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The biological fixation of nitrogen and the preservation of fodder in agriculture, and their importance to human nutrition

Nobel Lecture, December 12, 1945

Nitrogen is an essential constituent of proteins, the central building bricks of all living matter. Appropriate nitrogen nutrition is thus essential to both plants and animals. The molecular nitrogen of the atmosphere is the main supply of nitrogen for our earth and from it originates all the nitrogen found in living nature. Since all the higher and the majority of the lower plants, and the entire animal kingdom are unable to assimilate atmospheric nitrogen, it is a precondition for life that the nitrogen must be fixed in some way by other substances to form compounds which can be assimilated by plants. Nitrogen nutrition is frequently a minimum requirement for growth in the natural state. In effective agriculture, where we must of course try to achieve the maximum plant production per unit of area, one of the most important tasks is to provide plants with a sufficient supply of nitrogen.

In nature, molecular nitrogen is fixed to a certain degree by electrical discharges in the atmosphere. The main nitrogen fixation, however, is produced by the action of certain micro-organisms. Some of these fix nitrogen where they live freely in the ground and have suitable carbon compounds available, while others fix nitrogen only in symbiosis with a host plant, from which they obtain their carbon nutrition. Of the latter, the bacteria of leguminous plants are the most important. They are also predominantly the most effective nitrogen suppliers in agriculture, if we do but know how to make use of them.

Today it is also possible to fix atmospheric nitrogen industrially by ammonia synthesis based mainly on Haber's research. The idea that agricultural nitrogen requirements should largely be covered by this process is quite general even amongst chemists, but this is not the case. If we consider the agriculture of the entire world, the proportion of synthetic nitrogenous fertilizers in relation to the nitrogen contained in the crops is very low (possibly 0.3%). The following statistical calculation carried out in 1936 in con-

nection with the agricultural nitrogen balance in the U.S.A. gives us a clear picture of the situation.

According to this, the quantity of industrially manufactured nitrogenous fertilizers used annually in the U.S.A. was equal to 0.48 million tons of nitrogen or just a few percent of the total quantity of nitrogen supplied to the soil, 16.45 million tons. The quantity of nitrogen fixed by micro-organisms, on the other hand, was 9.87 million tons, of which 5.6 million tons was due to leguminous plant bacteria. It is also clear from these statistics that the nitrogen content of arable land in the U.S.A. drops annually, i.e., the plants

Table I. Nitrogen balance of arable acreage in the U.S.A. according to calculations carried out by Lipman and Conybeare in 1936.

Quantity of nitrogen annually supplied to arable acreage (millions of tons)		*Quantity of nitrogen annually taken from arable acreage (millions of tons)*	
In stable manure	2.57	In crops	4.61
In nitrogenous fertilizers	0.48	By erosion	5.00
By rain and artificial irrigation	3.57	By washing out	5.00
By biological nitrogen fixation (Symbiotic N-fixation 5.46; non-symbiotic N-fixation 4.37)	9.83	By grazing and stock breeding	9.05
Total	16.45	Total	23.66

are using up the soil's nitrogen reserve. The annual nitrogen loss in arable land in the U.S.A. according to the above calculation was 7.21 million tons.

With regard to *protein production,* the European countries, and particularly those carrying on intensive milk production, have to rely on imports. Although these countries have high crop yields, mainly due to intensive application of fertilizers, including nitrogen fertilizers, the ratio of protein to carbohydrate in the crops is not such that the fodder would be suitable for relatively high milk production without extra protein. The protein content is sufficiently high only in the case of the legumes, and in fact the protein content may even be much greater than necessary, so that the legumes occupy a special position in protein production. Protein production is thus closely related to biological nitrogen fixation.

Coverage of agricultural protein requirements, however, depends not only on the production of protein-rich fodder but also on the *preservation* thereof, since in the cold and temperate zones cattle cannot obtain fresh

fodder direct from the land during the cold seasons, while in the hot zones drought is a similar obstacle. The basic problem for intensive livestock production is therefore the problem of preserving the fodder. Enormous quantities of nutritive substances, both protein and carbohydrates, are annually lost as a result of preservation losses. The fact that *the protective substances* in food, such as the vitamins and certain amino acids, are very easily destroyed, is particularly detrimental to both man and beast. Research for the development of preservation methods is therefore equally as important as increased production.

After this introduction I should now like to mention those aspects of our research which relate to agriculture and human nutrition and for which the Swedish Royal Academy of Sciences has awarded this year's Nobel Prize in Chemistry. From the very outset I should like to stress the important contribution made by my colleagues in the results we have achieved.

In the spring of 1925 in the modest laboratory of Valio, the central association of the Finnish co-operative dairies, we began research in connection with the nodule bacteria of leguminous plants. These tests clarified for me the extraordinarily vigorous growth of legumes, which live in symbiosis with the bacteria of their nodules, solely by means of atmospheric nitrogen. Although at that time I had no close experience of agriculture, the experiments that had been carried out set my imagination in motion and led me to think how it might be possible to utilize atmospheric nitrogen to promote fodder cultivation and milk production by intensive and rational legume cultivation. It was self-evident that our knowledge of the activity of legume bacteria, the factors influencing nitrogen fixation, and its chemical mechanism, must be widened as much as possible if these remarkable bacteria were to be utilized effectively. The biological nitrogen fixation was therefore the central problem at the laboratory of Valio and later at the Biochemical Research Institute, which was founded in 1931.

When I studied the literature of the subject in 1925, I found that the difficulties in preserving protein-rich legume crops were a decisive factor in limiting the possibilities of utilizing these plants agriculturally. Such fodder can generally be dried to hay only once per summer and even then the weather conditions are a decisive factor. If the fodder is prepared for ensilage, the resultant fodder is of poor quality. High preservation losses (25 to 50% of the nutritive value) are also inherent in these two methods. *Inter alia,* a large part of the valuable protein is lost. For effective utilization of legumi-

nous plants, it was therefore necessary to prepare a reliable preservation method whereby the fresh fodder could yield a high-grade end product with low losses. The fodder preservation problem was therefore incorporated in the research programme of our laboratory. If it were possible to make fuller use of symbiotic nitrogen fixation and devise effective legume cultivation while successfully solving the problem of fodder preservation, hitherto unimagined possibilities would be opened to self-supporting milk production while it would also be possible biologically to increase the nitrogen content of arable land. That was the practical object of our work. Since the problems were also integrally bound up with such an important natural phenomenon as the assimilation of gaseous nitrogen, this research was also particularly interesting from the scientific aspect.

N-fixation in the root nodules of leguminous plants

As our research progressed, it soon became apparent that it was necessary for the experimental methods to be greatly improved. Since it had been found that the leguminous plant bacteria fix nitrogen only in symbiosis with their host plants, experiments had previously been carried out by cultivating test plants inoculated with legume bacteria in open pots in a nitrogen-free or low-nitrogen medium, for example quartz sand. Under such conditions, of course, there were also other micro-organisms than legume bacteria in the medium, particularly in the region of the roots. These bacteria could, of course, also include freely living N-fixing bacteria. The test plants could therefore obtain their nitrogen nutrition in some other way than just by the co-operation of the legume bacteria. We therefore prepared a *sterile cultivation technique,* in which the plant media were free of micro-organisms unless they had been inoculated with some particular bacteria, for example a strain of legume bacteria. In the summer of 1929 we carried out the first experiments using the sterile cultivation system and it was possible to prove positively that legumes with nodules could well utilize atmospheric nitrogen without the presence of other micro-organisms, and that the nodule bacteria in symbiosis with the leguminous plants therefore fix nitrogen as Hellriegel and Willfart had assumed in 1887 following upon their classic experiments in open pots. In the summer of 1930, by the use of the sterile cultivation technique in experiments with inoculated vetch we were able to prove the presence of nitrogen compounds diffused out of the root system. We also

found that this nitrogen was of an organic nature. After we had simplified and rendered the sterile cultivation technique effective (Fig. I), it was possible to study this secretion of nitrogen compounds more closely. We found that inoculated legumes can in certain cases secrete into the surrounding sand even greater quantities of nitrogen compounds than the host plant itself has assimilated. Special tests pointed to the proposition that the secretion was from the root nodules.

