phenomenon is perhaps, that although the stimulating factor or factors were necessarily diluted in the recipients' plasma, they exerted their effect over a longer period of time, causing a sustained and additive stimulation.

Conclusions and summary. 1. Once a day for 4 days 7 normal rabbits each received 50 ml of serum obtained from hypoxic rabbits, and 6 normal rabbits each received the same amount of serum obtained from normal rabbits. The hypoxic serum induced a reticulocytosis which was significantly different (p value<0.01) from the response induced by injection of normal serum. At the level of hypoxia used (10% oxygen for 48 hours) the response in the recipient animals was as great as that seen in the animals directly exposed to hypoxia. 2. These results bolster the hypothesis that the erythropoietic response to hypoxia, or to blood loss anemia involves the production of a humoral factor.

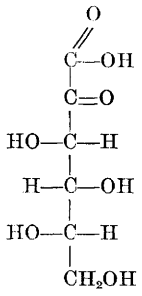
1. Krumdiek, Newton, PROC. SOC. EXP. BIOL. AND MED., 1943, v54, 14.
2. Hodgson, F., and Toha, J., *Blood,* 1954, v9, 299.
3. Erslev, A. J., *ibid.,* 1953, v8, 349.
4. Plzak, L. F., Fried, W., Jacobson, L. O., and Bethard, W. F., *J. Lab. and Clin. Med.,* 1955, v46, 671.
5. Stohlman, F. L., and Brecher, G., PROC. SOC. EXP. BIOL. AND MED., 1956, v91, 1.
6. Grant, W. C., and Root, W. S., *Physiol. Rev.,* 1952, v32, 449.
7. Erslev, A. J., *Blood,* 1955, v9, 954.
8. Stohlman, F. L., and Brecher, G., VI Congress of Internat. Soc. of Hematol., Boston, Aug. 27-Sept. 1, 1956.

Received November 21, 1956. P.S.E.B.M., 1957, v94.

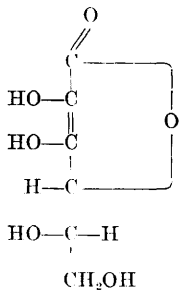
Fate of 2-Keto-L-Gulonic Acid in Rat and Guinea Pig.* (22922)

PETER G. DAYTON. (Introduced by J. Murray Steele)
Research Service Third (N. Y. University) Medical Division, Goldwater Memorial Hospital, Welfare Island, New York City.

The close structural relation between 2-keto-L-gulonic acid and L-ascorbic acid has prompted studies of 2-keto-L-gulonic acid as a possible precursor of L-ascorbic acid(1-6).



2-keto-L-gulonic acid



L-ascorbic acid

The recent finding that L-gulonolactone can be transformed by the rat to L-ascorbic acid(6,7) suggests that 2-keto-L-gulonic acid could be an intermediate in this transformation. For these reasons experiments were carried out in guinea pigs and rats with uniformly C^{14} labeled 2-keto-L-gulonic acid. The results of this study indicate no appreciable conversion of this compound to L-ascorbic acid in either species.

Methods. 2-Keto-L-gulonic acid C_6^{14} was synthesized from L-sorbose C_6^{14} according to modification of the method of Reichstein and Grüssner(8-11). The compound had a specific activity of 0.2 μc/mg and melted at 171°C. Titration of a 5 mg sample of the labeled substance with indophenol dye(12) showed that no more than 0.25% of L-ascorbic acid could be present. Radioactive purity was established by 2 criteria. The first consisted of finding no change of activity upon recrystallization with carrier from a solvent mixture consisting of methanol, acetone and ligroin. Secondly, L-ascorbic acid prepared from the labeled compound(10) had the same molar specific activity when counted as

* This study was supported in part by Josiah Macy Jr. Foundation.

