On the Role of Substances present in the Seeds and arising in them during Germination in the Growth of Plants[1]

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The significance of the substances present in the seed and arising in them during germination for the development and growth of plant is a question that has been very little explored. The conception prevailed that after the appearance of chlorophyll in the seedling the plant becomes autotrophic in respect to its metabolism, in other words, is able to form all carbon compounds by CO_2 assimilation. The importance of the reserve nutrition of the seed hereafter remains obscure.

In this laboratory the subject mentioned in the title has been investigated since the early part of the 1930's. We came upon this when following the formation of certain vitamins in green plants. Together with my collaborators V. HAUSEN and SAASTAMOINEN[3] we examined the development of vitamin C and carotene in different plants: pea, clover, and wheat, under different conditions of growth and noted that the vitamin content of the plant is generally highest during the period of powerful vegetative growth. Both the carotene and the vitamin C content fell in certain plants, e. g. wheat, already long before flowering, in others, e. g. pea, after the beginning of flowering. In general, it could be stated that the factors which prevent growth lower the vitamin content of plants. On the basis of these observations we concluded that vitamins are plant hormones necessary for normal metabolism and hence for growth. This concept was supported by the finding that the growth of the plant could be promoted in sterile cultures by adding vitamin C to the nutrient solution[4].

In her doctoral thesis V. HAUSEN[5] examined in detail the significance of vitamin C for the higher plants. The experiments showed that vitamin C formed in the seed during the germination period is necessary for the growth of the plant. This appeared distinctly when the experiment was conducted in the following way: After the seed had grown for some days—usually 5–9 days—its cotyledons were carefully removed, and the plant without cotyledons was aseptically transferred into a sterile nutrient solution. The plant grew at first weakly and formed some small leaves, but soon the growth stopped, and this the sooner the earlier the cotyledons were removed. The growth did not cease on account of possible injury to the plant through the removal of cotyledons, because if crystalline vitamin C was added to the nutrient solution the plant without cotyledons grew up to the flowering stage. Although it was not possible to induce by means of vitamin C—in the amounts used in the experiments—even approximately so good growth as in normal plants, the influence of vitamin C on growth was very distinct and strong. These experiments conclusively proved the significance of vitamin C formed in seeds during germination for the growth of plants.

The investigations have been continued in this laboratory after the war. SAUBERT-V. HAUSEN[1] reported recently on some results of the influence of vitamin-B complex, biotin, and vitamin K on the growth of cotyledonless plants. It appears from the experiments that also biotin and B complex have a slightly promotive effect on the growth. The influence of vitamin C is, however, far superior. It is interesting to note that the effect of different vitamins is largely additive as can be seen from Table 1.

Table I

Effect of various accessory growth substances on the growth of peas whose cotyledons were removed after a germination period of 5 days. Each figure represents the average value of the weight, the length, and the vitamin-C content of 20 plants.

Additions	Average fresh weight of seedling, mg	Average length of shoot, mm	Vitamin C per 100 g fresh matter, mg
Water (control)	85	170	24
Vitamin-B complex	181	190	52
Vitamin-B complex +biotin	756	540	64
Vitamin C	1450	680	154
Vitamin-B complex +biotin+vitamin C	2052	940	158
Normal plants	4746	1020	61

Our experiments with large amounts of vitamin C (100–120 mg ascorbic acid per pea plant, 1 l nutrient

[1] Lecture held at the Scandinavian Congress of Cereal Technicians in Helsinki, August 18, 1948.

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[3] A. I. VIRTANEN, S. V. HAUSEN, and S. SAASTAMOINEN, Ann. Acad. Sci. Fennicae, Ser. A., *38*, No. 7 (1933); Biochem. Z., *267*, 179 (1933).

[4] S. V. HAUSEN, Suomen Kemistilehti B, *8*, 27 (1935).

[5] S. V. HAUSEN, Ann. Acad. Sci. Fennicae, Ser. A., *46*, No. 3 (1936); Biochem. Z., *288*, 378 (1936).

[1] S. SAUBERT-V. HAUSEN, Physiologia Plantarum *1*, 85 (1948).

solution) have shown that a plant without cotyledons can grow nearly as well as a normal plant when vitamin C is furnished to it (Fig. 1). Amino acids and the expressed juice of plants also promote growth noticeably.

