Concentration of Ascorbic Acid in Human Adrenal Cortex Before and After ACTH Administration.* (20557)

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(Introduced by Philip E. Smith.)

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It has been demonstrated that there is considerable species difference in adrenal ascorbic acid content, the concentration in the rat adrenal being about 3 times that found in the guinea pig(1). A considerable variation has also been reported within the same species, a range of 30 to 154 mg % having been found in the dog by Peters and Martin(2), who also found that there was little post-mortem change in the adrenal ascorbic acid content of the dog up to 6 hours post-mortem at "Morgue temperature" (12°C). This observation appears to validate the findings of Yavorsky, Almaden, and King(3) on human adrenals from autopsy material. These investigators found an average adrenal ascorbic acid level of 55 mg %. Since the work of Sayers *et al.*(4-6) it has become apparent that much of the wide variation in ascorbic acid content of the adrenal is the result of depletion consequent to the action of adrenocorticotropic hormone, and that the lowering of adrenal ascorbic acid may be brought about through the action on the pituitary of noxious stimuli, such as cold, "shock", injection of toxins, hemorrhage, etc. The fall of adrenal ascorbic acid following stimulation does not occur in all species, (the chick(7) and the quail(8) failing to respond). Agate, Hudson, and Podberezec(9) have reported the fall of cortical ascorbic acid following ACTH in the human.

The opportunity was recently presented to gather some data on human adrenal ascorbic acid levels in surgically removed glands with only a relatively short time elapsed between surgical excision and determination of ascorbic acid content. It was also possible to obtain glands from patients who had been previously treated with ACTH or cortisone or both.

Materials and methods. The data presented here were secured from the surgically excised adrenal glands of 22 patients of both sexes, all of whom had metastases, in most cases from a prostatic or a mammary carcinoma. Only the data on the ascorbic acid content of the adrenal cortex are presented here. In one group (11 patients) bilateral adrenalectomy was performed at one operation. All patients in this group were treated for 24 hours pre-operatively with 350 mg of cortisone in 5 divided doses. The right adrenal was removed and in 8 cases immediately after excision 25 U.S.P. units of ACTH were administered intravenously. The second adrenal (the left) was removed after a variable interval of time ranging from 40 to 180 minutes. In 3 cases in this group *no* ACTH was administered and the second gland was removed after an interval of 75 to 90 minutes. In a second group the adrenalectomy was done in 2 stages; some patients were treated with cortisone preoperatively and some were not. Six patients received 25 U.S.P. units of ACTH intravenously at timed intervals ranging from 40 to 300 minutes prior to removal of the first gland. After a period of 14 to 30 days the second adrenal was removed from 8 of these patients after treatment with 350 mg of cortisone divided into 5 doses during 24 hours. No ACTH was administered prior to the removal of the second adrenal. In 3 patients, in this group, one ACTH treated and 2 treated with neither cortisone nor ACTH, death intervened before the scheduled time for the removal of the second gland. It should be mentioned that no clinical or laboratory evidence of adrenal insufficiency was found

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in any of these patients after the removal of a single adrenal. Immediately after excision, the gland was cut into slices about 2-5 mm in thickness. Some slices were fixed for routine pathological study. Several slices from each gland were used for ascorbic acid determination, slices adjacent to each of these being fixed for histological study. An outline was traced of each slice to be used for chemical determination. Small segments were then cut out ranging from 20-60 mg in weight and the outline of the removed segment was indicated on the section outline. By comparison of this map of the material used for ascorbic determinations with suitably stained microscopic sections it was possible to ascertain what regions of the gland, medulla, cortex, or even various zones of the cortex, were included in the determination. Only determinations on cortex are included, data from segments having medulary tissue, excessive quantities of connective tissue, or large blood vessels being excluded. The segment of gland used for ascorbic acid determination was weighed immediately and ground with acid washed Berkshire sand in 2 cc of 6% trichloroacetic acid. The total ascorbic acid was then determined by the method of Roe and Kuether. Determinations were done on at least 6 segments from each gland, usually on 10 or 12.