its 2,4-dinitrophenyl-osazone derivative(13). Male Wistar rats, weighing 275-325 g, were given a diet of milk for 5 days prior to and during each experiment. Guinea pigs obtained from Rockland Farms and weighing about 300 g were maintained on a Vit. C-free diet supplemented by 5 mg of L-ascorbic acid for 5 days before the experiment. The labeled compound was dissolved in 1-3 ml H_2O and injected intraperitoneally. Determination of C^{14} in respiratory CO_2 and urine was carried out as previously reported(14). The amount of C^{14} present in urine as unchanged 2-keto-L-gulonic acid was determined as follows: The urine was collected during 24 hours after the dose in the presence of 1500 mg of carrier 2-keto-L-gulonic acid† and diluted to 200 ml. The 2-keto-L-gulonic acid was then transformed to L-ascorbic acid by a modification of the method of Reichstein and Grüssner(10). To a 100 ml portion 3 ml 6N HCl were added and it was refluxed under N_2 for 2 hours at 100°C. The solution was then quickly cooled to room temperature. Titration of an aliquot with indophenol dye, indicated a yield of L-ascorbic acid of about 20%. The L-ascorbic acid was oxidized to dehydro-L-ascorbic acid with bromine water; excess bromine was removed by flushing the solution with N_2. The solution was then passed through a column of Amberlite IR-4B resin in the acetate form (a resin bed 15 cm long and 2.5 cm in diameter was found satisfactory). Dehydro-L-ascorbic acid being neutral passed through the column, whereas 2-keto-L-gulonic acid was adsorbed. The dehydro-L-ascorbic acid in the effluent was converted to its 2,4-dinitrophenylosazone derivative(13) which was crystallized from 1:1 acetone-ethanol. The derivative melted at 285-287°C (mixed melting point undepressed with reference compound) and its specific activity remained constant upon recrystallization. The conversion of 2-keto-L-gulonic acid to L-ascorbic acid by rats and guinea pigs was determined by a carrier dilution technic (7). In brief, the method involved determining the specific activity of tissue L-ascorbic acid 24 hours after administration of the labeled compound. The % conversion was calculated by multiplying the specific activity of the tissue L-ascorbic acid by the body pool for each species. In experiments with unlabeled 2-keto-L-gulonic acid and its methyl ester, the 0-24 urine was collected in 10 ml of 10% oxalic acid as a preservative. L-ascorbic acid was titrated with indophenol dye.

† The non-labeled 2-keto-L-gulonic acid and its methyl ester were kindly donated by Dr. R. Pasternack, Chas. Pfizer & Co.

TABLE I. Distribution of C^{14} 24 Hr after Intraperitoneal Administration of 2-Keto-L-Gulonic Acid C_6^{14}.

Animal	Wt (g)	Dose (mg)	% of admin. C^{14} found in: Respiratory CO_2	Urine	L-ascorbic acid*
Rat	275	4.00	11	78	—
"	325	18.00	7.9	80	<1.2
"	310	18.00	—	76	"
Guinea pig	300	5.84	3.4	102	<1.4
"	290	4.67	2.9	95	<1.3

* Calculated from data obtained by the method of Burns and Evans(7).

Results. It was found that less than 1.2% of the C^{14} administered to rats, as 2-keto-L-gulonic acid C_6^{14}, was present in the animal as L-ascorbic acid 24 hours after the dose (Table I). This compares to an average value of 8% obtained in similar experiments in rats with L-gulonolactone-1-C^{14}(7). The results showing that 2-keto-L-gulonic acid was converted to L-ascorbic acid to a lesser extent than L-gulonolactone suggest that this acid is not an intermediate in the conversion of L-gulonolactone to L-ascorbic acid. The most likely intermediates involved in this *in vivo* oxidation are either 2-keto or 3-keto-L-gulonolactone. These compounds, however, have not been isolated since they rapidly enolize to form L-ascorbic acid(1,15,16).

It was also found that less than 1.4% of the C^{14} administered to guinea pigs as 2-keto-L-gulonic acid-C_6^{14} was present in the animal as L-ascorbic acid 24 hours after the dose (Table I). The lack of appreciable conversion of 2-keto-L-gulonic acid to L-ascorbic acid in guinea pigs is in agreement with results showing that this compound has no vit. C activity in guinea pigs(1-4).

