conceivable that the animals used by Cori might have been so sensitive to inhibition that the progressive reduction in rate of emptying of the stomach with increasing concentration of solution fed might abolish the usual concomitant increase in amount absorbed.

Chemical determinations of phosphatase activity of intestinal homogenates showed that a considerable amount of the enzyme is present in the fluid contents of the small intestine (compare groups A and B, Table II). Oral administration of 25% glucose probably decreases the amount of enzyme in the tissues and increases the amount in the intestinal fluid. The effect is small. The cytochemical study, in agreement with the chemical findings, showed some translocation of the enzyme but no dramatic changes in concentration.

Conclusions. 1. Glucose absorption in the mouse has been studied. Increasing the concentration of administered solution results in an increase in the amount of glucose absorbed. 2. Glucose solutions of 75% concentration were absorbed somewhat more readily by mice of the C57 strain than by those of the A strain ($p = 0.05$). 3. Administration of 25% glucose solutions leads to some diminution of the phosphatase activity of the small intestine determined both chemically and cytochemically.

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Received April 4, 1952. P.S.E.B.M., 1952, v80.

Prolongation of Effects of Adrenal Cortical Secretion by Ascorbic Acid: Proposed Mechanism of Action.* (19533)

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(Introduced by C. E. Leese.)
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Systematic studies in this laboratory indicated an intimate relationship between ascorbic acid and the activity of the pituitary-adrenal axis. The alarm reaction (eosinopenia, lymphopenia, and adrenal cholesterol depletion) characteristic of acute exposure to stressors (*e.g.* epinephrin, trauma) was prevented by ascorbic acid treatment of rats, mice and guinea pigs(1-4). Although those data suggested that under such conditions the vitamin probably plays a compensatory role similar to cortical hormones, other observations indicated that the similarity is not complete. It was shown that the protective action of the vitamin against cold stress was not present in adrenalectomized rats(5) and that ascorbic acid *per se* failed to alter the adrenal cholesterol level, the blood leukocyte pattern, or the blood glucose levels(4). Further observations indicated that ascorbic acid is capable of enhancing the gluconeogenic activity of small or large doses of cortisone in adrenalectomized mice. These observations suggest a "synergism" between cortisone and ascorbic acid(6), since the vitamin *per se* does not alter the liver glycogen content(4).

The present communication presents data indicating that ascorbic acid treatment of rats prolongs the leukocytic effects of injected cortisone or of endogenously secreted cortical hormones (induced by ACTH).

Materials and methods. Female Wistar rats weighing about 205 g were used in 2

* This study was aided by a grant from the National Institutes of Health. The Cortone was supplied through the courtesy of Dr. E. Alpert, Merck and Co.; ACTH through the courtesy of Dr. I. Bunding, Armour and Co.; and Vit. C, injectable, through the courtesy of Dr. R. A. Lewis, Hoffmann-La Roche.

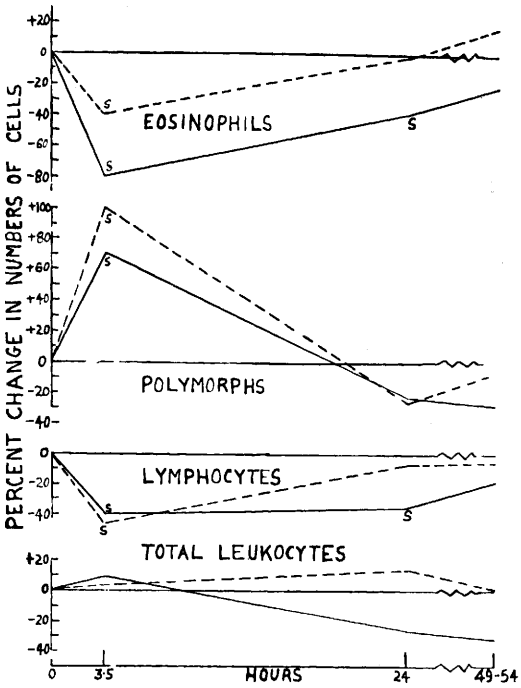

FIG. 1. Leukocyte response to cortisone in adrenex. Rats treated with ascorbic acid (——) or with saline (– – –). s = M.D. from count at 0 hr statistically significant.

experiments. For Exp. I the rats were bilaterally adrenalectomized (ether anesthesia) at least 2 weeks prior to use and received Purina Laboratory Chow and 0.9% NaCl solution *ad libitum*. For Exp. II adrenal-demedullated rats were used. The adrenals were enucleated (ether anesthesia) after the technic of Greep and Deane(7), at least 30 days prior to experimentation. Following operation, the animals received Chow and 0.9% NaCl for one week, and were then transferred to Chow and tap water. In both experiments tail blood was collected from all animals under nembutal anesthesia. The diluting fluid (eosin and methylene blue) was modified from that of Randolph(8). Total leukocytes, lymphocyte:polymorph ratio, and total eosinophils were determined by Randolph's method(8).

