Influence of Adreno-Cortical Extract, Cortisone and Desoxycorticosterone on Blood Levels and Urinary Excretion of Ascorbic Acid.*† (19011)

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Experimental evidence from these laboratories has been offered to show that adrenocortical hormones play an active role in the metabolism of ascorbic acid(1-3). In conditions of cold stress and in daily administration of ACTH, it has been found that the usual loss of ascorbic acid from the adrenals can be prevented greatly by adrenocortical hormones(4). Schaffenburg, Masson, and Corcoran(5) have recently called attention to the interrelationships between ascorbic acid and cortisone and ascorbic acid and desoxycorticosterone in disturbances of collagenous tissue. They have shown that, while cortisone will reduce the severity of scurvy in guinea pigs, desoxycorticosterone will increase the severity of disease in all of its aspects. Furthermore, they have reported that adrenal tissue from animals on a cortisone-ascorbic acid regime contained greater quantities of ascorbic acid than adrenal tissue taken from animals on ascorbic acid-desoxycorticosterone regime. They believe that the diseases of adrenal insufficiency and scurvy are similar.

In view of the very wide interest in the use of adreno-steroids in collagenous diseases and the fairly well established view that ascorbic acid is important in influencing metabolism and in maintaining integrity of collagenous tissue, it was thought well worthwhile and valuable to make a comparison of the adreno-steroids as they influence the metabolism of ascorbic acid. Such a study, we believe, may contribute to the understanding of the mechanism by which the adreno-steroids may be effective in certain collagenous diseases, and may define limits to which ascorbic acid is interrelated therein.

Methods. The following experiments were performed: 1. The plasma and cell ascorbic acid and 24-hour urinary excretion of ascorbic acid were studied in dogs on normal diet; dogs given ascorbic, 10 mg/kg daily, in addition to the normal diet; and dogs on normal diets given daily ascorbic acid as above and injected daily with one of the following adreno-steroids: adrenocortical extract, 5 dog units/kg; desoxycorticosterone, 2 mg/kg; and cortisone, 2 mg/kg. Eighteen daily experiments were performed on 3 dogs for each category, excepting the cortisone-ascorbic acid group. In this group, 12 daily experiments were performed. There were control dogs run with each experimental period, which covered 24 hours. Also each group was itself observed in control periods prior to the experimental period. 2. Four rats weighing an average of 225 g were placed in each of 5 metabolism cages and arranged on the following regimen for 4 days: group (A), control, normal diet; group (B), injected intramuscularly with 10 mg/100 g body weight of ascorbic acid daily; group (C), ascorbic acid as above plus 2 mg/100 g body weight desoxycorticosterone daily; group (D), ascorbic acid as above plus 5 dog units adrenocortical extract (Upjohn) per 100 g body weight of cortisone daily. Daily water intake-urine output records were kept. Ascorbic acid was determined on plasma and on blood cells(6)

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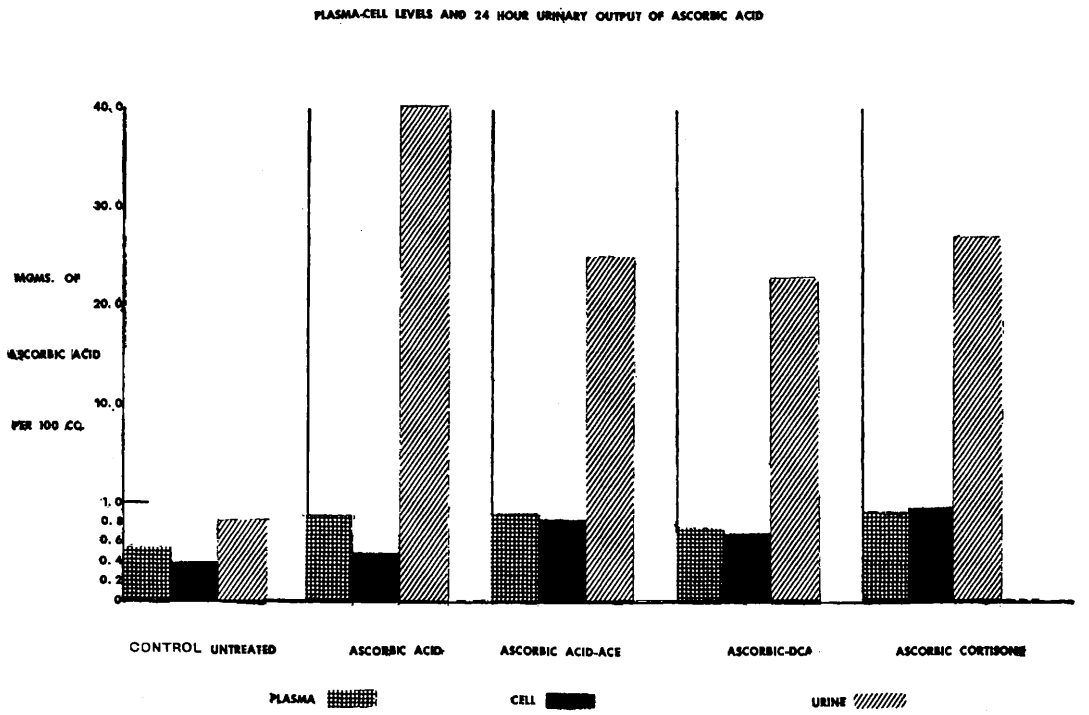

