with free access to water and without food. Im-

* This investigation was aided by a grant from the Division of Grants and Fellowships, National Institutes of Health, U. S. Public Health Service.

[†] Postdoctoral Fellow, American Cancer Society. Present address: University of Utah School of Medicine, Salt Lake City, Utah.

[‡] Present address: Chemical Specialties Co., Inc., New York, N. Y.

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

2. Roberts, S., and White, A., *J. Biol. Chem.*, 1949, v178, 151.

3. Gardner, W. U., Dougherty, T. F., and Williams, W. L., *Cancer Research*, 1944, v4, 73.

TABLE I.* Concentration and Total Content of Ascorbic Acid in Adrenals of Male CBA Mice Carrying a transplantable Lymphosarcoma.

Group	No. of mice	Tumor wt, mg	Body wt, g	Adrenal wt†	Ascorbic acid conc.‡	Total adrenal ascorbic acid, µg
Normal	25	—	22.8 ± 0.6	12.9 ± 0.4	235 ± 5	6.72 ± 0.15
Tumor 6 days	6	0	23.6 ± 1.6	13.3 ± 1.9	191 ± 11*	6.42 ± 0.28
11 "	8	100	24.5 ± 1.5	11.8 ± 0.9	229 ± 8	6.51 ± 0.46
14 "		500				
to 15 "	8	to 3000	23.3 ± 1.9	12.4 ± 0.8	214 ± 15	7.14 ± 0.49
21 "	6	1000 to 3000	25.9 ± 1.2	12.6 ± 1.6	230 ± 16	6.84 ± 0.40
Tumor (6-21 days)	28	0 to 3000	24.2 ± 0.6	12.2 ± 0.5	224 ± 6	6.57 ± 0.20

* Values shown are means and standard errors.

† mg of adrenal per 100 g body weight.

‡ mg of ascorbic acid per 100 g fresh adrenal.

§ Pooled values for all intervals after tumor implantation.

|| Represents days after inoculation of tumor transplant.

¶ Significantly lower than normal value ($p = < 0.01$) by "weighted" t-test.(7)

TABLE II.* Ascorbic Acid Concentration and Total Content in Adrenals of Normal and Lymphosarcoma-bearing CBA Male Mice Following a 48-hour Fast.

Group	No. of mice	Body wt	Adrenal† wt	Ascorbic acid conc.‡	Total adrenal ascorbic acid, µg
Normal, fed	25	22.8 ± 0.6	12.9 ± 0.4	235 ± 5	6.72 ± 0.15
Tumor (6-21 days)§ fed	28	24.2 ± 0.6	12.2 ± 0.5	224 ± 6	6.57 ± 0.20
Normal, fasted	10	20.2 ± 0.5	15.2 ± 0.6	247 ± 6	7.53 ± 0.30
Tumor (9-15 days)§ fasted	12	22.4 ± 0.9	15.7 ± 0.7	200 ± 10	6.93 ± 0.40

* Values shown are means and standard errors.

† mg per 100 g body weight.

‡ mg per 100 g fresh adrenal.

§ Days following inoculation of tumor transplant.

|| Significantly lower than mean value for normal fed group ($p = < 0.01$) by "weighted" t-test.(7)

mediately after sacrifice of each mouse, both adrenals were removed, trimmed of fat and connective tissue on saline-moistened filter paper, weighed to the nearest 0.1 mg on the Roller-Smith torsion balance, and extracted in a mortar with 3 ml of 6% trichloroacetic acid. The ascorbic acid content of a 2 ml aliquot of this extract was measured with the aid of a Coleman Jr. spectrophotometer by the method of Roe and Kuether(4).