Experiments and results. Exp. I. Two groups of 8 adrenalectomized rats each were used. Group I was treated with 100 mg of ascorbic acid (Na ascorbate, Roche) per 100 g body weight by intraperitoneal injection. Fifteen minutes later these animals were injected subcutaneously with cortisone (Cortone), 2 mg per 100 g body weight. Group II rats received intraperitoneal injections of 0.85% NaCl, followed by the same dose of cortisone. Blood was collected from the tails of all animals (nembutalized) at the following times: a) just before the inj., *i.e.* at 0 hr; b) 3½ hr following inj. of cortisone; c) 24 hr following inj.; and d) 49-54 hr following inj.

The data from this experiment are presented in Fig. 1, which presents the percent change of the numbers of circulating cells from levels at 0 hr. Statistical analysis was previously carried out on the absolute numbers of cells and the Mean Differences ± S.E. from the count at 0 hr were calculated. Statistically significant differences are indicated by the "S" in the Figure. No marked change was observed in the total leukocyte levels. The lymphocytes showed significant decreases in both groups of animals at the end of 3½ hr following cortisone ($P < 0.01$ in both groups). The lymphopenia persisted in the Group I animals at 24 hr ($P < 0.05$) but not in the Group II animals. The levels of lymphocytes were not significantly different from base levels by 49-54 hr.

The polymorphonuclear leukocytes are significantly elevated ($P < 0.01$ in both groups) in both groups of rats at the end of 3½ hr, but not at 24 or 49-54 hr. The eosinophil levels are significantly decreased in both groups of animals at the end of 3½ hours after injection ($P < 0.01$ in Group I, $P < 0.05$ in group II). The eosinopenia persisted in the Group I rats at 24 hours ($P < 0.05$) but not in the Group II rats. The levels are back to baseline values at 49-54 hr. The data demonstrate that the eosinopenic and lymphopenic actions of *cortisone* persist for a longer time in animals which received ascorbic acid plus cortisone than in control animals. While it was possible to detect an intensification of the effect of small doses of cortisone by ascorbic acid in mice(6), such a phenomenon is not observed here. It is possible that the dose of cortisone was so high in this case that the presence of the vitamin was of no marked consequence at 3½ hours, but served to prolong the effects

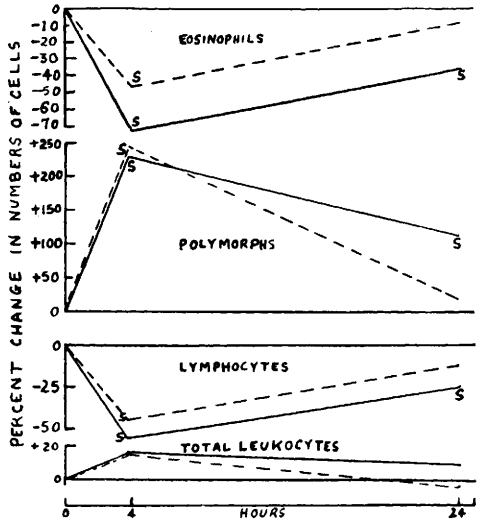

FIG. 2. Leukocyte response to ACTH in adrenal-demedullated rats treated with ascorbic acid (——) or with saline (– – –). S = M.D. from count at 0 hr statistically significant.

of the hormone. The effect noted here is not due to the vitamin independently since, as stated before, ascorbic acid does not alter the leukocyte pattern. The effect is due to a joint action of ascorbic acid and cortisone.

Exp. II was conducted on 2 groups of 6 adrenal-demedullated rats each. Group I rats received intraperitoneally 100 mg of ascorbic acid per 100 g body weight. Fifteen minutes later these animals were injected subcutaneously with 0.5 mg of ACTH per 100 g body weight. Group II rats received 0.85% saline (instead of ascorbic acid) and ACTH. Tail blood was examined at the following times: a) just before the inj. (*i.e.* at 0 hr); b) 3½ hr to 4 hr after ACTH inj.; and c) 24 hr after ACTH inj. The data are presented as percent change in numbers of circulating cells in Fig. 2.

