shown in Fig. 2. Evidently, the radicals are both formed and destroyed by the action of light. The ESR-spectrum, recorded in the dark immediately after the radical concentration had reached the maximum value, exhibited a hyperfine structure, consisting of at least 37 lines in three main groups. The spectrum was nearly identical with that reported by Hoskins [4] for the stable radical obtained when diphenylamine was oxidazed by molecular oxygen in a hot mixture of toluene and alkaline ethanol. This radical was considered to have the structure $(C_6H_5)_2NO\cdot$. It may be suggested that the hydrogen attached to the nitrogen atom of diphenylamine is removed in the photo-induced reaction here described yielding the radical $(C_6H_5)_2N\cdot$.

An ESR-spectrum probably derived from the substrate molecule was obtained in the eosine sensitized reaction of phenothiazine. Due to the instability of the radicals both when the sample was irradiated and when kept in the dark, it was impossible to obtain a complete spectrum under high-resolution conditions. It was observed, however, that there were three main lines separated by about 9.5 gauss, very probably produced by the interaction of the unpaired electron with the nitrogen atom of phenothiazine. The main lines exhibited a splitting into a large number of more or less completely resolved lines. Although the ESR-spectrum does not give precise information, it is believed that the hydrogen atom attached to the nitrogen atom is removed in the radical forming process. To get photo-induced radicals from phenothiazine the presence of this hydrogen seems to be essential, as it was found that no radicals could be detected when N-methylphenothiazine was used as a substrate. However, the radical reactions of unsubstituted phenothiazine are rather complex. Under certain conditions photo-radicals are formed even without eosine as a sensitizer [5].

Thus, the reaction mechanism of N,N-diphenylamine and phenothiazine probably involves an irreversible homolytic extraction of a labile hydrogen. The radicals are derived from the substrate molecules and are present even after the excitation of the sensitizer has ceased. In some other reactions it has been suggested that an addition complex [3] is formed between the excited sensitizer and the substrate. This complex decays rapidly after the excitation has ceased, in some cases obviously reversibly as seen from the kinetic behaviour of 1,4-dihydroxybenzene where the light-induced reaction can be repeated an indefinite number of times.

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1. Lagercrantz, C. and Yhland, M. *Acta Chem. Scand.* **16** (1962) 505.
2. Bubnov, N. N., Kibalko, L. A., Tsepalov, V. F. and Shliapintokh, V. IA. *Optics and Spectroscopy* **7** (1959) 71.
3. Schenck, G. O. and Wolgast, R. *Naturwiss.* **48** (1961) 737.
4. Hoskins, R. *J. Chem. Physics.* **25** (1956) 788.
5. Lagercrantz, C. and Yhland, M. *To be published.*

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Synthetic Ascorbigen as a Vitamin C Source for Guinea Pigs and Man

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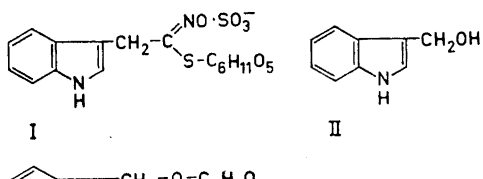
It was found in this laboratory that 3-hydroxymethylindole (II) is formed during the enzymic splitting of glucose and sulfate from the newly discovered thioglucoside, glucobrassicin (I), present in cabbage plants and isolated in pure form [1,2]. 3-Hydroxymethylindole reacts non-enzymically with the ascorbic acid present in cabbage to form the compound known as ascorbigen (III) [3].



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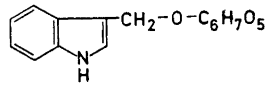

III

Ascorbigen can be prepared synthetically by heating a mixture of 3-hydroxymethylindole and ascorbic acid, or a mixture of indole, formaldehyde, and ascorbic acid in water [3]. On mild acid hydrolysis ascorbic acid is liberated from ascorbigen, as shown by Procházka *et al.*[4], who had isolated ascorbigen from cabbage some years before, considering it to be a genuine plant substance. The structure which these workers proposed for ascorbigen had to be modified, however, in the light of the results obtained by Gmelin and Virtanen. Although the details of its structure are not yet clear, we know that ascorbigen is formed by the splitting of one molecule of water from one molecule of 3-hydroxymethylindole and one molecule of ascorbic acid. Formula III gives an idea of the structure of ascorbigen.

