cc or any size desirable. This meant a reduction of the reading time by at least 10 times the original method. Leaving the system for 12 hours, *e.g.* overnight, yielded a satisfactory reading. No complete equilibrium was aimed at. In applying low microscopic magnification, estimation of differences less than 0.01 molarities can be obtained.

The instrument is constructed of lucite plastic. It can be used repeatedly for successive determinations. The transparency of the tubes allows the determination of the length of the fluid columns in the capillaries with the aid of low power magnification. The inner tube harboring the 2 capillaries possesses a recording grid. (The instrument may be obtained from Mr. V. L. Frykman at the Precision Instrument Shop of the University of Southern California in Los Angeles.)

Summary. The present paper describes an instrument constructed for determination of osmotic concentration in biological fluids. It estimates differences less than 0.1 molarities.*

* I take the opportunity to acknowledge the contribution of Dr. J. P. Meehn (Physiological Institute of the University of Southern California) in the design of this instrument.

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Potentiating Effect of Ascorbic Acid on Cortisone-Induced Gluconeogenesis. (19474)

HABEEB BACCHUS, MELVIN H. HEIFFER, AND NORMAN ALTSZULER.
(Introduced by Chester E. Leese.)
From the Department of Physiology, George Washington University, School of Medicine, Washington, D. C.

Previous work from this laboratory suggested that ascorbic acid treatment of rats, mice, and guinea pigs can prevent activation of the pituitary-adrenal axis in response to stressors(1-4). It was observed that the vitamin fails to alter the leukocyte response to cortisone and to adrenocorticotrophin. Furthermore, it was demonstrated that the vitamin does not possess any specific anti-epinephrin action to explain failure of the activation of the pituitary-adrenal axis(4). Those data suggested that the ascorbic acid prevents the activation of the axis by either directly or indirectly rendering the pituitary inert to certain stimuli. Preliminary data indicated that ascorbic acid prolongs the leukocytic effects, and intensifies the gluconeogenic actions, of cortisone. The present communication reports data which indicate that the gluconeogenic action of cortisone is potentiated by ascorbic acid.

Materials and methods. These experiments were conducted on young adult male Swiss mice. The animals were bilaterally adrenalectomized under ether or nembutal anesthesia at least 3 days, and not more than 3 weeks prior to experimentation and maintained on an *ad libitum* fare of Purina Laboratory Chow and 0.9% NaCl solution. At the end of the periods of experimentation the animals were killed by a blow on the head, and the liver extirpated and plunged into a 30% solution of potassium hydroxide; the glycogen was then hydrolyzed by the method of Somogyi *et al.*(6), and the glucose determined by the method of Folin(5).

Experimental designs and results. The first experiment was conducted on male Swiss mice which had been adrenalectomized 3 weeks previously, and were maintained on Chow and saline. Body weight range at time of experiment was 25-30 g. Group I mice received single intraperitoneal injections of ascorbic acid (sodium ascorbate, vit. C, injectable, Roche), 100 mg per 100 g body weight, on the previous evening. Early the next morning these animals received similar amounts of the vitamin. The first injection

TABLE I. Liver Glycogen Concentration in Adrenalectomized Mice Treated with Cortisone and Ascorbic Acid, and with Cortisone and Saline

Group	No. mice	Treatment	Liver glycogen, mg/1 g liver ± S.E.	P
I	4	A,* C	49.1 ± 3.1	I vs II <.05
II	"	S, C	37.3 ± 2.1	II vs III<.01
III	"	0	20.6 ± 1.75	I vs III<.01

* A = Ascorbic acid; C = Cortisone; S = Saline.

TABLE II. Liver Glycogen Content in Adrenalectomized Mice Maintained on Glucose and Treated with Cortisone and Ascorbic Acid, or with Cortisone and Saline.

Group	No. mice	Treatment	Liver glycogen, mg /10 g body wt±S.E.	P
I	7	A,* C, G	29 ± 2.7	I vs II<.01
II	7	S, C, G	17 ± 2.4	II vs III<.01
III	5	G only	3 ± 1.2	I vs III<.01

* A = Ascorbic acid; C = Cortisone; S = Saline; G = Glucose.

was made in order to prime the animals. Immediately following the second injection of ascorbic acid the mice received single subcutaneous injections of cortisone (Cortone, Merck), 4.0 mg per 100 g body weight. Group II mice received similar treatment as those of Group I except that in this case vit. C (ascorbic acid) was replaced by intraperitoneal injections of physiological saline. Group III mice received no treatment. The animals were all taken off food following the injections, but were permitted to drink 0.9% NaCl solution. All animals were killed at the end of 4 hours after the injection of cortisone, the controls at the corresponding time, and glycogen determinations conducted.