Results. Fig. 1 shows the mean cortical ascorbic acid content of all the glands in the entire series. The first column represents the mean plus or minus the standard error in mg of ascorbic acid per 100 g of cortex (135 ± 5) for males and females combined who were treated prior to operation with cortisone but *no* ACTH. The number in each column represents the number of glands included. The second column is the mean (129 ± 9.3) for those glands which received neither cortisone nor ACTH preoperatively. The third and fourth columns represent grouping according to sex of those glands which received no ACTH (131 ± 8 and 139 ± 5). The majority received cortisone but the 3 cases which received no cortisone are included. There appears to be no significant difference between the first 4 columns. The last 2 columns represent the cortical ascorbic acid of all glands in the series following ACTH treatment. The mean for the ACTH-treated males is 97.3 ± 9 mg % as compared with a value for the males with no ACTH of 131 ± 8. The mean for the ACTH-treated females is 98 ± 10, as compared with 139 ± 5 for the females with no ACTH.

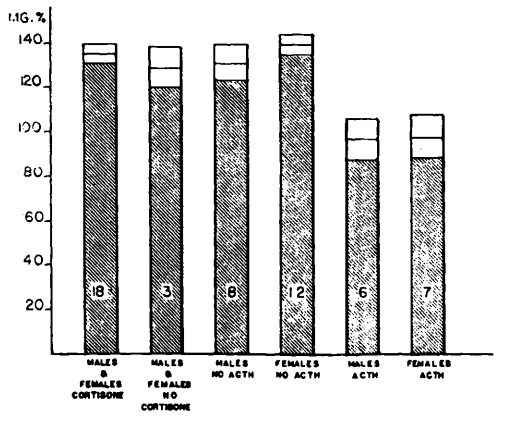

FIG. 1. Heights of the bars indicate cortical ascorbic acid content in mg/100 g fresh tissue. Unshaded area at top of bar represents range of plus and minus stand. error of mean, the mean being represented by the horizontal line in the center of this area. For further details see text.

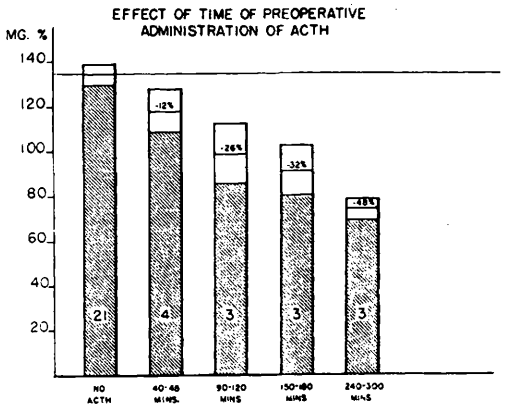

FIG. 2. Vertical coordinate is same as for Fig. 1. Numbers toward top of each bar represent % departure of mean from the mean of glands from patients not treated with ACTH. For further details see text.

Fig. 2 represents the effect of time of administration of 25 U.S.P. units of ACTH on the ascorbic acid content of the cortex. All figures are for males and females combined. The first is the mean of 134 ± 4.4 mg % for all untreated glands. Subsequent values (40-45 minutes, 90-120 minutes, etc.) show a

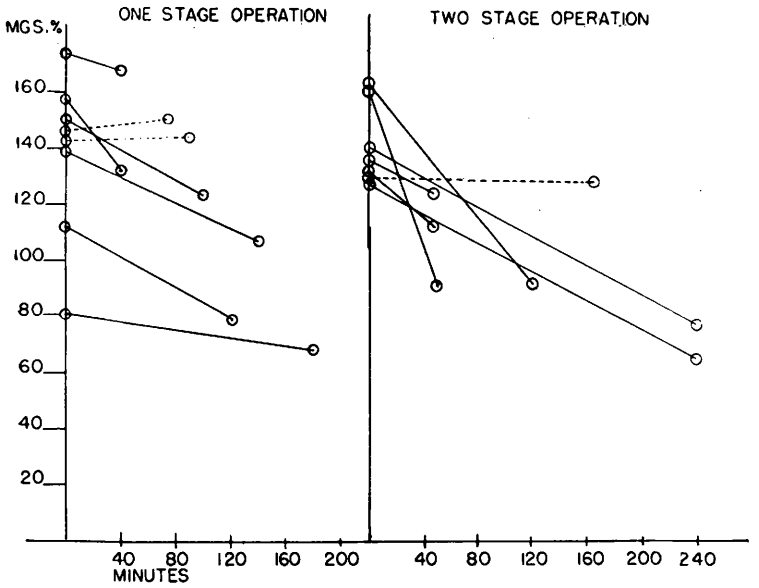

FIG. 3. See text.

steady decline to 91 ± 11 mg % at 150-180 minutes after ACTH. The last column at 240-300 minutes (74 ± 4.8 mg %) is complicated by the fact that 2 of these patients received an additional 50 U.S.P. units of ACTH during the operation making a total dose of 75 units.