Results. As is evident from the data of Table I, no progressive or consistent deple-

tion of ascorbic acid was observed in the adrenals of male mice autopsied at varying intervals after the implantation of lymphosarcoma tissue. At only 1 interval (6 days), at a time when the tumor had not yet become grossly visible even at autopsy, was there a statistically significant, although small, drop in adrenal ascorbic acid concentration below the control value. At this interval the mean value for total micrograms of adrenal ascorbic acid is not significantly different from the control. At all other intervals, even late in the course of the tumor, when the mice were moribund, the values both of adrenal ascorbic acid concentration and total content were

4. Roe, J. H., and Kuether, C. A., *J. Biol. Chem.*, 1943, v147, 399.

TABLE III.* Adrenal Ascorbic Acid Response to Histamine and ACTH in Male and Female CBA Mice.

Group	No. of mice	Body wt	Adrenal† wt	Ascorbic acid conc.‡	Total adrenal ascorbic acid, µg	Total adrenal ascorbic acid, µg/100 g body wt
Males—mg histamine per 100 g body wt.						
Control	25	22.8 ± 0.6	12.9 ± 0.4	235 ± 5	6.72 ± 0.15	29.2 ± 1.0
.25 & .50	4	21.2 ± 1.7	14.2 ± 0.7	202 ± 13	6.04 ± 0.32	29.3 ± 3.4
.75	6	23.9 ± 1.5	13.5 ± 1.6	193 ± 8§	6.15 ± 0.30	26.4 ± 2.6
1.0	6	24.8 ± 1.5	12.9 ± 0.8	182 ± 12§	5.80 ± 1.00	23.4 ± 1.1§
2.0	1	21.6	11.6	138	3.45	16.0
5.0	1	22.0	13.6	175	5.25	23.9
ACTH (1 mg)	4	21.5 ± 1.1	14.4 ± 1.0	142 ± 7§	4.35 ± 0.26§	20.0 ± 1.0§
Females—mg histamine per 100 g body wt.						
Control	9	20.0 ± 1.2	23.4 ± 1.1	234 ± 8	11.9 ± 0.2	54.8 ± 1.8
1.0	2	17.9	24.1	222	9.5	53.5
2.0	8	23.1 ± 1.3	21.2 ± 0.8	222 ± 10	10.9 ± 0.6	47.1 ± 3.3
5.0	4	24.3 ± 1.5	20.1 ± 1.1	201 ± 10	9.8 ± 0.8	40.0 ± 2.0§
ACTH (1 mg)	4	23.1 ± 0.9	21.3 ± 2.0	199 ± 5	9.7 ± 0.5	42.2 ± 3.6

* Values are means and standard errors.

† mg per 100 g body weight.

‡ mg per 100 g fresh adrenal.

§ Significantly lower than appropriate control value ($p = < 0.01$) by "weighted" t-test.(7)

remarkably similar to those of the control, tumor-free group.

In connection with observations of another nature, analyses of adrenals for ascorbic acid were also made in mice following a 48-hour fast (Table II). No significant change in concentration or total content of adrenal ascorbic acid was demonstrated, in confirmation of previously reported studies in fasted rats (5). However, a trend toward an increased total quantity of ascorbic acid was observed in the adrenals of fasted normal animals. On the other hand, it is of interest that the combined stress of a rapidly-growing tumor and a 48-hour fast resulted in a significant reduction in the concentration of adrenal ascorbic acid.

In Table III are presented data concerned with the response of adrenal ascorbic acid to the administration of varying doses of histamine and of 1 mg of ACTH. In agreement with data for the rat published by Sayers and Sayers(6), it will be noted that a progressive depletion of adrenal ascorbic acid results from

the intraperitoneal administration of increasing doses of histamine to male mice.

The refractivity of the adrenal ascorbic acid concentration of female CBA mice both to histamine and ACTH is also evident in the data of Table III. Histamine at a dosage-level of 0.75 mg of histamine base per 100 g of body weight was sufficient to produce a significant reduction of adrenal ascorbic acid concentration in the male mouse. In contrast, intraperitoneal doses of histamine base as high as 5.0 mg per 100 g of body weight administered to female mice failed to produce a significant drop in the concentration of adrenal ascorbic acid, although a significant fall in the total content of adrenal ascorbic acid per unit body weight was demonstrable following this dose. Similarly, 1 hour after the administration of 1 mg of ACTH to each of a group of male and female mice, the mean concentration value of adrenal ascorbic acid had fallen 40% in the males and only 19% in the females, from the appropriate control values.