Since the secretion was greatest in young plants in which the root nodules are still in growth, there was reason to assume that the nitrogen compounds collected in the medium were *intermediate products* in protein synthesis and not decomposition products of the proteins. It was therefore important to isolate and identify these nitrogen compounds.

Examination showed that over 90% of the nitrogen diffused out was amino-nitrogen. At an early stage of growth before the flowering of peas, the majority of this amino-N originates from *l*-aspartic acid. Some of the nitrogen belongs to β-alanine, which is formed by aspartic acid by decarboxylation. The reaction is caused by the leguminous plant bacteria. Since β-alanine is a secondary splitting product of aspartic acid, it was obvious that aspartic acid is the primary secretion product. Determination of the secretion phenomenon also provided an explanation of the disputed question concerning the way in which the nitrogen fixed in the root nodules is assimilated by the host plant and what N-compound or compounds make up this "fixed nitrogen", which serves as N-nutrition for the host plant. There was immediately reason to assume that the N-fixation took place on the surfaces of the bacterial cells in the root nodules and that the resultant amino acid, probably aspartic acid, is secreted in the plant sap and is thus assimilated by the host plant. Since the quantity of root nodules and, in particular, the quantity of active contents of the nodules did not increase substantially during the period of intensive nitrogen fixation to the same extent as initially when the nodules were formed, it was clear that the bacterial cells in this phase only slightly assimilate the fixed nitrogen to build up their cell protein and that the majority is therefore utilized by the host plant. The symbiotic N-fixation is thus very economical from the aspect of the host plant, since the bacteria use for their cell material only a comparatively small quantity of the carbon compounds of the host plant.

Bond arrived at the result that approximately 90% of the nitrogen fixed in the root nodules of the soya bean is assimilated by the host plant during the best growth period. Wilson and Umbreit have found similar values. In

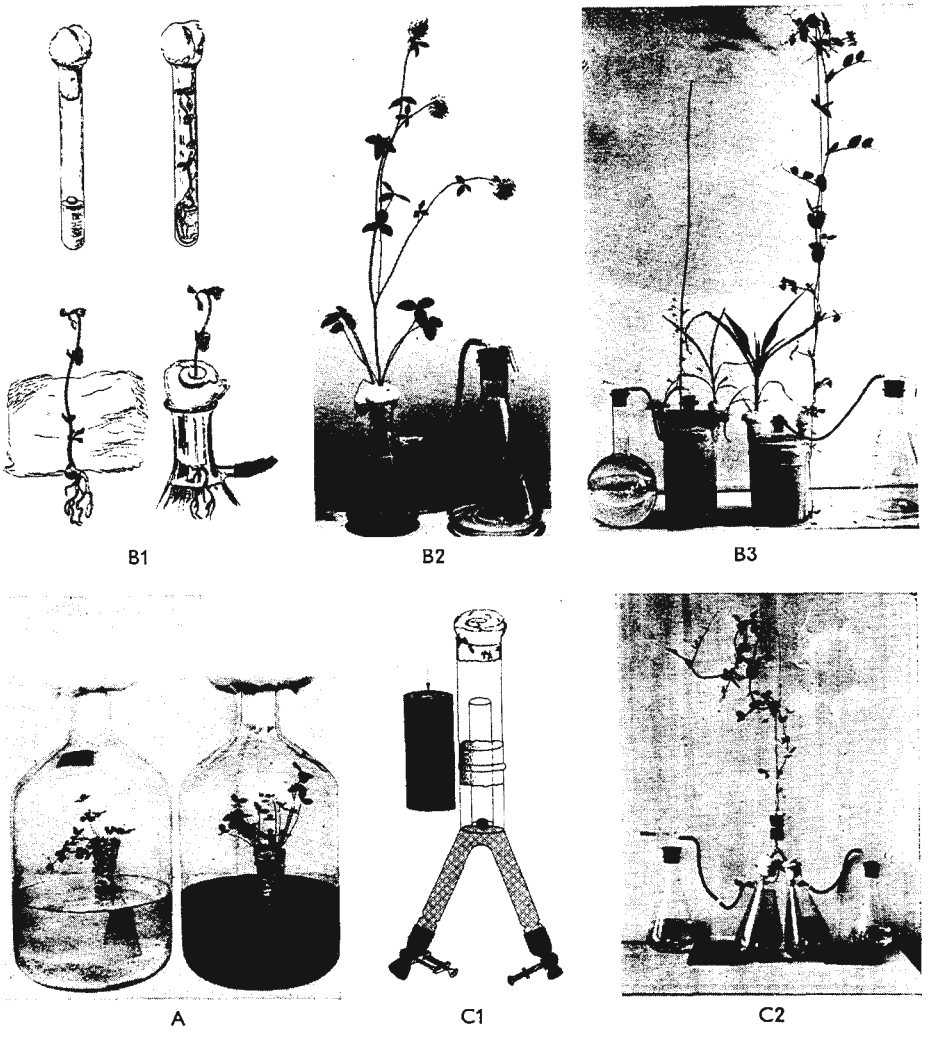


Fig. 1. Sterile cultivation system.

(*A*) An experimental arrangement from 1929. (The experimental plants grow entirely inside a 9-litre flask.)

(*B*) An experimental arrangement from 1931. (The root system of the experimental plant is in a sterilized bed, the green parts of the plant grow freely in air.)

B1: performance of the experiment; *B2*: red clover in a sterile system; *B3*: pea and maize growing in the same Woulfe flask without N-nutrition; non-inoculated pea on the left, inoculated on the right.

(*C*) "Breeches-tube" system, in which the experimental plant root system grows down in two flasks.

C1: experimental arrangement; *C2*: pea in breeches tube.

our tests with peas, the host plant proportion of the fixed nitrogen rose to 93% (Fig.2).

With regard to the chemical mechanism of N-fixation, I initially assumed that aspartic acid was formed from fumaric acid and ammonia by the action of aspartase ($HOOC\text{-}CH=CH\text{-}COOH+NH_3 \rightleftarrows HOOC\text{-}CH_2\text{-}CH(NH_2)\text{-}COOH$), but it soon proved that aspartase did not occur in the root nodules, so that this hypothesis had to be abandoned. Since small quantities of nitrite-nitrogen, the quantity of which increased during evapora-

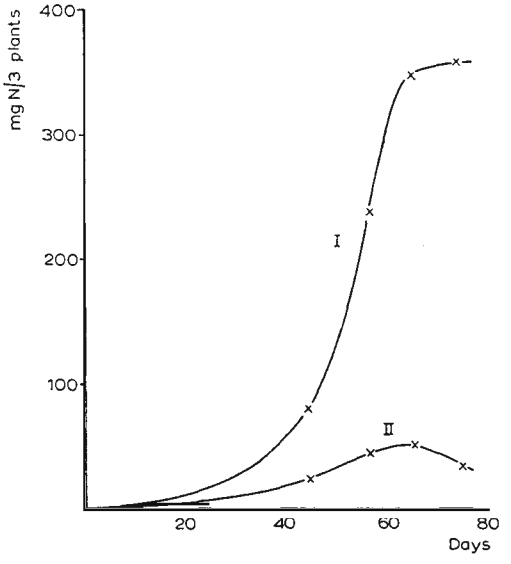


Fig. 2. Experiment with inoculated pea in quartz sand. Three plants in each pot. Three pots in each test series.
Curve I: N in host plants; *Curve II*: N in root nodules.