FIG. 1. Showing effects of adreno-steroids on plasma and cell ascorbic acid levels and on 24-hr urinary output of ascorbic acid in dogs. Values are mean values obtained from four experiments on each of three dogs.

according to the method of Mindlin and Butler(7). Two such experiments were performed involving a total of 40 rats. 3. From the above groups at sacrifice, the following tissues were removed from each animal and pooled for each category: Adrenals, liver, kidney, brain, skeletal muscle, tendon, fat and spleen. Homogenates of the pooled tissues were prepared in metaphosphoric acid for ascorbic acid determination.

Results. Fig. 1 is a representation of an average of 4 experiments on 3 dogs, showing changes in plasma and cell ascorbic acid and 24-hour urinary excretion of ascorbic acid under the regimen of ascorbic acid only, ascorbic acid plus adrenocortical extract, ascorbic acid plus desoxycorticosterone and ascorbic acid plus cortisone. Note that the plasma and cell ascorbic acid levels were increased by the adreno-steroids above the values seen where ascorbic acid only was administered. Note also that the increase in the plasma ascorbic acid where the adreno-steroids were administered along with ascorbic acid is not as striking as the increase in cell ascorbic acid. In the ascorbic acid-adrenocortical extract group the cell ascorbic acid increased 60% over the group receiving ascorbic acid only; while in the ascorbic acid-desoxycorticosterone group it increased 40%, and in the ascorbic acid-cortisone group, the ascorbic acid increased 80% above the group receiving only ascorbic acid. Regarding the 24-hour urinary excretion of ascorbic acid, it is of interest that the output of ascorbic acid was reduced 37% by adrenocortical extract; reduced by 42% by desoxycorticosterone, and in the cortisone group, the reduction was 30%, compared with the ascorbic acid alone group.

Fig. 2 presents the results of average values from rat experiments, showing the influence of the adreno-steroids on the 24-hour urinary excretion in rats. It can be observed that cortisone decreases the daily loss of ascorbic

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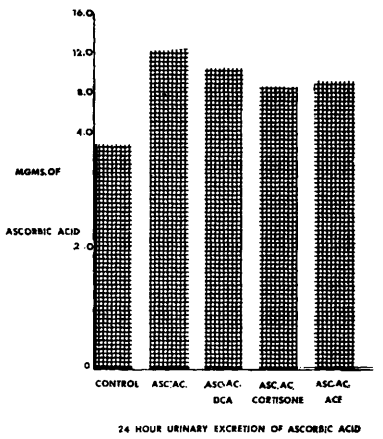

FIG. 2. Showing influence of adreno-steroids on 24-hr urinary excretion of ascorbic acid in rats. Values are representative of means of 8 animals in each category.

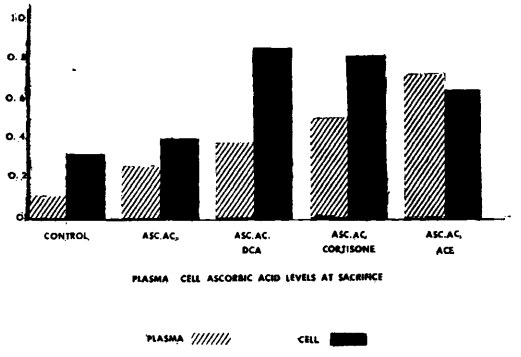

FIG. 3.

acid by 36%; adrenocortical extract by 23%; desoxycorticosterone by 15%. All values are compared with the group given ascorbic acid alone.