No marked change was observable in total leukocyte counts. The lymphocyte levels significantly decreased ($P < 0.01$) in both groups of rats at 3½ to 4 hr after ACTH. The lymphopenia persisted at 24 hours in the Group I ($P < 0.05$) rats, but not in the Group II rats. The polymorphonuclear leukocyte levels of both groups increased at 3½ to 4 hours ($P < 0.01$) after ACTH. This persisted in the Group I rats ($P < 0.01$) at the 24-hour count, but not in the controls. The circulating number of eosinophils significantly decreased in both groups ($P < 0.05$) at 3½ to 4 hours after ACTH, persisted in the ascorbic acid + ACTH group ($P < 0.05$) but not in the control group. Isolated counts showed that the cells were back to baseline levels within 50 hours after ACTH injection. The data established that the hematologic effects of ACTH in adrenal-demedullated rats are prolonged when animals received simultaneous ascorbic acid treatment.

Discussion. The data of the present study indicate that ascorbic acid treatment prolongs the hematologic effects of exogenous and of endogenous cortical hormones. These prolonged effects suggest that detectable amounts of hormones are probably present for a longer period in the animals which received ascorbic acid. While the mechanism of maintenance of cortical hormone levels is not known, certain data were obtained in this laboratory indicating that it may be due to diminution of excretion or diminution of inactivation of the hormones in the presence of the vitamin. It was observed that 17-ketosteroid excretion in the 24-hour urine was significantly decreased in cortisone + ascorbic acid-treated female adrenalectomized rats (unpublished data). This study is being expanded.

With these considerations in mind it is possible to describe the relationship of ascorbic acid to pituitary-adrenal axis activity. Ascorbic acid blocks the activation of the axis to non-specific stressors. The exact locus of this interference is not known, but it is not between the pituitary and the adrenal cortex, *i.e.*, the vitamin does not block the action of ACTH. Furthermore, it is not at the level between the adrenal cortex and the periphery, since ascorbic acid does not block cortisone action(4). Indeed, it has been shown that the vitamin may enhance cortisone-gluconeogenesis(6). The vitamin does not have a direct effect on the leukocyte pattern, or on gluconeogenesis (3,4). It may be added that the vitamin is capable of blocking the activation of the axis without having any demonstrable lytic action on non-specific stressors; it has been shown that ascorbic acid fails to inactivate injected epinephrin or, to block directly the actions of trauma(4).

In view of the above it seems likely that ascorbic acid blocks the activation of the axis by inhibiting in some manner the activation of the pituitary. Although the exact details of pituitary activation are controversial, it has been generally accepted that the titer of circulating cortical steroids plays an important role in the regulation of pituitary-adrenotrophic activity. Sayers and Sayers (9) have shown that high titers of cortical hormone in the blood depress pituitary ACTH release whereas low titers increase this activity of the pituitary. Data were obtained in this laboratory indicating that ascorbic acid probably decreases the inactivation of cortical hormones. (It was observed that the excretion of 17-ketosteroid metabolites of cortisone in adrenalectomized female rats is significantly decreased by simultaneous ascorbic acid treatment). By the above process the maintained levels of circulating cortical hormones would tend to depress pituitary-adrenotrophic function. Under such circumstances non-specific stressors fail to activate the pituitary-adrenal axis.

This proposed mechanism does not include the suggestion that ascorbic acid is necessary for the secretory activity of the gland. Previous observations(10-12) indicated that the adrenal of the scorbutic animal is capable of producing cortical hormones in response to ACTH. The decreased adrenal hormonal effects observed by Giroud in scurvy(13) were probably not due to a decrease of production of hormones, but to an absence of the post-adrenal synergism with ascorbic acid in scurvy. McKee *et al.*(14), for instance, observed that the gluconeogenic action of injected cortical hormones is diminished in scurvy. While Dugal suggested that: a) ascorbic acid *per se* may activate the pituitary adrenal axis(15), or b) that it synergizes with ACTH(16), data to that effect have not been observed in this laboratory. It may be added that in the later paper Dugal stated the vitamin *per se* does not activate the axis.

Summary. The influence of ascorbic acid treatment on the hematologic effects of cortisone and of ACTH was investigated. The vitamin (plus hormones) prolongs these effects beyond that in animals which receive saline + hormones. These data suggest that the circulating titer of cortical hormone is maintained for long periods by vitamin treatment. This observation is discussed in relation to previous findings, and a mechanism proposed for the relationship of ascorbic acid to pituitary-adrenal activity.

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