greater than one mg. per cc. As the vitamin is extracted from tissues by a procedure which usually involves a 10-fold dilution, and greater dilutions may be made, this possible difficulty with tissues containing free pentose is readily overcome. The method appears to be entirely specific for the estimation of vitamin C.

Osazone formation does not occur in the first few hours after mixing 2,4-dinitrophenylhydrazine with ascorbic acid but later crystals appear slowly. This behavior is interpreted as indicating that ascorbic acid does not react with the hydrazine under these conditions, but upon standing it is oxidized to dehydroascorbic acid which reacts to produce the compound described.

This procedure is particularly advantageous for the determination of vitamin C in urine, for which the indophenol and other oxidation-reduction methods lack specificity. Ascorbic acid added to urine is recovered quantitatively by this method.

In preparing this paper the report of Stewart, Scarborough and Drumm[2] came to our attention. These authors have made a 2,4-dinitrophenylhydrazine derivative from the ascorbic acid in urine. As the method of preparation was not stated and no analyses were reported, a comparison of their derivative with ours cannot be made. The melting point reported (269-271 uncorrected) is about 20 degrees higher than that of our product.

The details of our procedure for the estimation of vitamin C will be published later.

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Glycerol Toxicity and Hemoglobinuria in Relation to Vitamin C.

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In view of the several recent reports[1, 2, 3] of the curing of paroxysmal hemoglobinuria by the administration of ascorbic acid, it was thought that a study of ascorbic acid in relation to experimental hemoglobinuria might be of interest. Of the numerous substances which produce hemoglobinuria it was found that parenterally injected glycerol was the most practical.

2 Stewart, C. P., Scarborough, H., and Drumm, P. J., *Nature*, 1937, **140**, 282.

1 Armentano, L., *Nature*, 1936, **137**, 910.

2 Lotze, H., *Klin. Woch.*, 1936, **15**, 941.

3 Armentano, L., and Beutsath, A., *Klin. Woch.*, 1936, **15**, 1594.

When glycerol was administered subcutaneously to 150 gm. white rats of a homogeneous strain, it was found (Table I) that the

TABLE I.

Drug	Dose	Dose	Result		Hemoglobinuria	Color of Urine
Glycerol*	0.75 cc./Kg.		4/12†		Trace	Pink
"	1.00 " "		14/22		++	Red
"	7.00 " "		8/10	Lived	++++	"
"	8.00 " "		5/20	"	++++	"
" I.P.	4.00 " "		9/10	"	++++	"
" I.P.	5.00 " "		6/10	"	++++	"
Glycerol and ascorbic acid	1.25 " "	500 mg./Kg.	4/16		Trace	Green
Glycerol and ascorbic acid	1.50 " "	500 " "	26/36		"	"
Ascorbic acid and glycerol	500 mg./Kg.	1.5 cc./Kg.	2/6		"	"
Ascorbic acid and glycerol	500 " "	2.0 " "	8/12		"	"
Glycerol and ascorbic acid	8.00 cc./Kg.	300 mg./Kg.	2/4	Lived	++++	Brown
Glycerol and ascorbic acid	8.00 " "	500 " "	6/7	"	++++	"
Glycerol and ascorbic acid	8.00 " "	600 " "	3/4	"	++++	"

* All doses were administered subcutaneously unless otherwise designated.

† Should be read 4 out of 12 rats had hemoglobinuria. All rats had 10 cc. of 0.9% NaCl solution intraperitoneally for diuretic purposes. The glycerol was diluted to 10-50% with 0.9% NaCl solution before administration. All ascorbic acid was neutralized with 50% of its weight of $NaHCO_3$ immediately before administration.

smallest dose producing hemoglobinuria, as shown by spectrographic analysis of the urine, was 0.75 cc. per kg. where 4 out of 12 rats had hemoglobinuria, while 14 out of 22 rats (64%) had hemoglobinuria at 1.0 cc. per kg. When the glycerol was antidoted by a subsequent dose of ascorbic acid, 72% had hemoglobinuria at 1.5 cc. per kg. of glycerol. Because the glycerol is very rapid in producing hemoglobinuria it was thought that the prophylactic administration of the ascorbic acid might afford greater protection against the hemoglobinuria. Accordingly, when the ascorbic acid was given first, it was found that only 8 out of 12 (66%) had hemoglobinuria when a larger dose of glycerol (2 cc.) was injected.

That the ascorbic acid is useful in decreasing the percentage mortality at the minimal lethal dose (8 cc. per kg.) is shown by the reduction in mortality from 75% (5/20 lived) to 27% (11/15 lived) when 300 to 600 mg. per kg. ascorbic acid was used). The strong reducing action of the ascorbic acid is possibly responsible for the partial conversion of the oxy- and met-hemoglobin in the

urine to green degradation products, but this does not appreciably alter the typical spectrographic lines. Traces of hemoglobin in the presence of these green reduction compounds are easily overlooked unless spectrographic analysis is used.

The mode of action of this antagonism has not been determined. This study, however, adds to the list of toxins and toxic substances which ascorbic acid aids in detoxifying.[4-7] It is of importance to note that many of the biological compounds reported as antagonistic to vitamin C contain as much as 50% of glycerol.[8, 5, 7] Since glycerol compounds most probably occur in the animal body in the phagocytosis of tubercle bacilli,[9] this antagonism may be of importance in accounting for the increased ascorbic requirement of tuberculous patients.[10] The use of glycerol to produce more extensive tuberculosis in white rats[11] is also of interest in view of the above data.

Summary. The administration of ascorbic acid prior to a parenteral dose of glycerol will raise the dose necessary to produce hemoglobinuria by 100% or more. The ascorbic acid may be effective in lowering the mortality rate from toxic doses of parenteral glycerol.

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4 Greenwald, C. K., and Harde, E., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1157.

5 Steinbach, M. M., and Klein, S. J., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 151.

6 Heise, F. H., Martin, G. J., and Schwartz, S., *Brit. J. Tuberculosis*, 1937, **31**, 23.

7 Jungeblut, C. W., *J. Exp. Med.*, 1937, **65**, 127.

8 Fishberg, M., *Pulmonary Tuberculosis*, Lea and Febiger, Phila., 1932, p. 86.

9 Wells, H. G., and Long, E. R., *The Chemistry of Tuberculosis*, Williams and Wilkins, Baltimore, 1932, p. 89.

10 Heise, F. H., and Martin, G. J., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 642.

11 Long, E. R., and Vorwald, A. J., *Am. Rev. Tuberculosis*, 1930, **22**, 636.