Lately we have tried to find out the mode of action of vitamin C on plants. The idea that ascorbic acid would function as a regulator of the redox-potential in plants and so would have a decisive significance for growth does not seem impossible. In all previous experiments with cotyledonless plants the mineral solution contained nitrate as a N-source, i. e. a strongly oxidized nitrogen compound. Vitamin C noticeably lowers the redox-potential in such a solution. If a completely reduced nitrogen compound, viz. an ammonium salt, is used as a nitrogen source, the redox-potential of the nutrient solution is already as such lower. Whether vitamin C promotes growth in an ammonium salt solution to the same extent as in a nitrate solution was now an interesting problem. In order to solve it I have, together with SAUBERT-V. HAUSEN, carried out numerous experiments both with peas and with wheat in nitrate and ammonium nutrient solutions. An interfering factor in these experiments is that the p_H in an ammonium sulphate solution soon falls as growth advances, while in Ca-nitrate solution it remains at p_H 6·5–6·7 or slightly rises from p_H 6·5 to 7. As it is difficult to regulate the acidity of the ammonium sulphate solution more exactly in the course of the experiment, the varying acidity of the nutrient solutions may affect the results. In spite of that, the data obtained prove that a pea without cotyledons and a wheat embryo free of endosperm grow markedly better without vitamin C in an ammonium solution than in a nitrate solution. The following experiments show this (Fig. 2 and 3.)

Fig. 1. – Effect of various accessory growth substances on the growth of peas whose cotyledons were removed after a germination period of 5 days.

Additions	Fresh weight of seedling, g shoot	Fresh weight of seedling, g root	Length of shoot, cm
(1) No addition, $Ca(NO_3)_2$	0·128	0·217	23
(2) Inoculated with H7, no N	0·460	1·051	39
(3) Root extract, $Ca(NO_3)_2$	0·573	1·269	42
(4) Hydrolysed casein, $Ca(NO_3)_2$	0·903	0·644	53
(5) All amino acids of casein, $Ca(NO_3)_2$	1·120	0·929	55
(6) Plant extract, $Ca(NO_3)_2$	0·972	1·489	58
(7) Vitamin C, $Ca(NO_3)_2$	4·668	2·738	85
(8) Normal plant, $Ca(NO_3)_2$	4·855	5·032	92

The addition of vitamin C has strongly promoted growth in a nitrate solution but not in an ammonium solution. The results of the experiments with wheat are always very distinct. On the other hand, the experiments with peas occasionally give variable results due to the fact that a pea embryo without cotyledons often is destroyed in an ammonium solution to which vitamin C is added. This has occurred so frequently in our experiments that it cannot be ascribed to an incident. The reason for this phenomenon is not known more exactly. Since the plants died already in the beginning before the p_H of the nutrient solution had fallen below 6·5, the increasing acidity cannot be the cause for this phenomenon. It should be mentioned in this connection that also a normal pea plant which, to begin with, grows particularly well on ammonium sulphate, can in some experiments suddenly wither already before or in the beginning of flowering. This can depend on the fact that the plant takes up with its roots, ammonium ions much more rapidly than nitrates[1] and forms from ammonium salts large amounts soluble nitrogen compounds—in the first place glutamic and aspartic acids and their amides—as

[1] A. I. VIRTANEN and H. LINKOLA, Nature *158*, 515 (1946).