The data are presented in Table I in which the liver glycogen concentration is presented as milligrams of glucose per 1 g of liver. It is seen that the liver glycogen content of the animals receiving cortisone is higher than that in the animals receiving no treatment. The liver glycogen content in the animals which received cortisone and vit. C (Group I) is significantly greater than that of the animals which received cortisone and saline (Group II) ($P < 0.05$).

The second experiment was conducted on mice weighing 20 to 25 g. The procedure suggested by Venning *et al.*(7) for assay of corticoids was adopted in this experiment. Group I mice (adrenalectomized) received the previous evening the priming dose of ascorbic acid as those in the previous experiment, and were allowed to feed *ad libitum* overnight. The following morning these animals received 7 injections of a menstruum containing cortisone and glucose subcutaneously, the total dose of cortisone amounting to 70 μg, of glucose, 70 mg. These animals also received 2 injections of ascorbic acid, one at the time of the first cortisone injection, and the other at the time of the third cortisone-glucose injection, the amount of ascorbic acid being 100 mg/100 g body weight. Group II animals were treated like those of Group I, except that they received saline solution in place of the intraperitoneal injections of ascorbic acid. The animals of Group III received 7 injections of glucose, amounting to 70 mg, but received no cortisone, or ascorbic acid. The 7 injections mentioned above were given in the course of 6 hours, the animals being killed one hour after the last injection, *i.e.*, a total period of 7 hours after the first injection of the menstruum.

The data are presented in Table II in which the liver glycogen, as glucose, is presented as milligrams per 10 g of body weight of the animals. This is the method recommended by Venning *et al.*(7). It is observed that the liver glycogen content of Group II animals is 466% greater than that of the animals which received no cortisone (Group III); the livers of Group I mice contain 866% more glycogen than those of Group III animals. The liver glycogen content in the vitamin-cortisone mice is significantly greater ($P < 0.01$) than that of the cortisone-saline animals.

Discussion. The data obtained from the 2 experiments indicate that ascorbic acid treatment of adrenalectomized mice supplied with small and large doses of cortisone increases the deposition of glycogen in the liver. This deposition of glycogen in the livers of the cortisone treated animals was probably due to gluconeogenesis. It is unlikely that the glucose supplied to the mice in the second experiment was converted to glycogen stores in

the liver; it is observed that the glycogen values in the animals receiving glucose alone were extremely low. Venning(7) had shown that in such glucose-treated animals, the glycogen deposition following cortical hormones was due to gluconeogenesis. The ability of ascorbic acid to enhance the liver glycogen deposition must therefore be ascribed to an increase in the gluconeogenesis due to the ascorbic acid in association with the cortisone, since the vitamin alone fails to enhance gluconeogenesis in adrenalectomized mice (unpublished data). McKee *et al.*(8) reported that liver glycogen deposition following the injection of cortical hormones into scorbutic guinea pigs was lower than that in normal animals. This observation could conceivably have been due to the general debility in the scorbutic animals. In the present study it is observed that vitamin treatment to animals on otherwise good ascorbic acid balance (the mouse requires little or no ascorbic acid in the diet) is capable of enhancing the glycogen deposition. This finding may be related to the action of the vitamin in normal intact animals exposed to stressors, in which case the alarm reaction is prevented, and survival is increased (9). It was shown that this ability of the vitamin requires the presence of the adrenal cortex, or of circulating cortical hormones(10).

It is possible that the vitamin "synergizes" with the existent cortical hormones in intact animals, and in animals supplied with cortical hormones. Data supporting this postulate come from the observation that the vitamin is capable of prolonging the leukocyte effects of cortisone(11). This prolongation, in addition to the demonstrated potentiation, of the action of cortisone by ascorbic acid suggests a synergism of ascorbic acid with cortisone. This synergism may explain the non-activation of the pituitary-adrenal axis in ascorbic acid-treated intact animals exposed to stress. It is possible that through this synergism the titer of cortical hormones is maintained in the blood, hence increasing the threshold of the anterior pituitary, according to the mechanism proposed by Sayers and Sayers(12). The relation of this possible synergism to normal adrenal secretion is being investigated.

Summary. The action of ascorbic acid treatment on the gluconeogenic action of cortisone was investigated in adrenalectomized mice. The deposition of glycogen in the liver is enhanced in animals treated with ascorbic acid in addition to cortisone. This observation is discussed in relation to the action of the vitamin in preventing the activation of the pituitary-adrenal axis in animals exposed to stressors. It is suggested that the vitamin may be synergistic with cortisone in some of its actions.

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