and types of hemolytic streptococci are antigenically related. Group A streptococci not producing DPNase *in vitro* also failed to make detectable enzyme *in vivo*.

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Dietary Protein Levels, Vitamins, and Lactose on Depressant Effect of Sulfadrugs on Urinary and Liver Ascorbic Acid in the Rat. (28180)

N. CHANDRASEKHARA, M. V. L. RAO AND M. SRINIVASAN
(Introduced by E. L. Severinghaus)
Central Food Technological Research Institute, Mysore

It has been reported(1) that increase in dietary protein level enhances urinary excretion and liver ascorbic acid content in the rat. Preliminary observations on the depressant effect of sulfaguanidine in low protein diets and its counteraction on 50% protein diets were also recorded. This report concerns an investigation of the effects of sulfaguanidine and sulfasuccidine in relation to dietary protein levels and supplementation with lactose and certain vitamins.

Experimental materials and methods. As in the earlier investigations, these observations were made on adult male albino rats in the body-weight range 160-200 g. Composition of the diets and details of collection of urine were as described previously. The basal diet provided Vit. A and D and thiamine, riboflavin, pyridoxine, pantothenate, niacin and folic acid. In testing the effect of some of the other vitamins they were administered either orally or intraperitoneally. In the final test to ascertain the influence of supplements of all the vitamins omitted from the basal diet the vitaminized starch used at 1% level in the diet contained: 0.5 g menadione (sodium bisulfite), 0.5 g thiamine, 1.0 g riboflavin, 0.4 g pyridoxine, 4.0 g calcium pantothenate, 4.0 g niacin, 0.05 g folic acid, 0.02 g biotin, 0.002 g Vit. B_{12}, 200.0 g choline chloride and 25.0 g inositol per kilo, and the oil soluble vitamins were provided by feeding orally one drop of a suitably diluted stock solution of Vit. A acetate, calciferol and tocopheryl acetate in refined peanut oil so as to provide 20, 10 and 1 I.U. of Vit. A, D and E respectively to each rat daily. In this experiment the salt mixture originally used was also supplemented with 40 mg zinc carbonate per kilo to make up any deficiency of the diet in zinc.

Total ascorbic acid in urine and tissue was estimated by the Roe and Kuether method (2).

Results. 1. *Influence of dietary protein levels.* Data in Table I show results of ingestion of sulfaguanidine and sulfasuccidine at 2-2.5 and 4% levels in diets containing different amounts of protein. The findings fully confirm previous observations concerning the depressant effect of the sulfadrugs on urinary and liver ascorbic acid levels with low protein diets and the counteracting influence of higher levels of protein in the diet, the effect being completely nullified by 50% protein in the diet. The results obtained with the 2 sulfadrugs and at the 2 levels were similar. In the present series of experiments the effect of the sulfadrugs on liver ascorbic acid was not as pronounced as in the previous study(1), except in animals receiving protein-free diets.

Urinary excretions observed in these experiments corroborate the earlier finding(1) that prolonged ingestion, in contrast to short-term feeding, tends to equalize the differences

TABLE I. Effect of Sulfadrugs on Urinary and Liver Ascorbic Acid in Adult Male Rats Fed Diets of Different Protein Content.

Set*	Dietary Casein (%)	Dietary Sulfadrug (%)	Period of feeding (wk)	Urinary total ascorbic acid (mg/rat/day), avg		Liver ascorbic acid Total (mg), avg		Liver ascorbic acid mg/g fresh tissue, avg	
1.	0	0	2–3	.46 ±.11	†	2.40 ±.18	‡	.35 ±.015	‡
	0	SS—2	"	.23 ±.025		1.60 ±.10		.27 ±.017	
2.	10	0	6–7	1.30 ±.27	‡	3.22 ±.33	NS	.37 ±.026	NS
	10	SG—2.5	"	.49 ±.056		2.57 ±.21		.37 ±.007	
3.	10	0	8	2.91 ±.40	‡	3.74 ±.32	NS	.42 ±.008	NS
	10	SS—2	"	1.04 ±.55		3.69 ±.26		.39 ±.020	
4.	10	0	6	2.25 ±.38	‡				
	10	SS—4	"	.64 ±.11					
5.	20	0	4	3.11 ±.21	‡				
	20	SG—2	"	1.78 ±.27	NS	§			
	20	SG—4	"	1.42 ±.22					
6.	50	0	6–7	1.19 ±.098	NS	3.07 ±.22	NS	.37 ±.018	NS
	50	SG—2.5	"	1.06 ±.11		2.83 ±.18		.34 ±.002	
7.	50	0	6	1.63 ±.30	NS				
	50	SS—4	"	1.48 ±.39					

* The rats in each set were distributed into groups of 6 each by simple randomization.