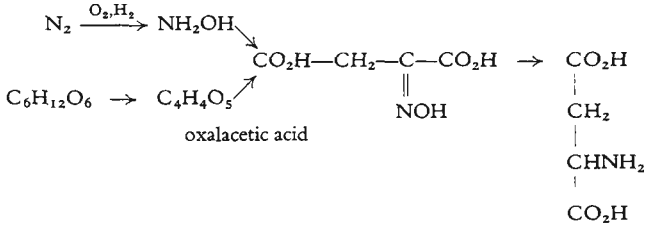
Days of growth	*Period of growth (days)*	*N-increase (mg)*		*N-increase (%)*	
		In the host plants	*In the root nodules*	*In the host plants*	*In the root nodules*
0–45	45	+ 80.6	+21.7	78.8	21.2
45–57	12	+158.5	+21.7	88.0	12.0
57–66	9	+107.8	+ 7.7	93.3	6.7
66–75	9	+ 11.8	—16.9	—	—
	Total	358.7			

tion, were found in the water extract obtained from the plant bed, my attention was drawn to another possibility.

At about the same time (1935) Endres had found oxime-N in *Azotobacter* cultures and we too were subsequently able to find it amongst the secretion products of the root nodules. The above-mentioned nitrite-N apparently occurs from decomposition of the oxime. The quantity of oxime-N was very small, about 1-2% of the total nitrogen in the extract.

It was therefore difficult to isolate and identify it. We finally obtained aspartic acid by reduction of the oxime fraction. As a result, we could conclude that oxalacetic acid oxime was included in the oxime fraction.

On the basis of these results I propounded the following hypothesis concerning the mechanism of N-fixation in the root nodules of legumes.



According to this hypothesis, the host plant would supply the carbon component, namely oxalacetic acid, with which the hydroxylamine formed from the molecular nitrogen reacts to form oxime which gives aspartic acid on reduction. It should also be mentioned that Blom had propounded a very interesting hypothesis at the beginning of the 1930's, according to which hydroxylamine would occur as an intermediate product in biological N-fixation.

The formation of aspartic acid as the primary amino acid in N-fixation is particularly interesting because according to earlier experiments (Braunstein and Kritzman, 1937) the amino group is transferred in animal tissues both from this amino acid and from its homologue, glutamic acid, to other α-keto acids by the enzyme transaminase. According to our experiments the transamination reaction with green plant material and root nodules takes place only in the system α-aminodicarboxylic acid (or ketodicarboxylic acid) + aliphatic α-ketomonocarboxylic acid (or α-aminomonocarboxylic acid) $\rightleftarrows$ α-ketodicarboxylic acid (or aminodicarboxylic acid) + α-aminomonocarboxylic acid (or ketomonocarboxylic acid). The reaction could not be brought about with α-amino or α-keto acids having a ring structure as the other com-

ponent, so that it is not possible to explain the synthesis of all amino acids by the transamination reaction.

According to our findings, growing pea plants contain about 50 γ and red clover contains about 100 γ oxalacetic acid per 1 g of fresh plant material if the analysis is carried out on a sunny day. The oxalacetic acid content drops rapidly in the dark. If pea or clover plants are left in the dark for 48 hours no oxalacetic acid can be found in them.

It is naturally not impossible that the N-fixation would only partly result in the formation of oxime nitrogen and that the reaction progresses mainly by way of ammonia and imino acid. The quantity of oxime found is very small indeed. If hydroxylamine is formed as an intermediate product in the ammonia synthesis ($N_2 \rightarrow NH_2OH \rightarrow NH_3$), the formation of oxine nitrogen in small quantities would be explained. However, there is still no direct proof that NH_2 appears as an intermediate product although one would expect a formation of ammonium nitrogen along with the hydroxylamine. If ammonia is formed in nitrogen fixation, glutamic acid would necessarily occur, since glutamic acid dehydrogenase, which was examined closely by Euler and Adler and which catalyzes the glutamic acid synthesis from ketoglutaric acid and ammonia, occurs both in the animal and plant kingdoms. We again demonstrated in our laboratory the occurrence of α-ketoglutaric acid in legumes in approximately the same quantities as oxalacetic acid. The absence of glutamic acid in the secretion products from the root nodules does not point to glutamic acid as the primary amino acid with aspartic acid, but the greater activity of glutamic acid in the transamination reaction may perhaps explain this.

Wilson and his colleagues in Madison (Wisc.), who some years previously were still very sceptical concerning the occurrence of aminodicarboxylic acids as primary amino acids in biological N-fixation, now (1945) consider from experiments performed with ^{15}N isotope that these amino acids are primary with the emphasis on glutamic acid.

We have no appreciable knowledge of the enzymes that catalyze the first stages of N-fixation ($N_2 \rightarrow NH_2OH$ or $N_2 \rightarrow NH_3$). Noteworthy experiments have, however, recently been carried out in connection with the red pigment found in root nodules. These experiments have shown that the pigment is closely bound up with N-fixation. According to Mothes and Pietz, the pigment is a derivative of dioxyphenyl alanine, while according to Kubo of Japan it is haemoglobin, which can store and transport oxygen. Kubo did not relate the pigment to N-fixation.

Owing to the war we were not able to examine the matter in our laboratory until the autumn of 1944. We first of all confirmed the accuracy of Kubo's observation concerning the nature of the pigment and proved that those root nodules that fix nitrogen always contain haemoglobin (a report to the meeting of the Finnish Academy of Sciences on January 12, 1945), which we now began to call leghaemoglobin to distinguish it from blood haemoglobin (Keilin and Wang in England also confirmed the haemoglobin nature of the red pigment in 1945). If the pigment is absent from the root nodules or if it is denatured, or if it has been changed to a green pigment, of which more later, no nitrogen fixation takes place.

Together with leghaemoglobin, brown legmethaemoglobin with trivalent iron also occurs in the root nodules. Contrary to conditions in the case of blood, the valence exchange $Fe^{II} \leftrightarrows Fe^{III}$ readily takes place in the root nodules. The oxidase enzymes in the root nodules apparently play an important part in oxidation of the iron to form leghaemoglobin. Oxalacetic acid, on the other hand, appears to be an important factor in maintaining equilibrium on the ferro-side.

When peas, soya and other legumes grow in strong light, the ferro-form is predominant in the root nodules, but when the lighting is reduced the balance gradually shifts towards the ferri-side.

Since the iron in leghaemoglobin is so easily oxidized and reduced, I began to speculate on the possibility that the N-fixation would be related to this valence exchange although the oxygen transport might possibly already explain the importance of the leghaemoglobin in nitrogen fixation. The observations by Wilson and his colleagues concerning the inhibiting effect of carbon monoxide on nitrogen fixation even in small concentrations can very easily be explained as being dependent upon the formation of a compound of the leghaemoglobin Fe^{II} iron and CO. In the first phase of N-fixation we probably have a complicated catalyst system which is not restricted to the leghaemoglobin system. However, in this context there is no reason to discuss in greater detail these questions which are still unsolved.

