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 subthreshold 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.

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

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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

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of the factors essential for the functioning of certain peripheral vasomotor mechanisms. Kasahara and co-workers(2,3) have described reduced pressor responses to intravenously injected epinephrine in guinea pigs receiving scorbutogenic diets. These reports have been substantiated by Freire(4). In addition, the perfused hind-limbs of scorbutic guinea pigs are reported to have a notably diminished vascular response to the vasoconstrictor effects of epinephrine(5). These findings could perhaps be explained at least in part by an epinephrine-protecting action of ascorbic acid contained either in the blood or the perfusion fluid in the control observations. This antioxidant property of the intravascular vit. C presumably would have little if any direct influence, however, on the peripheral vascular responses to epinephrine topically applied to the outside of the vessels observed. The direct method of testing epinephrine responsiveness therefore offered possibly more convincing evidence suggestive of vasomotor impairment in avitaminosis C. To supplement this evidence, we have examined the ability of vit. C deficient guinea pigs to vasocompensate in response to vascular stress. In addition, comparisons were made between the mean blood pressures and the pressor responses to intravascular injections of epinephrine prior to the stress period, in the deficient and in the supplemented animals. It was found that although the initial mean blood pressure and its rise following epinephrine (at the dosage used) were not significantly affected by vit. C deficiency, the capacity to withstand hemorrhage in such animals was greatly diminished.

Methods. Nineteen guinea pigs of 500-600 g body weight were given a synthetic ration free of ascorbic acid.‡ Ten of these animals received daily intraperitoneal injections of vit. C, 10 mg per 100 g body weight, neutralized with 0.5% sodium bicarbonate. Nine of these supplemented animals were pair-fed with the remaining nine guinea pigs who received no ascorbic acid. This program was continued for 24-26 days, then the following procedures were carried out on each animal. General anesthesia was induced with sodium pentobarbital (3 mg/100 g body weight) and the carotid artery was cannulated with a blunt No. 22 needle. Coagulation of blood was prevented by a previous intramuscular injection of Heparin, 100 units. The initial blood pressure and its rise following intravenous (or intra-arterial) epinephrine, .001 mg/100 g body weight, was recorded, using a Hurthle membrane manometer. Following the return of normotension, blood was slowly withdrawn from the carotid cannula over a 4-5 minute period, until the blood pressure had fallen to approximately 60% of its initial level. At intervals of 30 minutes, blood samples of 2 cc/500 g body weight were taken until the animal expired. The plasma from certain samples was refrigerated at 7°-8°C for 24 hours and was then tested for vasotropic properties by the Chambers-Zweifach rat meso-appendix test(6,7).

Results. It was noted early in the study that guinea pigs not supplemented with ascorbic acid were unusually susceptible to the hypotensive action of the anesthetic used. In the controls, satisfactory complete surgical anesthesia was obtained using 3 mg of sodium pentobarbital/100 g body wt, injected intramuscularly. The deficient animals were adequately anesthetized without relative hypo-

‡ This diet was composed of Vit. Test Casein—18%; cornstarch, 49%; lard, 5%; sucrose, 15%; salts (U.S.P. 12 salt mixture No. 2), 4%; CLO, 1%; WGO, 2%; corn oil, 1%; and vitamin supplements, 1%. These latter were (per 100 g diet): Thiamine HCL, .8 mg; Riboflavine, .8 mg; Pyridoxine HCL, .8 mg; cal. pantothenate, 1.5 mg; nicotinic acid, 1.5 mg; nicotinamide, 1.5 mg; choline chloride, 400 mg; and Inositol, 100 mg.

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TABLE I. Blood Pressure and Pressor Responses, Blood Loss, and Plasma Vasotrophic Activity in Rat Test of Representative Animals from Supplemented and from Deficient Groups. Findings in remaining animals are in close accord with the data given.

						Rat assay of plasma 1, 2		
No.	Initial B.P. (mm Hg)	B.P. level after epinephrine	Initial hemorrhage (cc/500 g body wt) cc	Total amt of blood loss (cc/500 g body wt) cc	Survival time, min.	Sample 1 (30 min.)	Sample 2 (60 min.)	Sample 3 (90 min.)
				Supplemented				
1	100	120	7	14	34	grde 1 VD	—	—
2	70	120	8	20	80	neutral	grde 1 VE	grde 3 VE
3	100	140	5	12	100	neutral	grde 1 VE	grde 3 VE
4	65	130	10	17	100	neutral	grde 2 VE	grde 3 VE
5	80	220	8	20	130	neutral	grde 2 VE	grde 3 VE
6	80	134			110			
Avg	83	146	7.6	16.6	92.0			
				Deficient				
1	84	135	5	7	50	lost	grde 1 VD	—
2	52	145	3.5	3.5	10	grde 1 VD	—	—
3	78	122	4	8	30	grde 2 VE	—	—
4	70	120	5	6	8	neutral	—	—
5	95	139	4	8	48	lost	neutral	—
6	82	190	5	11	90	neutral	grde 1 VD	grde 1 VD
Avg	77	143	4.3	7.1	39.0			
Table of x	P = 0.52		<0.01	<0.01				

