Nutritional Factors in Hemodynamics: III. Importance of Vitamin C in Maintaining Renal VEM Mechanisms.* (20063)

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It has been observed that the vit. C deficient guinea pig has a reduced reactivity of the terminal arterioles and precapillary sphincters to topically applied epinephrine(1) and a diminished capacity to withstand hemorrhage(2). Moreover, plasma from hemorrhaged, deficient animals is devoid of vaso-excitor material (VEM) as determined by bioassay of blood plasma by the Chambers-Zweifach rat mesoappendix test(2). To obtain further understanding of the relationship of vit. C and renal VEM, we have studied the ability of kidney tissue of ascorbic acid supplemented and deficient guinea pigs to form this principle *in vitro*. It was found that during the early stages of vit. C deficiency, there was a failure of the renal VEM mechanism in contrast to the absence of any such impairment in the pair fed supplemented control animals.

Methods. Thirty albino guinea pigs, selected at random, weighing 240-560 g, were given a stock ration known to be deficient in ascorbic acid. These animals were divided into 2 groups of 15 each, one of which was given a daily oral supplement of 20 mg of ascorbic acid (20 mg of ascorbic acid in 1 cc of 0.5% sodium bicarbonate). The vit. C supplemented animals were pair fed as controls with the remaining group of 15 animals which received no supplementation. This program was followed for a total of 21 days. At intervals of 7 days (on the 7th, 14th and 21st day), 5 guinea pigs from the deficient and 5 from the treated group were sacrificed by a blow at the base of the skull. The kidneys were removed immediately, decapsulated and placed in chilled saline. Slices of cortex were obtained from both kidneys (with one exception—one pair fed control had a single usable kidney, the second being hydronephrotic), and were washed in Ringer-phosphate solution. Two hundred to 300 mg of tissue were incubated in Ringer-phosphate solution of 37.5°C under 100% oxygen for one hour.† A second tissue sample from each animal, weighing 300-400 mg was incubated in similar manner under 100% nitrogen. In each case, the Ringer-phosphate medium was decanted and freed of suspended particles by centrifuging. Bioassays for vasoactivity were made on the first Ringer-phosphate wash of the fresh tissue slices and on both the aerobic and anaerobic incubation media by means of the Chambers-Zweifach mesoappendix test(3,4).

Results. Rat tests on the nitrogen incubation media revealed a sharp contrast between the kidneys of vit. C deficient guinea pigs and those of their pair fed controls. Renal tissue from the latter group invariably produced readily demonstrable VEM activity throughout the 21-day interval. Occasional moderate weight loss resulting from necessary restriction of dietary intake had no influence on the renal elaboration of this factor. A considerable difference was encountered, however, in the guinea pigs deficient in vit. C. Of the 5 animals sacrificed at the end of 7 days, the renal tissue of 2 animals was incapable of producing VEM activity; the remaining 3 animals' kidney tissue formed demonstrable amounts of this principle. Of the 5 deficient animals sacrificed after 14 days and the 5 sacrificed after 21 days, none were found whose renal tissues released VEM. The animals maintained for 21 days of vit. C deficiency lost a moderate amount of weight in contrast to their pair fed controls; the pigs maintained for a 14-day deficiency period showed no weight loss (with one exception), yet the renal tissue

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† Each tissue sample was incubated in Ringer-phosphate solution, which was proportioned one part tissue to 5 parts Ringer-phosphate by weight.

TABLE I. Result of Rat Mesoappendix Bioassay of Kidney Slices Incubation for Vasotropic Activity in Vitamin C Deficient Guinea Pigs and Their Pair-Fed Vitamin C Supplemented Controls.

No. of animals	Days on diet	Avg original wt	Avg final wt	% wt change	σ of % wt change	O_2 kidney incubation	N kidney incubation
			Vit. C deficient				
5	7–8	365.2	375.2	+ 2.7	7.4	N	1 neutral, 1 VD, 3 VE grade 1-3+
5	14–15	366	359.6	— 1.7	11.9	N	1 VD, 4 neutral
5	21	387.6	309.2	—20.2	5.0	N	1 " 4 "
			Pair-fed vit. C supplemented controls				
5	7–8	398.4	361.4	— 1.2	7.2	N	5 VE grade 1-3+
5	14–15	415.2	414	— .3	7.4	N	5 " " 1-2+
5	21–23	400.8	427.2	+ 6.8	7.7	N	5 " " 1-3+

VE = Vasoexcitor; VD = Vasodepressor; N = Neutral.

of both groups was similarly incapable of VEM production (Table I).