The haemoglobin system appears to be split in the root nodules in two different ways:

1. By denaturation, when the pigment group is separated from the protein part. The pigment is not obtained in aqueous solution from root nodules in which this change has taken place.
2. By conversion in approximately the same way as the formation of the bile pigments in the animal organism (Barkas, Lemberg). The porphyrin

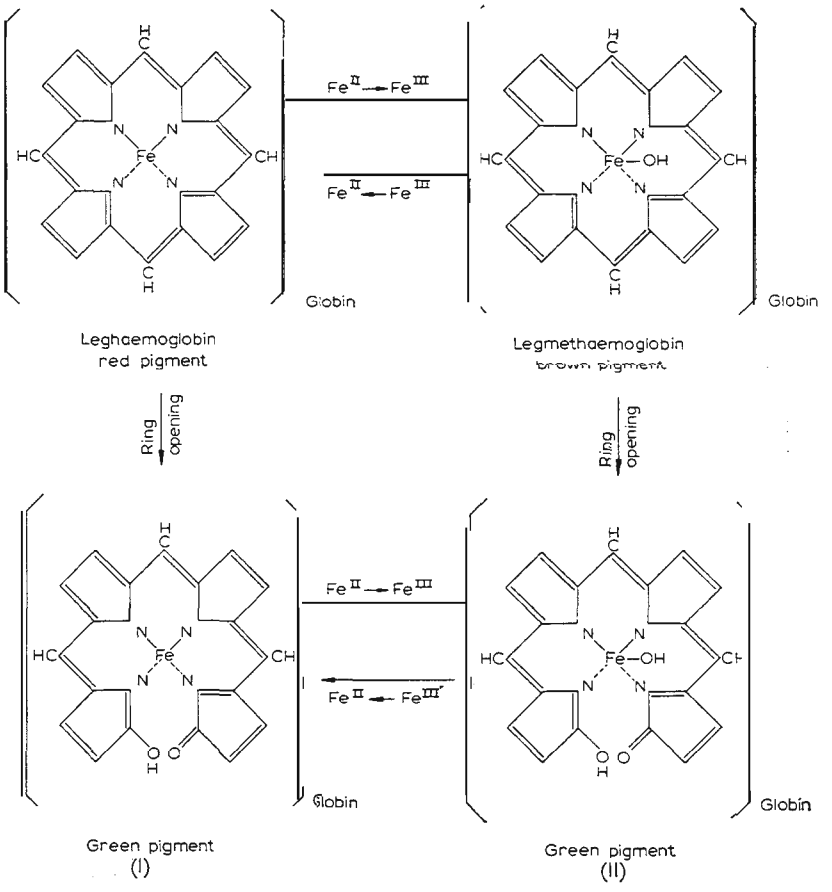
ring in these conditions is split between two pyrrol rings, whereby the combined CH-group is split off.

When the peas or soya beans ripen, the N-fixation ceases and the root nodules assume a green colour. The same change also takes place when vigorously growing legumes are kept in the dark for some days. The green pigment still contains iron although in a form easily split off with acids, and the pigment continues to be a chromoprotein although it can be easily denaturized. The iron content is the same as in leghaemoglobin. The properties of the green pigment are similar to choleglobin which was prepared by Lemberg from blood haemoglobin. Since the leghaemoglobin in the root nodules appears initially to pass over to methaemoglobin and it is only then that the green pigment appears, and since the green pigment occurs at a pH of 6.5 and less at room temperature from methaemoglobin (not from haemoglobin), if some ascorbic acid and hydrogen peroxide are supplied to the solution, it is possible that the green pigment is also formed in this way in the root nodules. The ascorbic acid content of the root nodules is sufficient to bring about this reaction. If we combine Lemberg's research with our own observations, we can provisionally give the mutual relationship shown on p. 85 between the red, brown, and green pigment in the root nodules.

The leghaemoglobin molecular weight determined by Dr. Pedersen in Svedberg's laboratory from a leghaemoglobin preparation which I sent him, and the degree of purity of which on the basis of its iron and hemin content was probably in the region of 85%, was approximately 34,000, i.e. half of the molecular weight in blood haemoglobin if there were no dissociation of the protein.

Apart from the chemical side of the nitrogen fixation problem, the more biological side also formed the subject of our continual research. Of this I will mention here only some of the problems of significance to practical agriculture.

The formation of leghaemoglobin in the root nodules is a result of symbiosis between the nodule bacteria and the host plant. The inability of the ineffective bacterial strains to fix atmospheric nitrogen is clearly due to the fact that root nodules formed by the same do not contain leghaemoglobin. Since the same bacterial strain in symbiosis with *one* host plant can form active root nodules containing leghaemoglobin, while with *another* host plant they form leghaemoglobin-free inactive root nodules (for example a bacterial strain isolated from the root nodules of *Trifolium alexandrinum* is effective in symbiosis with this species of *Trifolium,* but ineffective with



Trifolium pratense), the formation of the pigment is decisively dependent on the host plant as well. The leghaemoglobin is clearly not found in the bacterial cells themselves, because it dissolves as soon as the root nodules are crushed in a mortar. Whether the bacteria form the hemin component of the pigment and the host plant its protein component or *vice versa,* or whether the symbiotic formation of the pigment is explained in some other way is still obscure.

As a result of our sterile cultivation technique we were able positively to prove that the efficacy of individual bacterial strains is a highly constant property and that it does not change, for example, by successive passage through a host plant, as stated in the literature of the subject. The fact that several researchers arrived at this proposition is obviously due to the fact that other bacterial strains than the one in question have also been involved,

and this easily happens in experiments carried out in open vessels. This question is very important to agriculture, since the use of bacterial inoculation in the cultivation of the customary legumes on old cultivated land is based on the assumption that the bacterial strains used are very effective. If the efficacy of the bacterial strains were to vary to the extent indicated in literature, we could not refer to effective and ineffective strains. We have been able to prove positively the occurrence of quite ineffective legume bacterial strains, and that once they are allowed to penetrate into the root system they prevent the function of the effective strains. This demonstrates the importance of a correct choice of the bacterial strains used for inoculation.

Of the other results of practical importance in connection with leguminous bacteria and legumes I would give the following details:

The pH curve of the activity of legume bacteria has an optimum at pH 6.5-7.0, and this is also the optimum for the legume growth. On the acid side the activity of the legume bacteria ceases completely below pH 4.5 whereas the legume plants still grow to some extent at a pH of 4 if nitrate fertilizers are applied. In practice, however, the pH requirements of the bacteria determine the legume growth. If good leguminous plant crops are to be obtained in acid soils, the application of lime is therefore necessary.

The looseness of the earth, i.e., the air content, is of great importance to the activity of the root nodules. During rainy summers the lack of oxygen in stiff clay soils prevents the growth of peas, because the activity of the root nodules is paralyzed. By mixing a few percent of pumice stone in a stiff clay which was watered generously during the experiment, we have been able to increase the pea crop up to as much as 100% in pot tests. Also, 0.3-1% of chopped straw has in some cases resulted in a considerable crop increase. The conditions in the field are complicated because during dry weather such an addition may be detrimental as a result of excessive drying of the soil.