1. A dash indicates that no sample was obtained because of previous death of the animal.
2. VE = vaso-excitor; VD = vaso-depressor.
3. If the animal's death became imminent the sample was drawn immediately.

tension with approximately 1-2 mg of the agent/100 g body weight. Larger doses were frequently followed by a rapid and severe fall in blood pressure and a subsequent respiratory failure. The pigmented animals receiving the unsupplemented diet seemed possibly more susceptible than the albinos to this vaso-depressor property of the anesthetic agent.

Following anesthesia, the mean blood pressure levels before bleeding were slightly below those found in the control group (Table I). The p value of the difference, however, was greater than 0.5. Under these conditions, therefore, no significant difference existed between the mean blood pressures of the control and the deficient animals. Epinephrine injections were followed in all cases by rises in blood pressure that were comparable both in rate and in degree to those of the controls.

The deficient animals were not as capable as were the controls in withstanding blood loss. The removal of 5-10 cc/500 g body wt reduced the blood pressure of animals on a supplemented diet to approximately 60% of its normal level. Comparable amounts of blood withdrawn from non-supplemented individuals invariably lowered the pressure to about 25-35% of normal, and in some instances, was followed by a rapid death of the animal. For this reason, it was found necessary to remove only 3-5 cc of blood from them in order to induce the desired hypotension. The p value of the difference is less than .01 (Table I). In addition to this susceptibility to the initial hemorrhage, further blood loss by sampling was also poorly withstood by the deficient group (Fig. 1). They occasionally succumbed shortly after withdrawal of the first (30 min.) blood specimen, in contrast to the facility with which control animals readily endured repeated removal of blood. The average survival time of the deficient animals was 39 min., in contrast to 92 min. for the supplemented controls. This susceptibility to subsequent hemorrhage was also accompanied by a relative reduction in the total amount of blood obtained from the deficient animals (Table I).

Rat meso-appendix assays were made of the plasma samples obtained from 8 supplemented animals following the onset of hypotension.

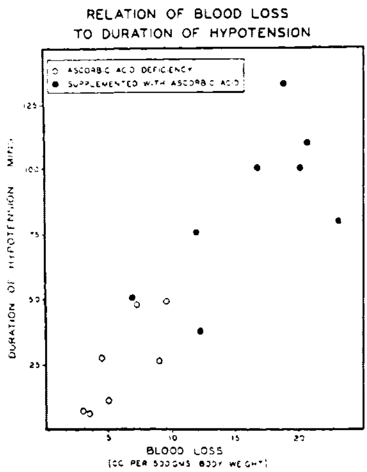

FIG. 1.
The total volume of blood loss is plotted against the duration of survival at hypotension for deficient and supplemented animals. It is evident that avitaminosis C is accompanied by a reduction of both features.

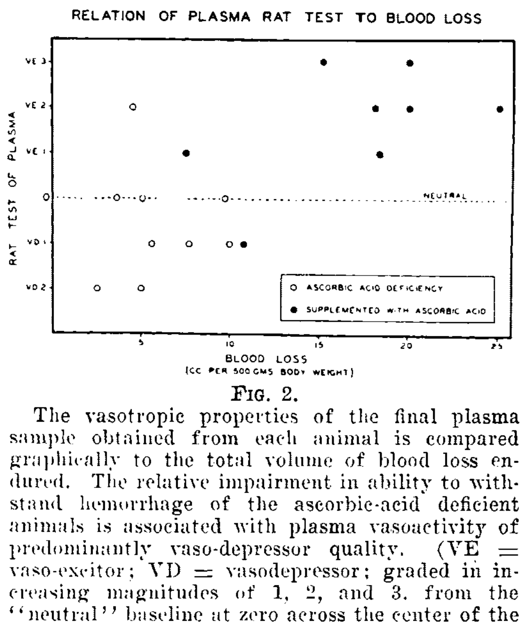

FIG. 2.
The vasotropic properties of the final plasma sample obtained from each animal is compared graphically to the total volume of blood loss endured. The relative impairment in ability to withstand hemorrhage of the ascorbic-acid deficient animals is associated with plasma vasoactivity of predominantly vaso-depressor quality. (VE = vaso-excitor; VD = vasodepressor; graded in increasing magnitudes of 1, 2, and 3. from the "neutral" baseline at zero across the center of the figure).

