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Administration of Ascorbic Acid to an Alkaptonuric Patient.*

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Recently it has been reported from the senior author's laboratory that artificial alkaptonuria may be readily produced in the guinea pig by the supplementation of a vitamin C deficient diet with extra tyrosine.[1] With the subsequent administration of the vitamin the homogentisic acid in the urine was promptly reduced in amount and within 48 to 72 hr was completely absent. These findings suggested that the relation of ascorbic acid to the excretion of homogentisic acid by the alkaptonuric patient should be investigated. However, in the course of the experiments with the guinea pigs, the results of two such investigations appeared. These reports by Monsonyi[2] and by Diaz, Mendoza and Rodriquez[3] indicate that ascorbic acid is without effect, but since, in our own more recent studies[4] it has been apparent that in the guinea pig the effectiveness of the dose of ascorbic acid is dependent upon the state of vitamin saturation in the tissues, it was considered imperative to investigate the effect of doses of the vitamin greatly in excess of the normal human requirement, and also of the relatively small amounts used by the above investigators.

Experimental. The individual who served as a subject for this study is an essentially normal 65-year-old white male who exhibited at the time of these experiments alkaptonuria and ochronosis—a deposition of pigment mainly in cartilage which is characteristic of these individuals in later years. Since the patient had previously been on an experiment in which he consumed a relatively high intake of protein, he was continued on this level, the diet being a mixed diet with considerable variety from day to day but so planned that it

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1 Sealock, R. R., and Silberstein, H. E., *Science*, 1939, **90**, 517.

2 Monsonyi, L., *Presse Med.*, 1939, **47**, 708.

3 Díaz, C. J., Mendoza, H. C., and Rodríquez, J. S., *Klin. Wchnschr.*, 1939, **18**, 965.

4 Sealock, R. R., Perkinson, J. D., and Silberstein, H. E., in press.

furnished approximately 130 g of protein per day. It included throughout the experiment 40 to 50 mg of vitamin C daily, an amount comparable to the previous daily intake of the patient. The urine was collected in 24 hr samples in bottles containing sufficient hydrochloric acid to make the sample slightly acid to congo red. The homogentisic acid was determined by the iodine-sodium thiosulfate titration procedure of Metz[5] as modified by Lieb and Lanyar.[6] The ascorbic acid determinations were made in the usual fashion by titration of the fresh sample with standardized 2,6-dichloro-benzenone-indophenol. Since homogentisic acid also reduces the dye under the conditions of the titrations the values were corrected for the amount of the hydroquinone derivative.

Results. Following a control period vitamin C† was given orally at the comparatively high level of 1.0 g per day. As shown in Fig. 1, this amount for 4 days proved to be without effect on the excretion of the metabolite, as was the further administration on the 12th and

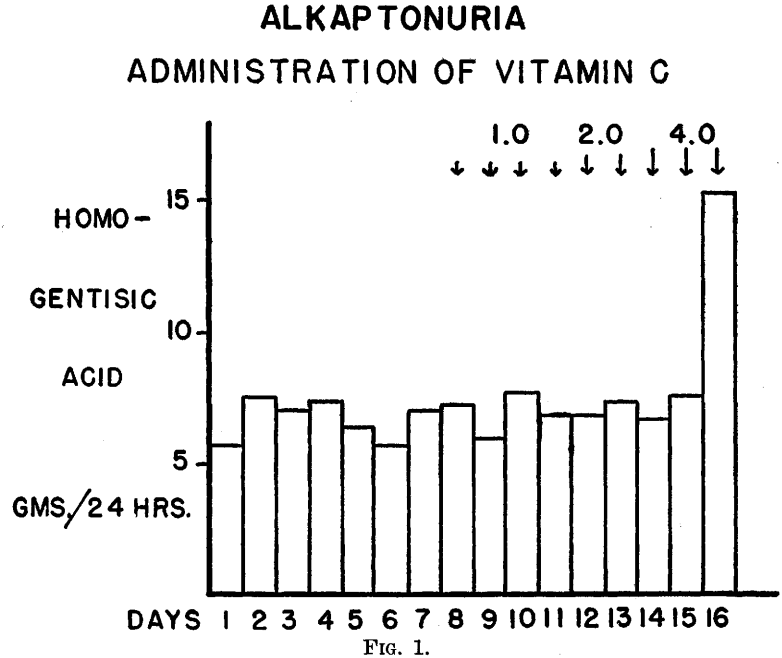

FIG. 1.