Determination of the importance of leghaemoglobin to the nitrogen fixation process makes it possible to obtain an idea of the activity of the root nodules in legume cultivation by examining the colours of the sections of the nodules after cutting. If the section is red, it shows that a vigorous N-fixation takes place, a brown colour indicates at least temporary reduction of the nitrogen fixation, while green sections show that the nitrogen fixation has ceased. It is not always easy to explain why chromoproteins vary from case to case, but an investigation of the question is important to the control of leguminous plant cultivation.

Research over the past 20 years has thrown a certain light on symbiotic

N-fixation. The improvements to the research methods and fundamental observations made hitherto will clearly accelerate the development of this field of research and thereby increase the possibilities of agriculture making even more effective use of atmospheric nitrogen than is now the case. Industry, which with its capital and technical resources can generally rapidly utilize scientific results and even promote research, is unfortunately not interested in biological nitrogen fixation because the latter does of course lead development "back to nature". This circumstance naturally acts as a brake on research to a certain extent.

Preservation of protein-rich crops

In the autumn of the same year (1925) as we carried out our first experiments with legume bacteria, I began to devise a reliable and effective method of preserving fresh fodder crops. The preparation of silage has undoubtedly been applied for thousands of years, as is apparent from certain Egyptian relics of antiquity, but in 1925 there was still no reliable theoretical basis for preparing silage despite comprehensive experiments, particularly during this century. The work in this field had largely been unfruitful because the tests were not based on theoretically grounded working hypotheses which could lead to generally valid results. Development was also greatly obstructed by an absence of clear principles for judging the quality of silage. The literature of the subject therefore frequently did not enable us to conclude what kind of results the method used really gives. Under such conditions it was self-evident that there would be a large number of processes which to some extent differed and that they all had their own advocates. In this connection it must be stated that G. Wiegner in Zurich in the 1920's carried out critical research work to clarify the losses of nutritive substance in various methods of preservation, and *inter alia* he found that the losses with the very much publicized electro-silo process were as high as 40-50%.

The following considerations formed the basis of our research into the preservation of fresh fodder.

The nutritive substances are broken down by the following processes in the preservation of fresh fodder:

I. By respiration of the plant cells, which first of all results in a loss of carbohydrates. This naturally takes place only at the beginning of the preservation when the plant cell respiration is still in progress.

2. By harmful fermentation processes caused by various micro-organisms. During such processes the carbohydrates are split into lower-grade or directly harmful products. Organic substance is also lost in these processes as a result of the splitting off of CO_2.
3. By the breaking down of proteins, whereby the amino acids are partially totally decomposed with the formation of NH_3. These decomposition processes are also caused by micro-organisms.

Our object when we began to take up the problem of the preservation of fodder was to prevent these three processes in such a way as not to make the fodder physiologically unsuitable.

At the beginning of the 1920's we had examined the decomposition of proteins and various bacterial fermentations in our laboratory. *Inter alia,* it was apparent that these processes with the exception of lactic acid fermentation can no longer take place at a pH below 4. Since we were also able to prove that the respiration of the plant cells, too, was greatly reduced at pH 4 and totally ceased at pH 3, effective fodder preservation could be expected if an acidity below pH 4 (Fig. 3) could be brought about in the fodder sufficiently quickly. With regard to protein-rich fodder, the preservation of which is the most important from the aspect of milk production, it was generally impossible to bring about such a high acidity by means of lactic acid fermentation in the fodder, owing to the high buffer action of the fodder. Since a method based on fermentation processes is also unreliable in other

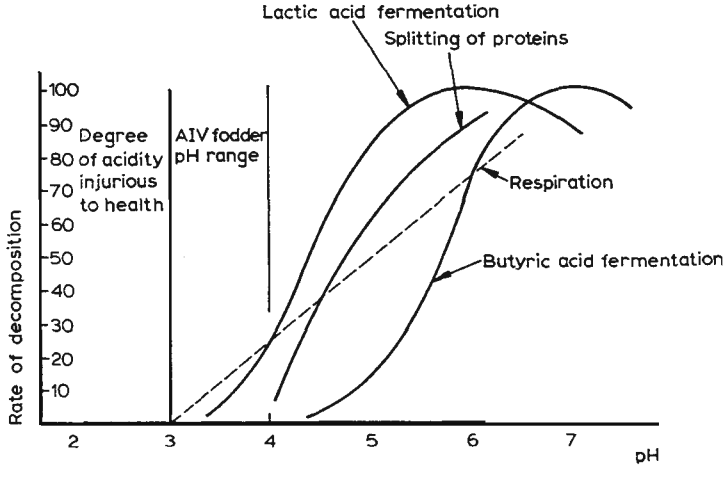


Fig. 3. Diagrammatic representation of importance of hydrogen ion concentration to various decomposition processes in compressed flesh fodder.

respects owing to the variations in the sugar content of the fodder, bacterial flora and other conditions, I considered it necessary to adopt the chemical method of controlling the fodder acidity in our search for a method of preservation reliable under all conditions.

On the laboratory scale using quantities of fodder of just a few hundred grams, we were initially able to prove the decisive feature that the acidity of the fodder was practically constant for an unlimited time if the pH of the fodder on preservation were adjusted to below 4 (pH 3-4) by means of an acid solution, and if the expressed juice formed during the compression of the fodder was allowed to run away. If, on the other hand, the quantities of acid added were such that the pH of the fodder was above pH 4, the pH of the fodder frequently rose during preservation as a result of the formation of ammonia and fermentation of the resultant lactic acid, so that an inadequate addition of acid did not prevent the harmful processes in the fodder. A pH of 4 proved to be the lowest acidity at which fodder can be reliably and effectively preserved.

Our research concerning the rate of diffusion of the added acids in the fodder showed that the penetration of the acids in the plant cells naturally

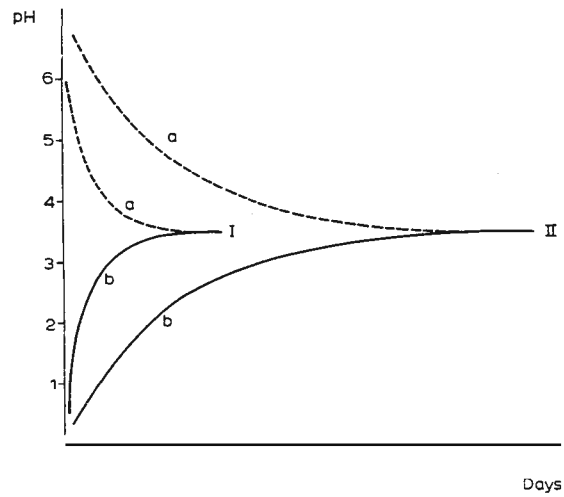


Fig. 4. Diagrammatic representation of equalization of acidity differences in fresh fodder to which sufficent acid has been added to reduce the fodder pH to 3-4.
Curves a: pH changes in the solid phase of the fodder.
Curves b: pH changes in the liquid phase of the fodder.
I: Tests with chopped fodder. *II:* Tests with unchopped fodder.

requires a certain time but is also relatively rapid with strong mineral acids Fig. 4). A rapid acidification of the fodder is very important, since in ordinary pressed fodder the splitting of both the proteins and the carbohydrates has already reached an advanced stage within two weeks (Table 2).

Quantitative experiments concerning the quantities of acids required for acidification of the fodder to a certain acidity show considerable differences for different types of fodder. The richer the plants in protein, the greater the quantities of acid required. The acidity and lime content of the soil also affects the quantities of acid. The quantities of the latter were of course also dependent upon the dry substance content of the plants and the degree of dissociation of the acids used.