Guinea pig kidney slices incubated in oxygen produced neither vaso-inhibitor nor vaso-excitor material regardless of the duration of the diet period and of the diet administered (supplemented or vit. C deficient). It was observed early in this study that the Ringer-phosphate wash of kidney slices from both the deficient and supplemented animals produced no vascular response in the rat test, irrespective of the humoral characteristics of the anaerobically incubated tissue. For this reason bioassay of the Ringer-phosphate wash of fresh kidney tissue was discontinued after 6 such tests revealed no vasotrophic activity.

Discussion. In a previously reported study, Lee and Holze have shown that vit. C deficient guinea pigs subjected to hemorrhagic shock had blood plasma in which VDM was the predominant vasotrophic material. This prevalence of VDM in the plasma was thought to have been of such concentrations as possibly to mask the presence of any significant amount of VEM. Plasma from other scorbutic animals showed a complete absence of vaso-excitor activity in the rat test ("neutral" reaction), thus extending as a second possibility the failure of any renal VEM mechanisms(2). The present studies show that early in deficiency of ascorbic acid, there develops an inability of the kidney cortex to form VEM *in vitro* during anaerobiosis. Other studies have shown that disturbances of VEM production are not limited to a single specific dietary deficiency, however; renal tissue from rats on a "cirrhotic diet" fails to elaborate this factor(5,6).

During aerobic metabolism, renal tissues release little, if any, VEM(7). With the onset of anaerobiosis, however, the renal cortical parenchyma in dogs, rats, rabbits and man begins to elaborate VEM in relatively large amounts(7,8). The release of this principle under such conditions has been compared favorably to the "Pasteur" effect, with the chief metabolic cycles utilizing primarily anaerobic enzyme systems(9). The absence of VEM formation by anaerobic renal cortex from vit. C deficient animals suggests very strongly that these enzyme systems in the kidney are greatly impaired by deficiency in ascorbic acid.

Conclusions and summary. 1. Compared with pair-fed vit. C supplemented controls, *in vitro* studies of renal tissue from guinea pigs on a vit. C free diet revealed a significant impairment of VEM forming capacity in 7 days. 2. Complete loss of *in vitro* renal VEM elaboration was found on the 14th day as well as on the 21st day of vit. C deficiency. 3. Impairment of renal VEM mechanisms was unrelated to loss of body weight. 4. The significance of these observations is discussed and related to other findings in hemorrhagic shock and vit. C deficiency in hemorrhagic shock.

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Origin of Dog Serum Cholesterol Esterase.* (20064)

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Recent reports(1-4) have presented evidence for the existence of esterifying and hydrolytic cholesterol esterase systems in pancreas, dog serum, and rat intestinal mucosa. The enzymes occurring in these tissues appeared to be identical in that they required bile salts for activity, were inactivated by heating at 65°C for 15 minutes and had the same optimum pH for the hydrolytic and esterifying systems. The identity of the enzyme in rat intestinal mucosa was further verified by the demonstration that 95% depancreatized rats showed a marked lowering of cholesterol esterase activity.

In studies by Sperry and coworkers(5,6) on the cholesterol esterase of serum, it was reported that the esterification of free cholesterol occurred when human or dog serum was incubated alone. The esterification reaction in these sera was inhibited by bile salts, but in dog serum as the bile salt concentration was increased hydrolysis of the cholesterol esters of serum took place. In a previous study(3), the serum of 5 species were examined for cholesterol esterase activity. In dog serum, but not in human, rat, rabbit or guinea pig, serum highly active cholesterol esterase systems possessing the properties of the pancreatic type enzyme could be demonstrated. The occurrence of this enzyme in the serum of the dog is unique. Since a considerable amount of controversy exists regarding the occurrence and nature of serum cholesterol esterase, it was felt that a study should be conducted to ascertain more conclusively the origin of dog serum cholesterol esterase. Depancreatized dogs were prepared and the esterifying cholesterol esterase activity was followed before and after the operation with and without the addition of raw pancreas to the diet.

Procedure. Three normal healthy mongrel dogs were used. The animals were depancreatized according to the technic of Markowitz(7). Following the operation the dogs were maintained on 10 units of insulin, ½ lb raw lean beef, 10 g sucrose and 50 g raw pancreas given twice a day. The dogs were allowed to recuperate for 2 weeks. The raw pancreas was withdrawn from the diet after this period and given again after 10 days. Blood samples were obtained before the operation and then at regular intervals. Cholesterol esterase activity was measured according to the technic previously described(2) using cholesterol-oleic acid as the substrate.

Results. The results of the experiment are shown in Table I. Prior to the operation all dogs showed the presence of an active choles-

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