From the above it appears that ascorbigen is an artefact, representing the "bound ascorbic acid" which is supposed to be present in cabbage. Because it is formed when glucobrassicin is split (*e.g.* when cabbage is crushed), it is important to know whether ascorbigen acts as a preventive and curative of scurvy in guinea pigs and in man, — in other words, if it exerts the vitamin C effect.

For the following animal experiments, ascorbigen was prepared synthetically from indole, formaldehyde, and ascorbic acid. 10 g of indole (about 0.085 mole), 30 g of ascorbic acid (about 0.170 mole), 5.1 g of formaldehyde (about 0.170 mole), and 400 ml of citrate buffer (0.05 M) were mixed. The mixture, the pH of which was about 4.0, was heated on a water bath at + 54°C with stirring for about 7 or 8 h. The mixture was filtered after cooling. The filtrate was extracted with 3 × 200 ml of ethyl ether and 5 or 6 × 500 ml of freshly distilled ethyl acetate. The ethyl acetate was evaporated, finally in a small evaporator. The residue was thoroughly dried in a vacuum desiccator. The yield, which according to paper chromatograms and the UV spectrum was pure ascorbigen, was 6 030 mg.

Twenty guinea pigs of a pure albino strain, weighing 250—300 g, were used as test animals. Ten of these were males and ten females. The animals were fed on dry timothy grass. Part of the ascorbic acid had been destroyed in the grass by keeping it in an oven at 40°C for 3 or 4 days. The ascorbic acid content had then decreased to 10 μg/g of grass. The consumption of grass was about 30 g per day and animal. In addition, the guinea pigs were given 10 g per day of a mixture of rolled oats and wheat embryos (4:1). This mixture contained no ascorbic acid at all. The animals were given tap water to drink.

The guinea pigs were weighed every other day. When their weights began to decrease, the animals were divided into the following groups on the 16th day of the experiment: (1) a control group, 4 males and 4 females, (2) an ascorbic acid group, 3 males and 3 females, (3) an ascorbigen group, 3 males and 3 females.

The animals in group 1 were given 1 ml of 50 % saccharose solution *per os* with a pipette daily. The animals in group 2 were given 15 mg of ascorbic acid, and the animals in group 3 26 mg of ascorbigen, likewise in 1 ml of 50 % saccharose solution *per os*. The ascorbigen solution was made up each day immediately before use. The ascorbigen contained 5 μg/mg of free ascorbic acid.

Results. The time course of the weights of the animals is presented graphically in Figs. 1—3. The weights of the control animals decreased continuously after the 10th or 12th day of the experiment. Symptoms of scurvy could be clearly seen as early as the 18th or 20th day of the experiment (lack of appetite, apathy, sore

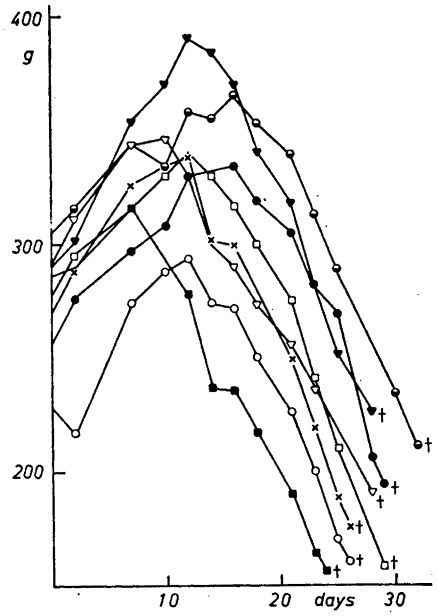

Fig. 1. No ascorbic acid administered to guinea pigs. Group 1.