Evidence has been presented for conversion of certain esters of 2-keto-L-gulonic acid to the vitamin. For instance, methyl 2-keto-L-gulonate has vit. C activity in guinea pigs and in addition increases urinary excretion of L-ascorbic acid when administered to man and dog(1-4). In the present study, data were obtained in the rat for transformation of methyl 2-keto-L-gulonate to L-ascorbic acid. This was shown as follows: 4 rats which excreted daily 0.9 ± .02 mg of L-ascorbic acid, received by intraperitoneal injection 90 mg of 2-keto-L-gulonic acid and a week later 90 mg of methyl 2-keto-L-gulonate. No appreciable increase in the daily urinary L-ascorbic acid excretion was observed following administration of the acid (.5 ± .1 mg) but following administration of the ester, the urinary excretion of L-ascorbic acid increased to 7.1 ± 1.0 mg/day. Control experiments in which 90 mg of the acid or the ester was added to the preservative, and urine was collected over 24 hours, showed less than 1 mg of each compound converted to L-ascorbic acid. The difference in the *in vivo* conversion of 2-keto-L-gulonic acid and its esters to L-ascorbic acid may be due to differences in the rates of enolization and lactonization of the compounds to L-ascorbic acid(1,5,10,16-18).

The data in Table I show that the major part of the C^{14} administered as 2-keto-L-gulonic acid C_6^{14} was excreted in urine which was found by an isotopic dilution method to be (within experimental error ± 5%) unchanged 2-keto-L-gulonic acid. During 24 hours after injection of the labeled 2-keto-L-gulonic acid, 3% of the C^{14} was recovered in respiratory CO_2 of guinea pigs and 10% in the case of rats.

Summary. Evidence has been obtained employing C^{14} tracer technics which shows no appreciable conversion of 2-keto-L-gulonic acid to L-ascorbic acid by the rat or guinea pig. From these results it is suggested that the most likely intermediate in the conversion of L-gulonolactone to L-ascorbic acid by the rat is 2-keto- or 3-keto-L-gulonolactone. In addition it was found that the major part of the administered 2-keto-L-gulonic acid was excreted unchanged in the urine.

The author wishes to thank Dr. J. J. Burns for his aid and valuable suggestions, which made this work possible.

1. Reichstein, T., and Demole, V., *Festschrift Emil Barell, Basel,* Hoffmann-LaRoche & Co. Ltd., 1936, 107.

2. Lorenzini, G., and Corbellini, A., *Arch. ist. Biochem. ital.,* 1938, v10, 131.

3. Gould, B. S., and Schwachman, H., *J. Biol. Chem.,* 1943, v151, 439.

4. van Eeckelen, M., and van der Laan, P. T., *Arch. neerland. physiol.,* 1943, v27, 182.

5. Yashima, K., *Kurume Med. J.,* 1954, v1, 1.

6. Isherwood, F. A., Chen, Y. T., and Mapson, L. W., *Biochem. J.,* 1953, v56, 1.

7a. Burns, J. J., and Evans, C., *J. Biol. Chem.,* 1956, v223, 897; b. Burns, J. J., Peyser, P., and Moltz, A., *Science.*

8. Bothner-By, A. A., Gibbs, M., and Anderson, R. C., *ibid.,* 1950, v112, 311.

9. Dayton, P. G., *J. Org. Chem.,*

10. Reichstein, T., and Grüssner, A., *Helv. Chim. Acta,* 1934, v17, 311.

11. Elger, F., *Festschrift Emil Barell, Basel,* Hoffmann-La Roche & Co. Ltd., 1936, 229.

12. Methods for Vitamin Assay, The Association of Vitamin Chemists, Inc., Interscience, N. Y., 1951, 42.

13. Jackel, S. S., Mosbach, E. H., and King, C. G., *Arch. Biochem. Biophys.,* 1951, v31, 442.

14. Burns, J. J., Dayton, P. G., and Schulenberg, S., *J. Biol. Chem.,* 1956, v218, 15.

15. Berends, W., and Konings, J., *Recueil,* 1955, v74, 1365.

16. Cox, E. G., Hirst, E. L., and Reynolds, R. J. W., *Nature,* 1932, v130, 888.

17. Regna, P. P., and Caldwell, B. P., *J. Am. Chem. Soc.,* 1944, v66, 246.

18. Petuely, F., and Künssberg, U., *Monatshefte,* 1952, v83, 80.

Received November 28, 1956. P.S.E.B.M., 1957, v94.