is highly significant. The results of the two experiments, by themselves, might not have statistical significance, but in the light of the large number of other experiments which can serve as legitimate controls we are convinced of the causal connection between the increase in plasma osmotic activity and the increase in gut fluid osmotic activity. The likelihood of the results occurring fortuitously is less than one chance in ten thousand, in other words, practically negligible.

It seems likely that the activity of the intestinal epithelium in the movement of water across it is influenced greatly by the osmotic activity on both the blood and lumen sides. In neither experiment reported did the increased osmotic gradient across the intestinal epithelium increase the net rate of water absorption. In one case, as noted, the hypertonic salt injection was associated with a negative net water absorption. Nevertheless the fact that gut fluid osmotic activities were changed by the procedure proves that the intestinal epithelium does not act independently of blood osmotic activity, unless the salt concentration itself has a specific effect, which seems to us to be unlikely, though possible. Comparable experiments with non-electrolytes are essential to a settlement of this question.

Summary and Conclusions. Experiments are reported in which the volume, osmotic activity, chloride and sulfate contents of plasma and ileal loop segments are measured, before and after raising the plasma osmotic activity by hypertonic sodium chloride solution injection by vein. The osmotic activity of originally isotonic sodium chloride-sodium sulfate solutions placed in the ileum rises above the pre-injection plasma osmotic activity after 100 cc of 5% sodium chloride solution was injected intravenously. In view of the fact that in more than a hundred control experiments with equiosmotic mixtures of sodium chloride and sodium sulfate the gut fluid osmotic activity has never exceeded the plasma osmotic activity except when poisons were present, the results of these experiments are believed to constitute satisfactory proof that the effect observed was due to the hypertonic salt solution injection.

After the elevation of the plasma osmotic activity the gut fluid values did not approach the former as closely as occurred before the elevation was produced. The net water absorption was not increased in proportion to the increase in osmotic gradient. In fact in one case the direction of net movement was reversed in spite of the larger osmotic gradient. These data add to previous evidence that although normal osmotic forces are probably contributing factors in the transport of water across the intestinal epithelium they are not the primary driving forces determining the direction and magnitude of net water transfer.

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Effect of Feeding Glucoascorbic Acid to White Rats, Chicks and Guinea Pigs.*

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Woolley and Krampitz[1] showed that cotton rats and mice failed to grow, developed severe diarrhea and showed hemorrhages in various parts of the body when fed a synthetic diet containing 10% glucoascorbic acid. The condition was prevented when plant ma-

* Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

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[1] Woolley, D. W., and Krampitz, L. O., *J. Exp. Med.*, 1943, **78**, 333.

terials such as cabbage or dried grass (Cerophyl) were added to the ration but synthetic vitamin C was without effect. In experiments with guinea pigs Woolley[2] observed the same effect of glucoascorbic acid as observed with cotton rats and mice, but in this case the condition was prevented by ascorbic acid. On this basis he viewed the disease in all the animals as an ascorbic acid deficiency.

Since diarrhea is not usually observed in scorbutic guinea pigs and monkeys the loss of body weight observed by Woolley after feeding glucoascorbic acid might have been the consequence of the diarrhea, and not the result of avitaminosis C. It was, therefore, considered desirable to study the effect of feeding glucoascorbic acid to young rats and chicks which like cotton rats and mice do not require an extrinsic source of vitamin C for their growth, and to guinea pigs for whom vitamin C is a dietary essential.

Experiments with rats. Twenty-one day old male rats were divided into 4 groups. The first group was fed a basal diet consisting of sucrose 75 g, casein 18 g, salt mixture[3] 5 g, corn oil 2 g, thiamine 300 γ, riboflavin 500 γ, pyridoxin 400 γ, Ca *d*-pantothenate 1.5 mg, nicotinic acid 5 mg, choline chloride 100 mg, biotin 20 γ, *i*-inositol 50 mg and 2-methyl-1,4-naphthoquinone 200 γ. For the remaining 3 groups the following were added to the basal ration to give the concentration indicated: glucoascorbic acid (10%), ascorbic acid (10%), or a mixture of glucoascorbic acid (10%) plus ascorbic acid (10%), respectively. The animals were weighed every other day and the symptoms noted. All the animals received a concentrate of vitamins A, D, and E in corn oil once a week.

All the animals except those on the basal diet suffered from diarrhea and failed to grow (Fig. 1). The symptoms were most pronounced in the group receiving both glucoascorbic acid and ascorbic acid and least marked in the group receiving ascorbic acid only. In the group receiving glucoascorbic acid plus ascorbic acid one animal was dead on the eighth day and the other 3 were lethargic and cold to touch.