SS—Sulfasuccidine
SG—Sulfaguanidine

Differences significant at: † 5%, ‡ 1%, § 0.1% level. Since variances are not homogeneous the t-test has been done by approximate method suggested by G. W. Snedecor in Statistical Methods of Analysis, 1950, (Iowa State Coll. Press, Ames, Iowa), p. 83.

observed between animals receiving high and low protein diets.

2. *Influence of lactose.* The effect of the sulfadrugs suggested possible involvement of intestinal microflora in biosynthesis of ascorbic acid in the rat. The influence of inclusion of lactose, known to promote high proliferation of intestinal microflora, in the different diets was therefore examined. In these experiments the sucrose moiety (10%) of the diet was replaced by an equal amount of lactose. From the results (Table II) it is evident that in both high and low protein diets lactose inclusion did not in itself elevate either urinary excretion or liver levels. However, 10% lactose in the diet served to abolish the adverse effects of 2% sulfaguanidine which could again be reversed by increasing the sulfadrug level to 4%.

3. *Influence of vitamins.* Since biotin and Vit. B_{12}, 2 of the important vitamins known to be synthesized by intestinal microflora in the rat, had not been provided in the basal diet, their influence on the effect of sulfa-

TABLE II. Urinary Excretion and Liver Content of Ascorbic Acid in Rats Fed Diets Containing 10% Lactose and Different Levels of Protein and Sulfaguanidine. 6 Rats in each group. Period of feeding: 2 wk.

Set	Dietary Casein (%)	Dietary Sulfaguanidine (%)	Urinary total ascorbic acid (mg/day), avg		Liver ascorbic acid (mg/g tissue), avg	
1.	10	—	1.66 ± .23	NS	.38 ± .04	NS
	10	2	1.30 ± .05		.37 ± .02	
2.	10	—	2.03 ± .33	†		
	10	4	.74 ± .08			
3.	30	—	1.88 ± .18	NS	.41 ± .04	NS
	30	2	1.84 ± .11		.35 ± .028	

Difference significant at: * 5%, † 1%, ‡ 0.1% level. NS = Not significant.
See footnotes, Table I.

TABLE III. Urinary Excretion of Ascorbic Acid in Adult Male Rats Fed 10% Casein Diet Containing All Vitamins and 2 and 4% Sulfaguanidine.

6 Rats in each group. Period of feeding: 4 wk.

Group	Diet	Urinary total ascorbic acid (mg/rat/day), avg		
1.	10% casein diet containing vitamins A, D, E, K, B_1, B_2, B_6, pantothenate, niacin, folic acid, biotin, B_{12}, choline and inositol	2.99 ± .28	‡	
2.	*Idem* with 2% sulfaguanidine	1.02 ± .19		‡
3.	Same as 1 with 4% sulfaguanidine	.47 ± .13	*	

Differences significant at: * 5%, † 1% and ‡ 0.1% level. See footnotes, Table I.

guanidine was ascertained. Two groups of rats whose excretion of urinary ascorbic acid had been depressed to very low values by maintaining for 3-4 weeks on the 10% protein diet containing 2% sulfaguanidine subsequently received supplements of biotin (4 μg/rat/day orally) and Vit. B_{12} (5 μg/rat/day intraperitoneally) respectively for 12 days with no enhancement in urinary ascorbic acid. In view of the reported involvement of Vit. E in biosynthesis of ascorbic acid in the rat(3, 4) the influence of Vit. E was also examined similarly with negative results. Finally rats distributed into comparable groups by simple randomization were fed for 4 weeks a 10% casein diet containing all the vitamins and 2% and 4% sulfaguanidine. Total Vit. C excretion in the urine of these rats is presented in Table III. Supplementation with all the vitamins simultaneously also failed to counteract the effect of the sulfadrug.