Fig. 5 shows an experiment carried out with young clover (dry substance 17%) showing the effect of different acids on the degree of acidity of the fodder.

From experiments carried out with strong acids it was apparent:

1. That the quantity of strong acids required to increase the acidity of protein-rich plants to a pH of between 3 and 4 was not so great that it would be unacceptable a priori from the nutritional physiological or economic aspects. The quantity of weak acids required, such as lactic acid, phosphoric acid, and so on, on the other hand, was so great that their use did not seem economically worth-while at least at that time.

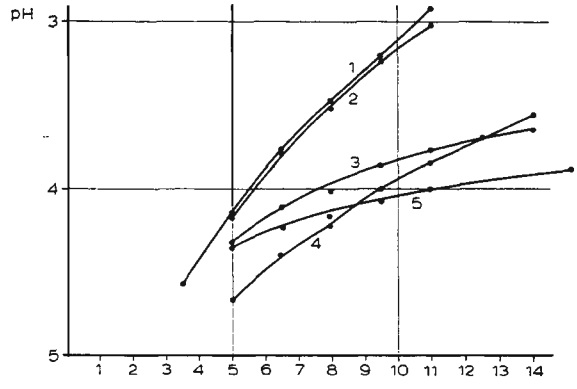


Fig. 5. Influence of various acids on the acidity of the fodder prepared from young clover. The curves show the quantities 2 *N* acid in ml per 100 g fresh clover.
Curve 1: hydrochloric acid.
Curve 2: sulphuric acid.
Curve 3: lactic acid.
Curve 4: phosphoric acid.
Curve 5: formic acid.

2. That a fodder acidified with strong acids (hydrochloric and sulphuric acid) to a pH of between 3 and 4 did not contain these acids in the free state. In the plants the cations (K, Ca, Mg, etc.) are bound by organic acids, such as oxalic, malic, fumaric, and citric acid. These plant acids are liberated by the action of mineral acid and therefore result in the acidity of the fodder. At the same time, the dissociation of the proteins is also changed, so that they also influence the final acidity of the fodder.
3. That with a fodder of a pH of between 3 and 4 we therefore did not need to take into account the influence of the free mineral acids on the state of health of the livestock. On the other hand, it was important to determine by tests with livestock whether the quantity of chloride and sulphate formed as a result of the addition of the acid might be injurious to the animals and whether the reduced base surplus might ultimately have a detrimental effect on the health of the animals.

Table 2. Preservation of chopped clover in glass cylinders, about 6.5 kg in each cylinder. The solutions are carefully mixed with the fodder, which is covered with a wooden lid and loaded with a weight of 10 kg.

	Fresh fodder	*AIV fodder*	*Ordinary pressed fodder (without additives)*	*0.7% sugar addition*	*1% NaCl addition*
Storage time 15 days (20.IX – 5.X.1934):					
pH	6.5	3.74	4.95	4.57	4.9
NH_3—N (% of total N)	0.7	2.9	13.7	10.8	14.3
Storage time 40 days (5.X – 14.XI.1934):					
pH	6.6	3.60	4.99	5.02	5.11
NH_3—N (% of total N)	0.8	1.9	14.1	15.2	16.7
CO_2-evolution (g/l kg dry substance)	—	24.5	94.8	139.9	105.3

When we had carried out the basic scientific experiments and gone over to the actual fodder preservation tests, initially only on a laboratory scale, we found that the test results agreed with the theory. It was found without exception that if the acidity of the fodder is raised to pH 3-4 from the very beginning, the harmful fermentations and splitting of ammonia from amino acids is practically completely inhibited, the formation of butyric acid is completely prevented and the evolution of carbon dioxide is reduced to a minimum.

The curves shown in Figs. 6 to 8 illustrate experiments from later years and clearly show that the splitting processes in silage are dependent upon the pH of the silage of the type of preservation method applied, and that these processes are completely controlled and reduced to a minimum only below pH 4. Above pH 4 the splitting processes increase as the pH rises and the fermentation products in the various tests will change considerably. The curves show that only pH 3-4 represents the pH range guaranteeing reliable and effective preservation.

On the basis of these results, assessment of silage quality became simple and clear: a pH analysis gives a clear idea of the fodder quality, namely fodder preserved with acid. In the case of preservation methods based on fermentations in which the extent of the decomposition processes is also dependent on how quickly the acids are formed, it is frequently necessary to determine not only the pH but also the NH_2 nitrogen expressed as a percentage of the total nitrogen.

Since the formation of ammonia in fodder acidified to pH 3-4 is minimal, we can already conclude that the preservation of the amino acids in the fodder must be excellent. Analysis of various amino acids and amino

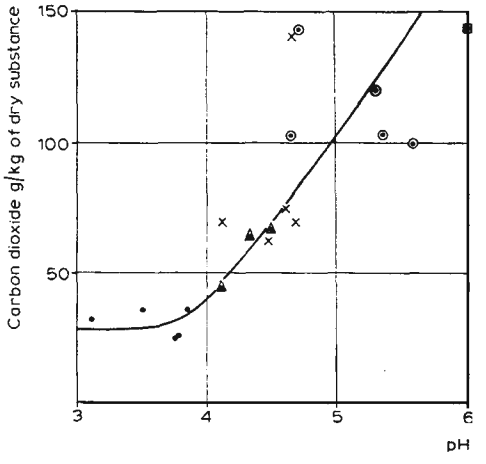


Fig. 6. The development of carbon dioxide in fodder preserved in various ways. (Five parallel tests.)

• AIV fodder.
x Amasil fodder (normal quantity of formic acid).
△ Amasil-2-fodder (double the amount of formic acid).
⊙ Ordinary pressed fodder.
⊡ K.S. fodder.

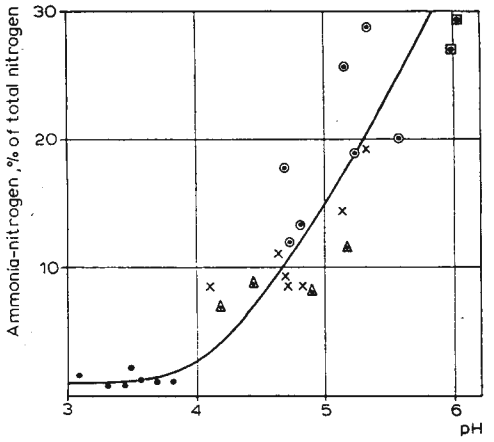


Fig. 7. Formation of ammonia in fodder preserved in various ways. (Six parallel tests.)
• AIV fodder.
x Amasil fodder (normal quantity of formic acid).
△ Amasil-fodder (double quantity of formic acid).
⊙ Ordinary pressed fodder.
□ K.S. fodder.

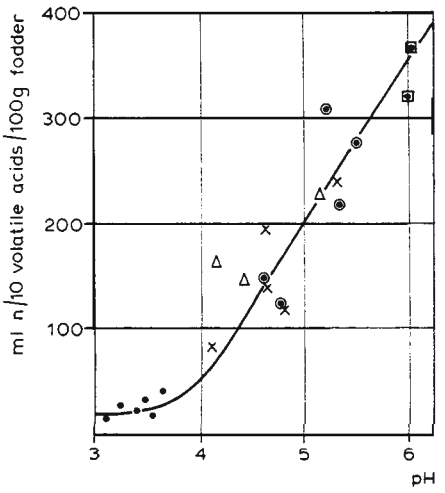


Fig. 8. Formation of volatile fatty acids in fodder preserved in various ways.
• AIV fodder.
× Amasil fodder (normal quantity of formic acid).
△ Amasil-2-fodder (double quantity of formic acid).
⊙ Ordinary pressed fodder.
□ K.S. fodder.

acid groups before and after preservation has shown that this is the case.