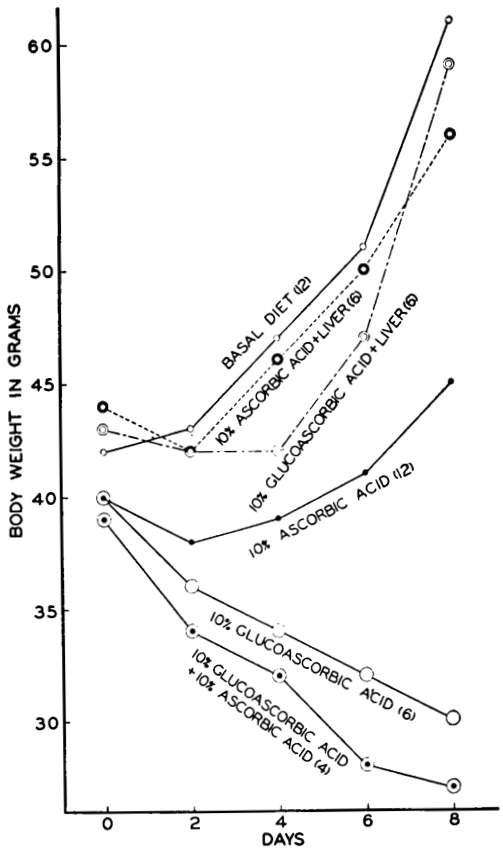

FIG. 1.

Growth curve of rats fed high levels of glucoascorbic acid and ascorbic acid. (Figures in parentheses indicate the number of rats used.)

The surviving rats were killed on the eighth day of the experiment and examined; no hemorrhages or other macroscopic pathological changes were observed in any part of the body. Extracts of the liver and kidneys were prepared by the method of Bessey[4] and titrated with 2,6-dichlorophenol indophenol which was standardized according to method of Menaker and Guerrant.[5] Since glucoascorbic acid also reduces the indophenol dye it is difficult to estimate how much of the reaction depended upon the gluco-

[2] Woolley, D. W., *Fed. Proc.*, 1944, **3**, 97.

[3] Hegsted, D. M., Mills, R. C., Elvehjem, C. A., and Hart, E. B., *J. Biol. Chem.*, 1941, **138**, 459.

[4] Bessey, O. A., *J. Biol. Chem.*, 1938, **126**, 771.

[5] Menaker, M. H., and Guerrant, N. B., *Ind. Eng. Chem., Anal. Ed.*, 1938, **10**, 25.

ascorbic acid. It is evident that the total activity in both the liver and kidney was slightly higher in the rat receiving glucoascorbic acid than in those receiving the basal diet alone. When both compounds were added there was a very significant increase in both tissues. Thus, there is no indication of a vitamin C depletion such as occurs in scorbutic guinea pigs.[6,7]

To study the effect of smaller doses of glucoascorbic acid for a longer period of time, groups of four rats were fed glucoascorbic acid mixed with the basal ration at levels of 10, 5, 1 and 0.5%, respectively, for 18 days. As previously observed, the rats receiving 10% glucoascorbic acid failed to gain in weight during the period but when they were transferred to the basal ration they grew as well as normal animals and the diarrhea disappeared. Though the animals receiving the 5% and 1% levels showed diarrhea they grew well. The basal ration produced an average gain of 3.2 g per day. The increases in weight of the other groups during the same period were as follows: 5%, 2.7 g; 1%, 3.7 g; 0.5%, 4.0 g.

Since the animals receiving 10% ascorbic acid also showed diarrhea and failed to grow it was considered that either 10% glucoascorbic acid or 10% ascorbic acid might alter the intestinal flora in such a way that necessary factors for growth were not available. Therefore a group of six 21-day-old rats (male) were fed the basal diet containing 10% glucoascorbic acid plus 2% liver 1:20 powder and another similar group, the basal diet containing 10% ascorbic acid plus 2% liver 1:20 powder. The rats of both the groups had diarrhea but not as severe as that of the groups receiving no liver powder. The animals of both the groups grew almost as well as rats on basal diet alone (Fig. 1). The liver powder did not contain any ascorbic acid but supplied some essential factors which the rats need in the presence of 10% ascorbic acid or glucoascorbic acid.

A group of 4 rats when fed a basal ration containing 10% citric acid grew normally like rats on basal diet alone. This indicated that the action of 10% ascorbic acid or glucoascorbic acid was not directly due to acidity of the rations.