Fig. 3 shows some individual cases. The left half of the chart shows cases of the first group which were bilaterally adrenalectomized in one stage. The points at the left represent the cortical ascorbic acid value of the first adrenal which was removed prior to ACTH administration. Each point is connected by a solid line with the ascorbic acid value for the second adrenal removed after 25 U.S.P. units of ACTH, the horizontal axis representing the time between administration and excision of the gland. The 2 dotted lines represent 2 cases in which no ACTH was given. In these cases the time indicated is that elapsed between the removal of the first and second glands. On the right half of Fig. 3 are shown values for the 2-stage operation. In these cases the values at the left represent the second gland which was removed with no ACTH treatment. The value for the first gland removed 2 weeks to a month earlier is shown toward the right, the time represented being the time elapsed between the administration of 25 U.S.P. units of ACTH and excision of the gland. The dotted line shows values for a case which received no ACTH prior to the removal of either gland and the time indicated is the duration of surgery up to the time of excision of the first gland.

Discussion. The primary purpose of cortisone administration prior to adrenalectomy was to protect the patient from the effects of sudden cortical steroid deficiency. However, we felt, in view of the finding of adrenal atrophy in humans following prolonged cortisone administration(10), that cortisone in the dosage used probably inhibited the release of endogenous ACTH from the patient's hypophysis during the course of the operation.

This appears to be substantiated by 2 cases at a one-stage operation and one case at a 2-stage operation, which received cortisone but no ACTH (Fig. 3, dotted lines).

The usual dosage of ACTH used in this series of humans ranged from 300-550 milliunits per kg of body weight. The mean fall in cortical ascorbic was only 12% at 40 to 45 minutes, but reached 26% at 90-120 minutes and 32% at 150 to 180 minutes. Under the conditions in our laboratory, using 100 g male rats at 24 hours after hypophysectomy a 30% depletion is obtained in adrenal ascorbic acid at 60 minutes after intravenous injection of

50 to 70 milli-units per k body weight. As demonstrated by Sayers(4), the ascorbic acid response in the rat reaches a maximum at about 20-30 minutes after ACTH injection.

In evaluating the data in this series it is important to bear in mind that all the adrenals included in this series were from patients with relatively advanced carcinomata and no comparable data are as yet at hand from human adrenals of noncancerous patients.

Summary. 1. The mean ascorbic acid content of the adrenal cortex of human cancer patients prior to ACTH treatment was found to be 131 ± 8 mg per 100 g tissue in the male and 139 ± 5 mg % in the female. 2. A 32% depletion in cortical ascorbic acid was found at 2 to 3 hours after intravenous injection of 25 U.S.P. units of ACTH. 3. The dosage of ACTH required to produce this depletion appears to be about 7 to 8 times that required per kg of body weight for the male hypophysectomized rat and the time elapsed between ACTH injection and maximal ascorbic depletion appears to be greater than 3 hours in the human.

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Effect of Continuous Injection of Epinephrine upon the Glycosuria of Partially Depancreatized Rats. (20558)

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In acute experiments on intact animals, epinephrine causes hyperglycemia, redistribution of glycogen stores and occasionally a mild glycosuria of short duration. The effects of the prolonged injection of epinephrine are less well studied. Epinephrine was administered continuously to normal and to partially depancreatized rats for periods of 3 and 4 weeks. Increased amounts of glucose were excreted during the injection of epinephrine.

Methods. Male rats of the Sprague-Dawley strain were maintained on Archer Dog Pellets until they were partially depancreatized at a weight of 275 to 280 g. Depancreatized rats and unoperated rats were thereafter force-fed a medium carbohydrate diet(1) by stomach tube each morning (8:30 a.m.) and afternoon (4:15 to 5:00 p.m.). Each 24-hour dose of epinephrine hydrochloride (Upjohn) was contained in 1 ml of physiological saline with 1 mg of ascorbic acid added as an antioxidant. The control rats received an equal amount of saline and ascorbic acid. Each rat was placed in a metabolism cage which restricted its activity so that the animal was unable to reverse its position. A 21-gauge needle, having a small barb attached to its shank to prevent its withdrawal, was placed subcutaneously. Sterile needles were used for replacement every 48 hours. The solutions were administered to 6 rats simultaneously by a continuous injection machine. Three of the 6 rats received epinephrine and 3 received control injections. The room temperature was 74° to 78°F. Urine was collected at the same hour (8:00 to 8:30 a.m.) each day for the quantitative determination of glucose(2).

Experiments and results. Exp. 1 (Fig. 1)