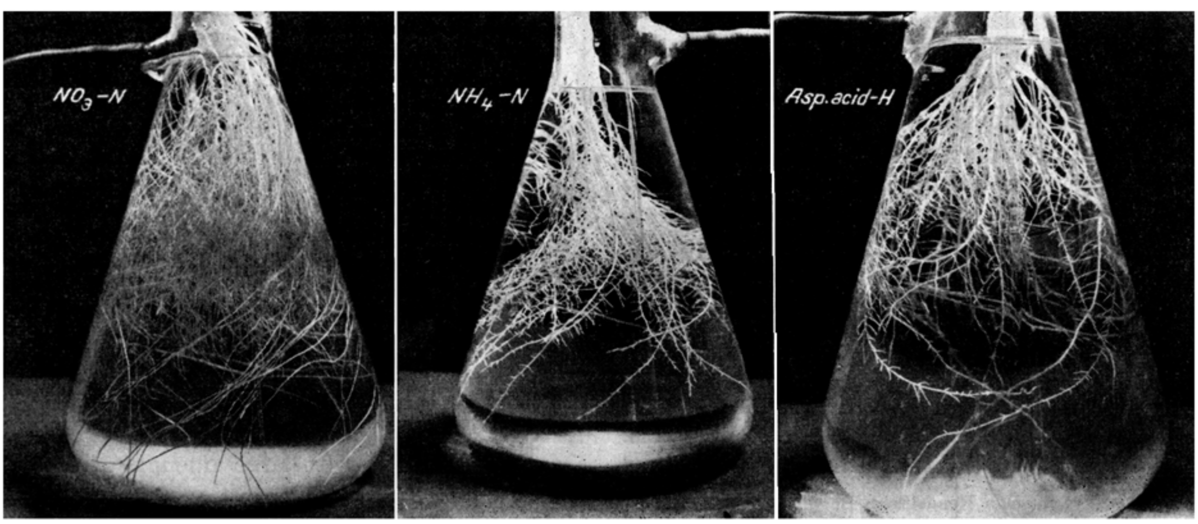

Fig. 1.

RAUTANEN[1] has shown in this laboratory. In the case that protein synthesis does not take place in a corresponding degree, the soluble nitrogen compounds, including ammonia, may, when their concentration has become sufficiently high, poison the plant. That this might cause the small cotyledonless plants to die right

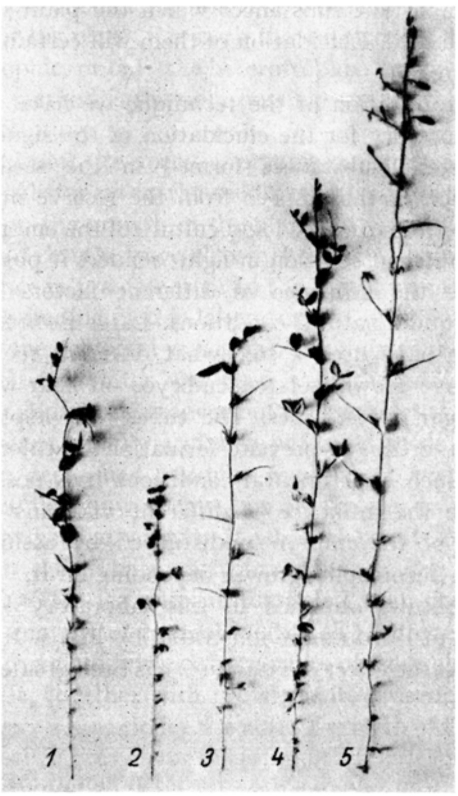

Fig. 2. – Effect of ascorbic acid and different sources of N-nutrition on the growth of peas whose cotyledons were removed after a germination period of 5 days.

Additions	Dry weight of seedling, mg	Length of shoot, cm	N, %
(1) No addition, $(NH_4)_2SO_4$	396	61	3·79
(2) No addition, $Ca(NO_3)_2$	132	44	3·66
(3) Vitamin C, $(NH_4)_2SO_4$.	410	65	3·13
(4) Vitamin C, $Ca(NO_3)_2$. .	663	89	3·57
(5) Normal plant, $(NH_4)_2SO_4$	867	113	2·40

at the start seems, however, scarcely likely, in particular as an increase of vitamin C causes the poisoning. Rather can a too low redox-potential in the nutrient solution be a cause for the phenomenon.

The above results concerning the different effect of vitamin C in nitrate and ammonium sulphate solutions support strongly the conception that the significance of vitamin C is at least partly dependent on its influence on the redox-potential. Continued investigations will disclose whether this explanation is sufficient.

[1] N. RAUTANEN, Acta Chem. Scand. 2, 127 (1948).

In this connection I wish to mention some observations regarding the influence of ammonium and nitrate nitrogen on the form and structure of the roots of normal plants[1]. It has consistently appeared in all experiments that the roots formed in a nitrate solution have a much finer structure than those grown in ammonium salts or amino acids. The small root branches of nitrate roots are sparse and long and grow parallel to the main root. The small root branches of the ammonium root are again short and lie perpendicularly to the main root. Fig. 4 illustrates the conditions in the pea. The nature of the nitrogen source thus affects markedly the structure of the roots.