Experiments with chicks. Groups of 4-day-old chicks were placed in electrically heated cages with raised screen bottoms and fed respectively a synthetic ration (486k[8]) containing 2% solubilized liver, the same ration containing 10% ascorbic acid, and the synthetic ration containing 10% glucoascorbic acid, for a period of 15 days. Animals of all the groups grew at equal rates and showed no diarrhea or any other symptoms. Thus, on the basal diet containing 2% solubilized liver no adverse effect of glucoascorbic acid was demonstrable.

Experiments with guinea pigs. Groups of 4 guinea pigs weighing between 190 g and 320 g were fed respectively a ground natural stock ration (Maritime Milling Co. pellets) and the stock ration containing 10% gluco-

TABLE I.
Ascorbic Acid Values of Tissues.

	Liver ascorbic acid (mg/g)	Kidney ascorbic acid (mg/g)	Adrenal ascorbic acid (mg/g)
Rats			
Basal diet	0.32	0.21	
" " + 10% glucoascorbic acid	0.37	0.23	
" " + 10% ascorbic acid	0.39	0.26	
" " + 10% glucoascorbic acid + ascorbic acid	1.12	1.09	
Guinea pigs			
Stock ration	0.07	0.02	0.23
" " + 10% glucoascorbic acid	0.21	0.47	0.40

[6] Banerjee, S., *Ann. Biochem. Exp. Med.*, 1943, **3**, 165.

[7] Banerjee, S., *Nature*, 1944, **153**, 526.

[8] Briggs, G. M., Jr., Luckey, T. D., Mills, R. C., Elvehjem, C. A., and Hart, E. B., PROC. SOC. EXP. BIOL. AND MED., 1943, **52**, 7.

ascorbic acid. The animals receiving glucoascorbic acid had severe diarrhea from the second day and did not grow like the other group. After 1 week 2 of the animals receiving glucoascorbic acid were fed the same diet which contained in addition 10% ascorbic acid to see if the diarrhea and loss in weight could be counteracted. Two of the guinea pigs receiving glucoascorbic acid diet died on the 9th and 12th days respectively. Two of the guinea pigs receiving 10% ascorbic acid in addition to the glucoascorbic acid diet died on the 14th and 20th day respectively. Ascorbic acid at the level of 10% could not prevent the action of glucoascorbic acid.

To study the possible inhibiting effect of added liver powder on the glucoascorbic acid action observed in experiments with rats, one guinea pig was given a diet consisting of stock ration 84 parts, liver 1:20 powder 6 parts and glucoascorbic acid 10 parts. The animal had no diarrhea and grew well. Unlike rats, the guinea pigs suffered no deleterious effect from the ascorbic acid at the 10% level.

No hemorrhages were observed in any of the guinea pigs which died. The ascorbic acid contents of the liver, kidney and adrenal glands of the guinea pig on glucoascorbic acid were determined and compared with those of one of the animals on stock ration alone. The results are given in Table I. It will be seen that the ascorbic acid activity of the tissue of the glucoascorbic acid fed guinea pigs was very high.

The results of these experiments indicate that the unfavorable effect of glucoascorbic acid in the diet of rats and guinea pigs may not be due entirely to an antivitamin C action as suggested by Woolley but to a lack of some factor other than vitamin C which is normally supplied by the intestinal flora and which is present in the liver 1:20 powder.

Summary. Rats fed a synthetic diet containing 10% glucoascorbic acid lose weight and develop severe diarrhea. Similar but less severe symptoms develop when 10% ascorbic acid is used. Post-mortem examination revealed no hemorrhages in any part of the body. Animals receiving 2% liver powder in addition to either 10% glucoascorbic acid or ascorbic acid grew as well as normal animals although they showed moderate diarrhea. Glucoascorbic acid at levels of 5, 1 and 0.5% had no effect on the growth of rats but the 5 and 1% levels produced diarrhea.

Chicks fed a synthetic ration containing 10% glucoascorbic acid and 2% solubilized liver showed no deleterious effects.

The severe diarrhea and loss of weight in guinea pigs fed a natural diet containing 10% glucoascorbic acid could not be prevented by the addition of 10% ascorbic acid but was prevented by the addition of 6% 1:20 liver powder.

We are indebted to Wallerstein Laboratories, New York, for a liberal supply of glucoascorbic acid; to Merck and Company, Rahway, New Jersey, for the crystalline vitamins; and to Wilson Laboratories, Chicago, Ill., for liver 1:20 powder.