Since concentration values for adrenal ascorbic acid are closely similar in male and female mice, while the weight of both adrenals in the female is roughly twice that in the male, it is evident that the female mouse possesses a total quantity of adrenal ascorbic acid approximately twice that of the male. It is of

5. Sayers, G., and Sayers, M. A., *Recent Progr. Hormone Research*, 1948, v2, 81.

6. Sayers, G., and Sayers, M. A., *Endocrinology*, 1947, v40, 265.

7. Snedecor, G. W., *Statistical Methods*, Iowa State College Press, Ames, 1946, p. 83 ff.

interest to note that when a comparison is made of the total quantity of ascorbic acid disappearing from the adrenals of male and female mice following the administration of ACTH, the respective values are closely similar—2.4 and 2.2 micrograms in the case of males and females, respectively. A similar relationship with respect to adrenal ascorbic acid values is suggested following the administration of larger doses of histamine, although the data for corresponding doses in males and females are insufficient to establish this point.

Discussion. The data described in this study demonstrate that the adrenal ascorbic acid response of the host to the growth of a tumor(1) is not necessarily a general phenomenon independent of such variables as species, sex, strain and tumor type. When it occurs, the depletion of ascorbic acid in the adrenal, generally recognized as a consequence of severe stress(5), may be associated not with the growth of a given tumor *per se* but with incidental nonspecific tissue changes such as inflammation or necrosis. The failure to demonstrate lability of adrenal ascorbic acid in an animal with a rapidly growing lymphosarcoma is of some interest in connection with the possibility mentioned previously that a reciprocal relationship may exist between lymphoid tissue and the pituitary-adrenal cortex system. This hypothesis, however, cannot be even tentatively supported

solely from these data, unless further observations show lymphoid tumors to be unique in this respect.

It may be suggested that the growth of the tumor studied represents a stress of sub-threshold effectiveness, since when combined with a period of fasting, significant reduction of the adrenal ascorbic acid concentration results, although neither stimulus is effective alone.

Finally, the data concerning differences in the response of male and female mice to graded chemical stresses suggest that whatever the role of ascorbic acid in the production or release of adrenal cortical steroids, its utilization expressed as the disappearance of an actual quantity of ascorbic acid from the gland has a more constant quantitative relationship to the stimulus than when expressed as a given decrement in its concentration.

Summary. The progressive depletion of ascorbic acid previously reported to occur in the adrenals of female mice carrying a transplantable sarcoma has not been confirmed in the case of male mice of another strain bearing a transplantable lymphosarcoma. A pronounced sex difference exists in the adrenal ascorbic acid response of CBA mice to histamine and ACTH, although relatively constant total quantities of ascorbic acid may disappear from the glands of each sex following similar stimuli.

Received January 9, 1951. P.S.E.B.M., 1951, v76.

Nutritional Factors in Hemodynamics: Dissociation of Pressor Response and Hemorrhage Resistance in Avitaminosis C.* (18479)

RICHARD E. LEE† AND ELIZABETH A. HOLZE.†

From the Department of Pharmacology, Syracuse University Medical College, Syracuse, N. Y.

Direct microscope observations of the mesenteric capillary bed in vit. C deficient guinea pigs have revealed that in addition to slowed flow and "increased fragility" of the collecting

venules there was also present a reduced reactivity of the terminal arterioles and precapillary sphincters to topically applied epinephrine(1). This latter phenomenon suggested that ascorbic acid was perhaps one

* This study was supported in large part by the Nutrition Foundation, New York. We are also grateful to the Upjohn Company of Kalamazoo, Mich., for supplies of certain vitamins.

† Present address: Department of Medicine, Cornell University Medical Center and The New York Hospital, N. Y. City.