As clearly appears from the above, vitamin C promotes the growth of the cotyledonless pea as well as that of wheat free from endosperm in a nitrate solution. Between these plants there exists, however, a noteworthy difference. In the pea the influence of vitamin C is strong when the cotyledons are removed 5 days or still longer after the beginning of germination. But the endosperm must be removed from the wheat germ within a few hours or at least one day after moistening

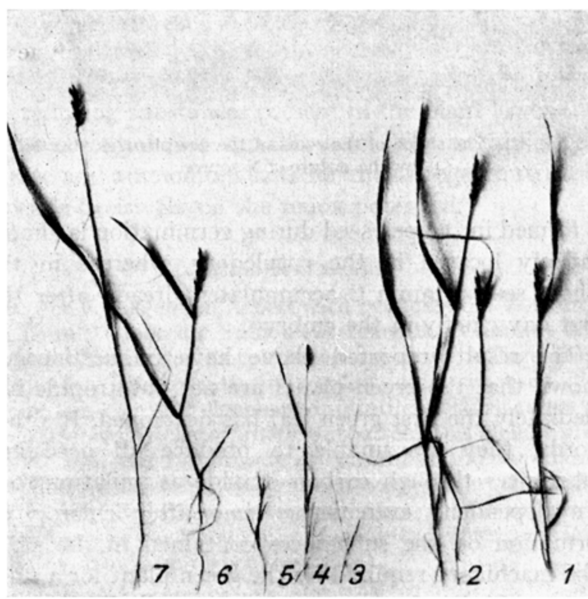

Fig. 3. – Effect of ascorbic acid and different sources of N-nutrition on the growth of isolated wheat germs.

No. of experiment	Additions	N-nutrition	Dry weight of seedling, mg	Length of shoot, cm
1	None	$(NH_4)_2SO_4$	520	37
2	None	$(NH_4)_2SO_4$	720	46
3	None	$Ca(NO_3)_2$	11	11
4	None	$Ca(NO_3)_2$	123	28
5	None	$Ca(NO_3)_2$	38	12
6	Vitamin C	$(NH_4)_2SO_4$	370	37
7	Vitamin C	$Ca(NO_3)_2$	542	56

[1] A. I. VIRTANEN, Kemiantutkimus-Säätiön vuosikertomus 1946, p. 7 (Helsinki 1947). (Annual Report for 1946 of the Foundation for Chemical Research, in Finnish).

of seed in order to secure a distinct effect from the addition of vitamin C. This difference depends apparently on the fact that the amount of vitamin C that is formed in the pea seed during germination is almost entirely located in the cotyledons, whereas in the wheat seed vitamin C accumulates already after the first day chiefly in the embryo.

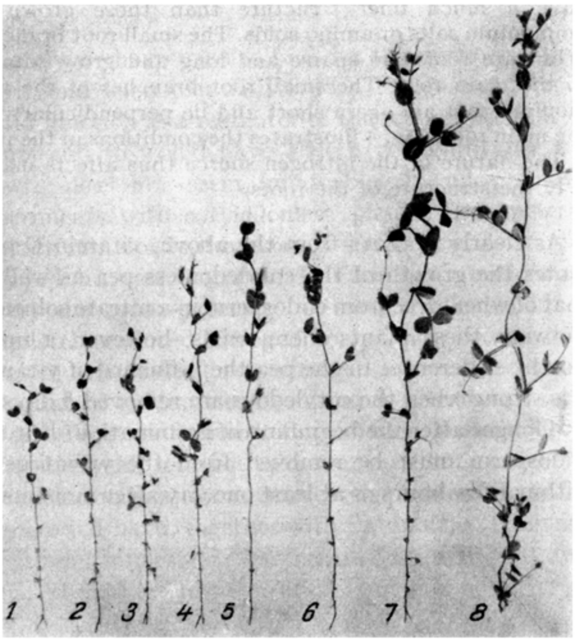

Fig. 4. – The shape of the roots of the pea-plant as affected by the nature of N-source.