By the laboratory tests we also showed in various ways that from the preservation aspect the lactic acid fermentation in fodder acidified to pH 3-4 no longer has any appreciable significance. Although a slow lactic acid fermentation still takes place in such fodder - and there is no reason to prevent this fermentation since the nutritive value of lactic acid is practically the same as that of sugar - the quantity of lactic acid formed has no appreciable influence on the fodder acidity at such a low pH. If, for example, 1% of lactic acid is added to fodder acidified to pH 3.55 by the addition of lactic acid, the pH of the fodder drops only to 3.40. This change of the fodder acidity is insignificant from the preservation aspect. Since certain researchers still assumed at the end of the 1930's that the addition of mineral acid was important only because it ensured lactic acid fermentation, this hypothesis should be regarded as incorrect.

The new method was investigated on a large scale in 1928-1929. A particularly important question now was whether in practical fodder preservation it would be possible at all to mix the acid solution sufficiently uniformly throughout the fodder. Results in this respect were good. In these experiments the greatest attention was paid to the physiological effects of the fodder apart from certain technical questions. To begin with, we observed the cows' appetite for the artificially acidified fodder. We found that cows eagerly ate fodder prepared from clover-rich grass whose pH was between 3.4 and 3.9, in quantities equal to those that can occur in practice. If the fodder pH was below 3, the cows ate only small quantities thereof and then reluctantly. If hunger drove the cows to eat larger quantities of such fodder, their appearance quickly showed that the fodder was not wholesome (acidosis). Thus a pH of 3 could be regarded as the physiological top limit for acidity in the new process. This limit is not sharply defined but depends on the quantities of fodder eaten and the acid requirements of the preserved green fodder.

Since any harmful effects will be shown most clearly if the test animals are given large daily portions of the fodder in question, a number of cows were given 40-60 kg daily of the fodder acidified to a pH of 3.5-3.7. A test cow was slaughtered after one month's feeding and the internal organs were examined. Chemical analyses were carried out on the teeth and various tissues and also on various parts of the skeleton. The blood Ca, P, and Cl content were determined. No abnormalities were found. Similar results were obtained with two cows respectively given 4,500 and 5,600 kg of

acidified fodder during 5 and 6 months respectively, together with a daily amount of 40 g of chalk, and were slaughtered after the test period. No physiological disturbances whatever were found in many of the test cows which for months were fed with a daily portion of 23-46 kg of acidified fodder. Only the urine reaction changed during feeding from basic to acid, the CO_2 content being reduced and the NH_3 content increased. These changes can be considerably counteracted by the addition of a mixture of soda and chalk to the feeds. In the spring of 1929, when the method was put into practice, I therefore suggested the use of such an alkaline soda-chalk mixture in the case of a considerable diet of acidified fodder. In the case of a moderate diet of the fodder together with hay I did not consider it necessary to use soda. In this respect nothing has changed since then. During the past 16 years experience has dispelled any doubts as to whether fodder acidified to a pH of 3-4 really would be wholesome in practice.

To determine preservation losses in ensiling, we developed a method which makes it possible to determine these losses exactly. The method is based on the performance of tests in air-tight containers and on a quantitative observation and analysis of the gases formed and of the expressed juice running out. Since substance losses cannot occur in any other way, the method is completely reliable. *The substance losses* in fodder acidified to pH 3-4 proved to be only a few percent if the dry substance content of the raw material is so high that no pressed juice runs out of the fodder. The nutritive substance losses then do not exceed 5%. When the quantity of expressed juice rises, the nutritive substance losses also increase. If fodder having a dry substance content of 18-20% is preserved, the nutritive substance losses will not exceed 10%, as against 30-40% in ordinary silage.

The preservation method described has been used in Finland since 1929 under the name of the AIV method. In the 1930's the method was also applied in many other countries, and gradually, after much violent opposition and doubt, the theoretical principles of the method were approved more and more. It should be mentioned here that the first to confirm our experience concerning the hydrogen ion concentration significance to the preservation of fodder by their own research were Sjöberg and Köhler here in Sweden.

Before I leave the fodder preservation problem I should like to draw your attention to a central problem concerning the utilization of the AIV method and protein production in leguminous plant crops. Under the climatic conditions prevailing in our latitudes in Europe, clover-rich pastures give the richest leguminous plant crops per hectare. Farther south, blue lucerne on

suitable ground is more advantageous. Since an effective cultivation of pastures with a predominant legume stock gives the cheapest crops and the richest in protein with the minimum of work, and is also irreplaceable in the battle against weeds and in increasing the soil fertility, the endeavour should be to base milk production on this cultivation to a greater extent than previously. In this connection the AIV method offers quite new possibilities. By means of cutting time tests extending over many years in Southern Finland we have found that the best result is obtained from first- and second-year clover-rich fields - and this applies to fodder units, protein and production value of the fodder - if the fields are harvested three times per summer, the first time before the clover buds, and if all the crops are preserved by the AIV method unless the second crop is fed direct to the cattle during dry summers owing to lack of pasturage. If this procedure is adopted, one's own resources give a fodder which satisfies requirements for a fairly high milk production. This process also promotes N-fixation in comparison with the hitherto conventional practice of harvesting hay from the clover-rich fields only when it is in full bloom. As far as the labour aspect is concerned, the new system is even more profitable than the hitherto conventional.

According to one of our tests, the same clover field harvested at different times gave the following results, taking into account the losses in various methods of preservation.

Table 3. Clover-rich field, yield per hectare. First-year field 1936.

	Test Series II (cut 15/6, 24/7, 6/9)		*Test Series II (cut 1/7, 28/8)*	
	As green material	*Preserved as AIV fodder (lime)*	*As green material*	*Dried to hay (lime)*
Fodder (units/ha)	5,367	4,830	4,641	3,249
Nitrogen (kg/ha)	222	—	157	—
Digestible raw protein (g/ha)	1,055	1,002	706	529
Dry substance per fodder unit (kg)	1.27	1.3	1.37	1.7
Digestible raw protein per fodder unit (g)	197	207	152	160

On many farms in Finland 2-3 clover crops have already successfully been taken per summer for many years from clover-rich fields in connection with the AIV method. On my own test farm which I purchased in 1933 and

where this system has been applied in an extreme form since then, agriculture has been carried on without the purchase of nitrogen either in the form of nitrogenous fertilizers or protein-rich concentrates. During the past years, therefore, the entire nitrogen requirements on the farm have been covered by the use of atmospheric nitrogen. The milk production during this time has been fairly high, both per cow (about 3,500-4,000 kg of 4% milk per annum) and per hectare (over 2,000 kg of 4% milk per annum). By this practical test on a large scale, therefore, it has been proved that the use of atmospheric nitrogen in agriculture by effective legume cultivation combined with the AIV method *can* be taken to lengths such that intensive cattle farming is possible. It is in no way my intention on the basis of my experiments to try to discredit the use of nitrogenous fertilizers for non-legumes, but simply to show to what extent it is possible to dispense with the use of nitrogenous fertilizers in agriculture in cases where livestock production is the predominant feature.