Discussion. The adverse effects of sulfadrugs in animals maintained on low protein diets confirm some of the observations of Schwartz and Williams(5) and their coworkers(6), during their studies on the interrelationship between Vit. C and folic acid. They recorded decreased excretion and liver levels of the vitamin in aminopterin-fed rats and also reported lower liver levels in sufasuccidine-fed rats. It is noteworthy that we have obtained similar results on diets adequately provided with folic acid. The important additional finding is the annulment of the depressing effect of sulfaguanidine on urinary and liver ascorbic acid in rats by high levels of protein in the diet. This protective effect may be ascribed to the effect of excess protein *per se* on the biosynthetic mechanisms or on the precursors, or indirectly on the microflora.

The effect of dietary lactose in reversing the unfavorable effects of sulfaguanidine appears to indicate the possible intervention of intestinal microflora in the biogenesis of the vitamin. In this connection, Evans, Conney, Trousof and Burns(7) reported that galactose is a better precursor for Vit. C than even glucose. Whether lactose serves such a purpose, through the galactose component, has to be critically examined.

The results obtained with animals receiving the vitamin supplements preclude that any deficiency of these vitamins, caused either directly by their absence from the diet, or indirectly, through inhibiting the proliferation of microflora producing these vitamins, is responsible for the effects observed with sulfadrugs.

In addition to the influence of sulfadrugs on the intestinal microflora, their direct effects have also to be considered. Whether these drugs, even in the small concentrations in which they may reach the blood, would exert appreciable influence on liver enzymes directly concerned with Vit. C synthesis is not clear. Another possible effect of these drugs is their influence *per se* on the intestine. Morphological changes in the intestine induced by feeding of such drugs have been reported(8). The counteracting influence of high dietary protein levels and of lactose would then have to be understood in terms of preventing such damage. If the effects of these drugs on Vit. C biogenesis arise through such changes in the intestinal wall, the intestinal tissue would itself be importantly implicated in synthesis of either the vitamin or its precursors.

Experiments are now in progress to ascertain the influence of antibiotics and the direct correlation, if any, between intestinal microflora, as measured by fecal and cecal counts, and the ascorbic acid status of the rat on diets containing different levels of protein, sulfadrugs and also lactose.

Summary. 1. The adverse effect of sulfadrugs on urinary excretion of ascorbic acid in the rat and its counteraction by high levels of dietary protein have been substantiated. 2. Lactose, known to enhance proliferation of intestinal microflora, did not in itself raise ascorbic acid levels in the liver or urine when added to high and low protein diets at 10% level. However, it abolished the adverse effects of the sulfadrugs at 2% level which again was reversed by increasing the sulfadrug level to 4%. 3. Supplementation of the diet with all the known vitamins failed to reverse the effect of the sulfadrug.

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Effects of Epsilon-Amino-Caproic Acid on Prolactin-Inactivating and Fibrinolytic Activities of Streptokinase-Activated Plasminogen.* (28181)

THOMAS F. HOPKINS† AND JOSEPH MEITES‡
Department of Physiology and Pharmacology, Michigan State University, East Lansing

Previous reports have indicated that several protein hormones could be inactivated by blood plasma(1-4) or serum(5) from various mammalian species, and that the degree of inactivation, in some instances, could be increased by streptokinase (SK), a product of bacterial origin(3). SK has been found to be a highly effective activator of the fibrinolysin (plasmin) system of blood, but has not been found to activate other enzyme systems. Thus, some degree of specificity exists between SK and fibrinolysin. Fibrinolysin(5,6) and SK-activated plasminogen(3) have likewise been reported to inactivate protein hormones. The purpose of the studies reported here was to determine the relationship of fibrinolytic activity(FA) to prolactin-inactivating activity (PIA) of SK-activated plasminogen, and to determine whether either activity could be altered by (a) e-aminocaproic acid (EACA), a specific inhibitor of fibrinolytic activity of SK-activated plasminogen, or by (b) bovine antifibrinolysin (AFL), without influencing the other activity.

Methods. Purified ovine prolactin§ with an activity of 15 IU per mg was used. The prolactin was dissolved in distilled water to a concentration of 5.0 mg prolactin per ml solution. One-tenth ml (0.5 mg) prolactin solution was added to each flask which contained the incubation medium in Krebs-Ringer phos-

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