The results reported above have unquestionably shown that the green plants are not autotrophic immediately the first green leaf has developed. In other words, they are unable to produce all necessary substances through carbon dioxide assimilation, but are dependent even some time after chlorophyll formation on the substances contained in the seed. The machinery required by the green plant for a fully autotrophic life does not develop to completion without a longer or shorter heterotrophic time, a period during which the plant receives necessary specific substances from the seed. This time is variable for different plants. The growth of the pea, for instance, is weakened if the cotyledons are removed even as long as a fortnight after germination has commenced, whereas the growth of wheat is little influenced if the endosperm is removed several days after the beginning of germination. It is the task of future research to find out what deficiencies arise in the metabolism of the plant without these substances.

The decisive significance of the chemical composition of the seed for the development of the plant is revealed in a new light through the results obtained. Since vitamin C, which is formed during germination, affects the growth of the plant in a high degree, it can be supposed that, the more abundant the formation of vitamin C in the seed, the stronger the future plant. If this be true, it would be possible to advance plant breeding to a great extent by giving due attention in the selection of the seed material to the quantity of vitamin C formed during germination. Besides vitamin C there are, as appeared from the above results, many other important substances which the plant receives from the seed. Elucidation of them will certainly need much work.

The application of the technique we have used in this laboratory for the elucidation of the significance of different substances formed in the seeds, i. e. separation of the embryo from the reserve nutrients in the germinating seed and culture of the embryo in a sterile nutrient solution in light, renders it possible to examine the influence of different factors on the growth under natural conditions. Later BONNER[1] and BONNER have used a somewhat different technique. They have grown isolated embryos on agar with 4% cane sugar in test tubes. The tubes were kept in the dark in order to prevent formation of chlorophyll. Under such experimental conditions it is possible to examine the influence of different vitamins on the growth of the embryo undisturbed by assimilation and the autotrophic growth depending on it.

The results obtained in this laboratory[2] by the method of BONNER are illustrated by Fig. 5. It can be seen that the effect of vitamin C has been greatest even

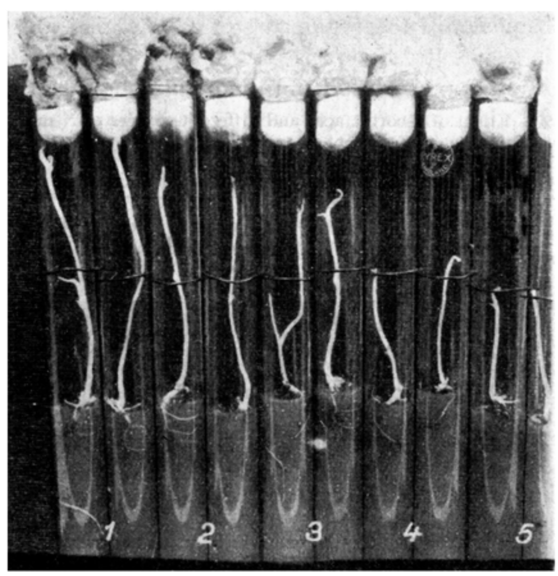

Fig. 5. – Embryo cultures, supplied with various growth substances.

1 Vitamin-B complex + biotin + vitamin C
2 Vitamin C
3 Vitamin-B complex + biotin
4 Vitamin-B complex
5 Control, no additions

[1] J. BONNER and D. BONNER, Proc. Nat. Acad. Sci. U.S. *24*, 70 (1938).

[2] S. SAUBERT-V. HAUSEN, Physiologia Plantarum *1*, 85 (1948).

then. However, biotin and B-complex have had a greater influence respectively than in the experiments performed in the light. This suggests that the factors of B group are synthesized in such high degree by assimilation that their effect in light remains slight, as is evident from the above results. Which of the two methods is most suited for experimenting depends on whether the aim is to examine the natural growth of the plant, i. e. both the autotrophic and the heterotrophic, or only the heterotrophic. In the former case our method is the only possible one, in the latter that of BONNER.

In addition to specific organic substances, also mineral substances in the seed naturally have a great bearing on the growth of the plant. In particular, the content of certain trace elements seems to be of great importance in practice, too. In the 1930's we made the following finding in this laboratory[1].