Fig. 3 presents data on ascorbic acid in plasma and in blood cells of rats at sacrifice, following 4 days on the respective regimen described. It is obvious that, as in the case of dogs, the adreno-steroids as a group increased the levels of ascorbic acid in blood. Note that desoxycorticosterone and cortisone are more effective in increasing cell ascorbic acid than adrenocortical extract, and that adrenocortical extract is more effective than either cortisone or desoxycorticosterone in building up plasma ascorbic acid.

Tissue distribution studies did not reveal any consistent trend regarding the influence of adreno-steroids on the deposition of ascorbic except at the adrenal gland. Here, desoxycorticosterone increased the ascorbic acid content of the gland by 10%; cortisone by 30%, and adrenocortical extract by 40%. In brain, those animals on ascorbic acid which received also adreno-steroids, showed a decrease of the ascorbic acid content of more than 100% below that of animals receiving ascorbic acid only. In muscle, adrenocortical extract and cortisone decreased ascorbic acid storage by 450% and 250%, respectively, below that of animals receiving ascorbic acid only. In fat, where the ascorbic acid content of the ascorbic acid treated group was surprisingly high (1.4 mg % on the average), cortisone and adrenocortical extract decreased the ascorbic acid levels 200 and 300%, respectively, but desoxycorticosterone increased the ascorbic acid by 50%. In tendon, the ascorbic acid treated group showed 10 times as much ascorbic acid as the untreated controls, while the cortisone-ascorbic acid and desoxycorticosterone-ascorbic acid group showed an increase of only 290% and the adrenocortical extract-ascorbic acid group showed an increase of 500%.

Discussion. The foregoing experiments show that adrenocortical extract, cortisone and desoxycorticosterone act to increase the plasma and cell ascorbic acid and to lower the urinary excretion of ascorbic acid. The influence of cortisone upon the permeability of the blood cells to ascorbic acid is in agreement with the works of others pointing out the value of cortisone in this connection(8,9). Cortisone is more effective in lowering urinary excretion of ascorbic acid than cortisone or adreno-cortical extract; although in rats cortisone controls the urinary output of ascorbic acid better than either of the above. The greater influence of adrenocortical extract and cortisone on storage of ascorbic acid in the adrenal gland than that of desoxycorticosterone is, in our opinion, significant. We have described experiments in another communication(10) showing that ascorbic acid storage

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at the adrenal gland is enhanced by adrenocortical extract. If adrenocortical function and ascorbic acid storage are related, then it is understandable that adrenocortical extract and cortisone would be more effective than desoxycorticosterone in ameliorating the symptoms of scurvy as shown by Schaffenburg, Masson, and Corcoran (referred to above). These workers point out that the symptoms of experimental scurvy and of adrenal insufficiency are similar. It is of interest in this connection that some workers have found that in ascorbic acid deficiency, the adrenocorticosteroid is markedly lowered (11), and that the symptoms of scurvy can be partially corrected with adrenocortical extract. It is of further interest that desoxycorticosterone will aggravate scurvy and other collagenous diseases. As we see the problem, it is not enough to raise the kidney threshold to ascorbic acid and to build up high levels in blood cells without at the same time increasing the storage of ascorbic acid at the adrenal gland. It is likely in this regard that desoxycorticosterone may differ from cortisone and adrenocortical extract in its influence on the course of collagenous diseases. This is a point, however, that should remain open until further work has been done. Indeed, Schaffenburg, Masson, and Corcoran have expressed the view that vit. C deficiency in arthritis of scorbutic animals leads to deficiency of carbohydrate-active cortico-steroids, the lack of which may underlie collagen degeneration. The results of this study and data from unpublished experiments lead us to agree in principle with the above work, although the mechanism is still unexplained. Certainly, we believe that adreno-cortical function is important in the maintenance of normal blood levels of ascorbic acid. On the other hand, it appears that normal adrenocortical function is dependent to some extent upon proper storage of ascorbic acid at the adrenal gland.

Summary and conclusion. (1) Experiments have been described comparing the effects of adrenocortical extract, desoxycorticosterone and cortisone on blood levels and urinary excretion of ascorbic acid in dogs and in rats. 2. The data show that all of the above substances reduce the urinary excretion of ascorbic acid. In this connection, cortisone is more effective in rats, while desoxycorticosterone is the most effective in dogs. 3. All the compounds caused increased plasma and cell ascorbic acid in dogs and in rats. 4. Inconsistent patterns were seen in the influence of the adreno-steroids on tissue distribution of ascorbic acid. In many instances, the adreno-steroids reduced markedly the tissue content of ascorbic acid. 5. Adrenocortical extract and cortisone were more effective than desoxycorticosterone on storage of ascorbic acid at the adrenal gland.

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