Mr. G—130 g protein per day. Beginning of last day also received 10 g of *l*-tyrosine. Ascorbic acid, g. —

[5] Metz, E., *Biochem. Z.*, 1927, **190**, 261.

[6] Lieb, H., and Lanyar, F., *Z. physiol. Chem.*, 1929, **181**, 189.

† The vitamin used in this study was very kindly supplied by Merck and Company, Incorporated, and Chas. Pfizer and Company, Incorporated.

13th days of 2.0 g in 0.5 g portions. For the following 3 days the ascorbic acid was again doubled, 2 of the 4 g being given by intravenous injections of 1 g each. That a high level of tissue saturation had been attained was indicated by the urinary excretion of 1.08, 1.62, 1.82 and 2.67 g of ascorbic acid on the 12th to the 15th days and a blood ascorbic acid of 2.89 mg per 100 cc. In view of the failure of even 4.0 g of the vitamin to effect the homogentisic acid output, the possibility yet remained of establishing a relationship between the two by flooding the metabolic processes with an extra dose of the precursor. In order to test this possibility 10 g of *l*-tyrosine were given at the beginning of the 16th day and subsequently four 1 g doses of ascorbic acid. The experiment resulted negatively, for in the 24 hr urine sample there were present 15.2 g of homogentisic acid, which when compared to the previous average represents a recovery of 88% of the theoretical.

The administration of the vitamin was not entirely without advantage, albeit the advantage was unrelated to the metabolism of the alkaptone substance. Whereas the urine samples of the control period showed the usual tendency to darken on standing, with excretion of extra ascorbic acid there was no longer any discoloration even after several days, a finding which again illustrates the well known protective action of this substance against the oxidation of ortho- and para-diphenolic compounds by atmospheric oxygen.

It should be pointed out that the large doses of ascorbic acid were without visible effect on the ochronosis exhibited by the individual. However, these results do not preclude the possibility of a continued high intake of the vitamin in early life preventing the deposition of melanotic pigment in later years. That such a possibility is of some importance is evident from the fact that perhaps the only unpleasant feature of the condition is the appearance of melanin pigment in the cartilage of the ears and nose as discussed by Garrod.[7]

The ineffectiveness of ascorbic acid on the abnormal metabolism of the alkaptonuric individual indicates a real difference between this type of experiment and the one with the guinea pigs[1,4] or in other words between hereditary and experimental alkaptonuria. When one recalls that the majority of metabolic reactions are chain reactions proceeding under the influence of many different factors and enzyme systems, the above difference is not surprising. In the guinea pig the missing factor is ascorbic acid while in the alkap-

[7] Garrod, A. E., *Inborn Errors of Metabolism*, Oxford University Press, 1923, p. 58.

tonuric patient it is not the vitamin but some other factor as yet unknown.

Summary. The administration of from 1 to 4 g of ascorbic acid per day has been found to be without effect on the excretion of homogentisic acid by an alkaptonuric individual excreting an average of 7 g of the metabolite daily. The ineffectiveness of this excessive amount of vitamin is a finding which is in agreement with results with smaller doses obtained by other investigators.

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Relation of Pantothenic Acid to Dermatitis of the Rat.*

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Richardson and Hogan[1] demonstrated that rat dermatitis (acrodynia) is prevented or cured by an aqueous extract of yeast or rice bran, and by certain oils. The activity of the aqueous extracts was ascribed to the presence of a vitamin, presumably the one later designated as vitamin B_6. Since this communication is not immediately concerned with oils our observations, and those of other workers,[2] on their protective action will not be described. Birch[3] reported that two factors are required to prevent or cure acrodynia. One is vitamin B_6, the other is one of the essential unsaturated fatty acids. György and Eckhardt[4] obtained complete protection with a

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1 Richardson, L. R., and Hogan, A. G., *Mo. Agr. Exp. Sta. Res. Bul.*, 1936, No. 241.

2 Salmon, W. D., and Goodman, J. G., *J. Nutr.*, 1937, **13**, 477; Schneider, H., Steenbock, H., and Platz, Blanche R., *J. Biol. Chem.*, 1940, **132**, 539.

3 Birch, T. W., *J. Biol. Chem.*, 1938, **124**, 775.

4 György, P., and Eckhardt, R. E., *Nature*, 1939, **144**, 512.