The first sample from animals No. 2-9 was neutral, but that of No. 1 was slightly vaso-depressor. This animal was one which had failed to adapt well to the diet, and it expired shortly after the sample was withdrawn. The second sample (at 60 min. of hypotension) was slightly to moderately vaso-excitor in character in No. 2, 3, and 5, and still neutral in No. 3, 7 and 8. The third plasma sample (90 min. of hypotension) was strongly vaso-excitor in all surviving animals.

In sharp contrast to plasma of the control animals, the plasma from deficient animals, with one exception, showed a complete absence of vaso-excitor activity in the rat test (Table I) (Fig. 2). Instead, despite the brief duration of their hypotension and only moderate blood loss, the plasma of many of the animals which survived a period sufficiently long to permit a 60 min. sample was vaso-depressor in character. The one deficient animal with vaso-excitor properties prevalent in the plasma samples was also very resistant to anesthesia (ultimately it received 3x the usual dose) and had no recognized symptoms of vit. C deficiency.

Discussion. It is of interest that with poor resistance to hemorrhage, plasma samples from the deficient animals did not exhibit the vaso-excitor property in the rat test (contain VEM) that was uniformly observed in the plasma of control animals under apparently similar circumstances. During and subsequent to such procedures as hemorrhage, trauma, etc., the elaboration of VEM is considered possibly to constitute a humoral vaso-compensatory process, probably supplementing neurogenic vasoconstrictor tone(8). It is known that the presence of vaso-excitor material is dependent at least in part on adrenal cortical secretion(9). Vit. C deficiency may prevent VEM formation or release by interfering with the availability of adrenal factors or perhaps other essential substances. Disturbances in VEM production are not related to one specific dietary deficiency, however. Payne and her co-workers have shown conclusively that rats on a low protein diet are relatively incapable of forming this principle(10).

Greatly reduced or absent pressor responses to intravenous injections of epinephrine in vit. C deficient guinea pigs have been de-

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scribed by several workers(3,4). Our data revealed no essential differences between the deficient and the control groups, with regard either to rate or degree of such a pressor reaction. The dose of epinephrine used in this study, however, was somewhat greater than that used by the previous workers. This undiminished pressor response to epinephrine of the deficient animals contrasted greatly with their impaired capacity to compensate for blood loss. This finding militates strongly against the use of such acute pressor tests as attempts to predict vaso-compensatory capacity, at least in response to hemorrhage. It is not known whether such a similar dissociation exists between the ability to raise blood pressure in response to epinephrine injections and the power to resist or survive other prolonged hypotensive procedures such as trauma, burns, etc.

Previous direct microscopical observations of the capillary bed in avitaminosis C revealed undiminished constrictor responses to topical epinephrine in the larger more proximal arterioles (greater than 90-100 μ in diameter). This reactivity of the metarterioles, however, (terminal arterioles supplying the capillary bed directly) was greatly diminished(1). It is conceivable that these observations are related to the apparent dissociation between acute pressor ability and the capacity to resist hemorrhage in vitamin C deficiency. The vasoconstrictor component in pressor response of *acute* and transient nature may depend in large part upon the maintained responsiveness of these larger arterioles. If this be true, then what is the role, if any, in the pressor mechanism, of the smaller terminal arterioles? Their *hypo*-reactivity to epinephrine, along with other phenomena, accompanies reduced resistance to hemorrhage. On the other hand, in such conditions as early shock, and experimentally induced hypertension, these arterioles in animals on fully supplemented diets become greatly and persistently narrowed, and gain and maintain an *augmented* reactivity to epinephrine. This state is believed to be mediated in large part by humoral factors, and perhaps to serve during vascular strain as an important vaso-compensatory mechanism(8). Thus by supplementing the peripheral resistance produced by the larger vessels, the capacity for vasoconstriction in these smaller terminal arterioles may be of relatively greater importance in effecting more *sustained* attempts at blood pressure elevation.

Conclusions and summary. 1. As compared to pair fed controls, guinea pigs on a vit. C free diet showed no obvious differences in blood pressure or in ability to respond in pressor manner to intravenous or intra-arterial injections of epinephrine at the doses employed. 2. The animals were bled by a standard procedure of hemorrhage. Avitaminosis C was accompanied by a significant reduction in the survival time, in resistance to anesthesia, and in the ability to withstand comparable amounts of blood loss. In addition, all deficient animals, with one exception, were unable to elaborate renal VEM as determined by rat assays of the plasma. 3. The significance of these observations is discussed. The suggestion is offered, in relation to earlier findings, that the larger arterioles with undiminished constrictor responses to epinephrine in avitaminosis C may function primarily in maintaining normotension and in producing the vasoconstrictor component of *acute* pressor reactions. The smaller, terminal arterioles, with greatly diminished epinephrine reactivity during vitamin C deficiency, may act chiefly in the pressor mechanism to supplement the larger vessels in acute stress states and during *prolonged* attempts at blood pressure elevation.

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