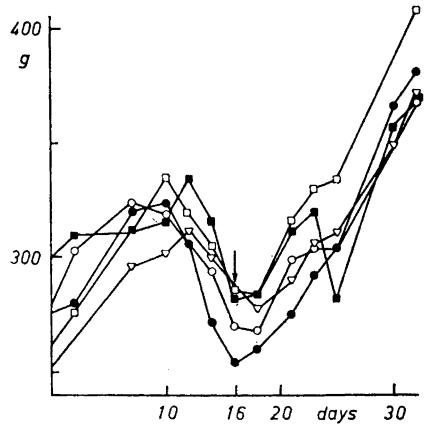

Fig. 2. 15 mg/day of ascorbic acid administered to guinea pigs from the time pointed out by the arrow. Group 2.

joints). These animals died between the 24th and 32nd day of the experiment.

26 mg of ascorbigen per day prevented the development of scurvy, as did 15 mg of ascorbic acid, and the weights of the animals began to increase again at the same rate in both groups. No symptoms of scurvy could be observed in the guinea pigs in the ascorbigen group. In group 2, one animal was taken ill with diarrhea and died on the 27th day of the experiment.

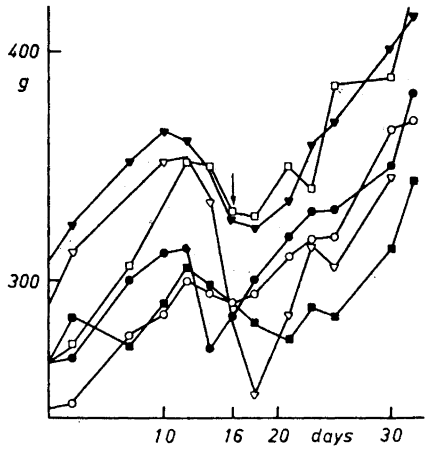

Fig. 3. 26 mg/day of ascorbigen administered to guinea pigs from the time pointed out by the arrow. Group 3.

When investigating the vitamin C effect of ascorbigen on man, the ascorbic acid was determined in the urine of two test persons after the eating first of ascorbic acid and then of the equivalent amount of ascorbigen. The determinations were performed according to the method described by Roe [5].

The test persons were on no special diet during the test, but they avoided to eat fresh fruits. When the vitamin C level in the urine had been followed for some days, they ate 500 mg of ascorbic acid. When the level had sunk again, they ate 875 mg (= 500 mg ascorbic acid) of ascorbigen.

The vitamin C excretion in the urine of both test persons rose sharply after the eating of ascorbic acid, as well as after that of ascorbigen. After the eating of ascorbigen, the vitamin C content in the urine of the first person was about 15 % of the amount excreted after the eating of ascorbic acid. In the second case it was 250 %. Accordingly, the vitamin C effect was established with both persons, even if the excreted amount of ascorbic acid differed greatly. Determinations with several test persons on controlled diets are needed to find out if the ascorbic acid bound in ascorbigen is split quantitatively in the organism, and if there are variations among different individuals in this respect.

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1. Gmelin, R., Saarivirta, M. and Virtanen, A. I. *Suomen Kemistilehti B* **33** (1960) 172.
2. Gmelin, R. and Virtanen, A. I. *Ibid.* **33** (1961) 15.
3. Gmelin, R. and Virtanen, A. I. *Ann. Acad. Sci. Fennicae A II*, **1961** No. 107.
4. Procházka, Z., Šanda, V. and Šorm, F. *Collection Czechoslov. Chem. Communs.* **22** (1957) 654.
5. Roe, J. H. *Chemical Determination of Ascorbic, Dehydroascorbic, and Diketogulonic Acids* in Methods of Biochemical Analysis, Vol. 1, pp. 127–132 and 136. Interscience Publishers Inc., New York 1954.

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