When the pea plant was grown in a nutrient solution for which tap water was used with the addition of ordinary mineral substances but no trace elements, the plant grew normally and also produced seeds. When we again performed a similar experiment with these seeds, the pea grew at first but began later to wither. It did not form any seeds. If ashes of burnt soil were added to the nutrient solution, the pea grew quite normally. This experiment suggested that the trace element content of normal seeds was high enough to provide the plant with such amounts of trace elements that these, together with the elements present in tap water, were adequate for the normal growth of the pea. In this way, however, the seeds produced were impoverished in respect to the particular element, so that they no more contained the same amount of trace elements as the normal commercial seeds. What the lacking trace element was did not yet appear from our preliminiary experiments. Later ERKAMA[2] in this laboratory has examined the role of copper and manganese in the growth of higher plants. These investigations revealed that the lacking trace element in our experiments had been copper.

The results recorded show clearly that the trace element content of the seeds can have an especially great importance if the substrate contains too little of some trace element. If the content of the particular element in the grain is high, the plant can thrive well even if the soil is poor in that respect, whereas grain that contains less of the said element than normal can give a much poorer crop in such a soil.

As a summary of the above I wish to state in conclusion that the decisive significance the chemical composition of the seed has on the development of the plant is a fact, and that the research in this respect has so far taken the first steps only. It is likely that in this field extensive research activity will go on, which will help to widen our knowledge of the chemical composition of seeds and plants.

[1] A. I. VIRTANEN and S. v. HAUSEN, Planta *31*, 263 (1940).

[2] J. ERKAMA, Ann. Acad. Sci. Fennicae, Ser. A., II. Chem., No. 25 (1947).

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Later experiments have confirmed the view that the reducing properties of vitamin C are of high significance for the plants grown in nutrient solutions with nitrate. In addition to vitamin C many other substances, such as reductone, cysteine, and glutathione in suitable concentrations promote growth in nutrient solutions with nitrate. With ammonium salts these reducing substances are either ineffective or, in somewhat higher concentrations, injurious. Our measurements have also shown that vitamin C as well as other reducing substances added to the substrate lower the redox potential within the plant. The redox potential within the plants was determined by sticking two platinum electrodes and a HCl-agar-bridge into the stem of the growing plant and by measuring the potential in the course of ten days.

Our experiments have thus revealed that the reduction of nitrate does evidently not take place unless the reducing substances present in the plant lower the redox potential to a suitable level. The suitability of nitrate and ammonium salts for the nitrogen nutrition depends decisively on the redox potential.

Zusammenfassung

In der vorliegenden Arbeit wird zunächst die Wirkung der beim Keimen der Samen entstehenden Vitamine behandelt: Wenn die Kotyledonen aus etwa 5 Tage lang keimenden Erbsen entfernt werden, dann wachsen die Keimlinge nur sehr langsam in sterilen Nährlösungen, die *Nitrat* als Stickstoffquelle enthalten. Das gleiche gilt für Weizen, der 1–2 Stunden gekeimt hat und dem dann das Endosperm herausgenommen wird. Wenn in diesen Fällen der Nährlösung Ascorbinsäure zugesetzt wird, dann beginnt ein Wachstum, das unter günstigen Umständen dem normalen nahekommt.

Bei der Untersuchung der Ascorbinsäurewirkung konnte festgestellt werden, daß die kotyledonfreien Erbsen, ebenso wie auch der endospermfreie Weizen, *ohne* Ascorbinsäurezusatz wachsen, wenn *Ammonium*salze als Stickstoffquelle dienen. Durch eine größere Konzentration von Ascorbinsäure wird das Wachstum gehemmt. Diese Befunde stützen die Arbeitshypothese, daß die Ascorbinsäure durch eine Herabsetzung des Redoxpotentials wirkt. Das Nitrat kann ohne diese Potentialsenkung offenbar nicht reduziert werden. Für diese Annahme spricht auch, daß die Ascorbinsäure durch bestimmte Mengen von Redukton, Glutathion und Cystein ersetzt werden kann.

Durch Ascorbinsäure und durch die anderen erwähnten reduzierenden Stoffe wird das Redoxpotential sowohl in den Lösungen wie auch in den wachsenden Pflanzen herabgesetzt. Die verschiedene Wirkung des Nitrat- und Ammoniumstickstoffs ist demnach vor allem auf das Redoxpotential zurückzuführen.