The influence of the AIV method on human and animal nutrition

From the vitamin aspect, milk and milk products should be regarded as the most important foods of all. In the Scandinavian countries the use of these foods has been very great and the generally used nutrition in these countries in its best form has also been good qualitatively according to our present knowledge. A very general lack, however, has been associated with this nutrition in winter and particularly in the spring, namely a lack of vitamin A.

This is due to the fact that the vitamin A effect of milk depends entirely on the carotene content of the fodder and during the stable feeding period this content is much lower than during the summer. In connection with the work of the National Nutrition Committee of Finland we made an examination in 1936 to 1937 of the diet of the relatively poor sector of the population and fixed our attention also on the vitamin A status. We arrived at the results shown in Table 4.

Therefore, 52% of the families examined undoubtedly received insufficient vitamin A. If the milk and butter used had had the same vitamin A effect throughout the year as in the summer, the situation would have been quite different, as shown by the figures in parentheses. Only 10% would then have had too little vitamin A and then only because the quantities of milk and butter were exceptionally small.

Table 4. Vitamin A status expressed in international units in the daily food of families under examination (48 families, 318 persons) in tests carried out in spring 1936.

	Persons over 10 years at least 3000 I.U. and persons under 10 years at least 1500 I.U.	*Persons over 10 years 2000 to 3000 I.U. and persons under 10 years 1000 to 1500 I.U.*	*Persons over 10 years under 2000 I.U. and persons under 10 years under 1000 I.U.*
Percentage of persons examined	25 (73)	23 (17)	52 (10)

The figures in parentheses apply to cases where the food remains unchanged in respect of its composition but the milk and butter used had had the same vitamin A effect as in the summer.

This result illustrates the enormous significance in human nutrition of the changes in the vitamin content of milk during various seasons. This disadvantage is all the more striking since the value of milk and milk products as a vitamin source is if anything even greater during the winter and spring than during the summer. According to investigations made in our laboratory, only a small part of the carotene in carrots is resorbed in the intestinal canal, so that the importance of milk and milk products as a source of vitamin A has become all the clearer. Van Eekelen found practically no resorption of the carotene of carrots in man.

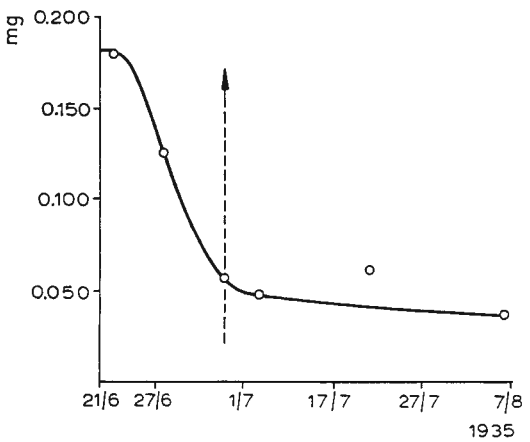


Fig. 9. Carotene content of red clover.
(The vertical broken line shows the beginning of flowering. Carotene expressed per 1 g of dry substance.)

At the beginning of the 1930's we began to investigate the formation and importance of vitamins in plants and the preservation of the vitamins in silage. From the experiments relating to the formation of the vitamins, it was apparent that at least carotene and vitamin C, which were the object of our attention, are bound up with the growth of the plants to such a degree that I considered there to be reason for assuming that the said vitamins should be regarded as plant hormones. All the factors which promoted the plant growth, such as the application of fertilizers and optimum acidity of the soil, also increased the plant carotene and vitamin C content. The observation that the vitamin content of grass plants and legumes dropped rapidly when the plant approached the flowering stage (Fig. 9), became particularly significant. This observation showed that the quantity of vitamin A received by livestock in their feed varies enormously depending upon the stage of development of the fodder plants.

The experiments whereby we examined the effect of crystalline vitamin C on plant growth using the sterile cultivation system clarified the importance of vitamins to plants. The test results given by Synnöve v. Hausen in her doctorate thesis positively proved the absolute necessity of vitamin C as a growth factor for plants. When she removed the cotyledons from peas at a suitable growth stage - the cotyledons contain practically all the vitamin C formed during germination of the seed - the pea could no longer grow. By the addition of vitamin C to the culture solution of such plants the pea could be made to grow again. This important observation in conjunction with our previous research concerning the ability of plants to assimilate organic compounds of the soil shows that carbon compounds present in the soil may also have great significance for plant growth.

Our research concerning the clarification of the preservation of the vitamins in silage showed that the AIV method is absolutely ideal in preservation of the vitamins. Carotene is preserved practically quantitatively, and the vitamin C preservation - in comparison with other methods of preservation - is excellent, and the same also appears to apply to other vitamins. When hay is dried and stored in barns, on the other hand, the carotene deteriorates to a great extent. During spring the carotene content drops, according to circumstances, to 10 to 25% of the original value. The vitamin C disappears practically quantitatively during drying. These changes are important to the animals' health and the utilization of the feed, at least insofar as concerns carotene. In tests carried out with various livestock the AIV fodder proved extremely important if the other feeds were deficient in carotene.

This research fully explained the enormous drop in the vitamin A effect of milk during the stable feeding period. As an immediate result of the low carotene content of hay, during the stable feeding period the cow receives in its feed only a fraction of the quantity of carotene than during pasturage. The low carotene content of hay is due partially to the fact that grass crops when harvested as hay are not cut until the beginning of flowering or in full flower, and partly because of oxidation of the carotene during drying and storage of the hay.

If the AIV method is used, the carotene is retained practically completely. Since the method also includes cutting the grass at an early stage of growth, the AIV fodder corresponds largely to the summer feed of the cattle. If a cow receives daily about 30 kg of AIV fodder prepared from young clover-rich grass during the stable feeding period, the vitamin A effect of the milk is practically the same as during summer pasturage. Table 5 shows the research result in 1937 to illustrate the vitamin A effect of milk of various breeds of cow under different feeds.

A considerable increase in the vitamin A effect of milk as a result of AIV feeding has been found by many other researchers, such as Peterson, Brouwer, etc. both in the U.S.A. and Europe. The value of the vitamin A effect of milk produced during stable feeding as compared with pasturage milk depends partly on the quantity of AIV fodder given and partly on the raw material from which the fodder was prepared.

The AIV fodder has a favourable effect not only on the milk vitamin A content but also on the quantity of vitamin B_2. We have found that if cattle are given a plentiful supply of AIV fodder throughout the year, the lactoflavin content is 25-30% higher than with ordinary stable feeding. Since vitamin B_2 is formed in the rumen and the cow therefore can manage with a feed free from vitamin B_2, the influence of the feed on the lactoflavin content of milk is not immediately clear.

Elvehjem, Peterson, and colleagues in the U.S.A. have shown that the milk produced on pasturage or with AIV fodder contains much more so-called "grass sap factor" than ordinary winter milk. The effect of this factor can be demonstrated if rats are fed solely with milk supplemented by Cu, Mn, and Fe salts.

The AIV fodder is rich in vitamin C which again is practically completely lacking in hay. The vitamin C content of milk is not affected by the fodder, but a fodder rich in vitamin C does improve the quality of the milk. If the fodder does not contain sufficient vitamin C, the milk acquires a tallowy

taste which is quite usual in milk resulting from hay and concentrated feeds. This defect never occurs in pasturage or AIV milk. It is not known why the oxidase enzymes increase or why the factors which inhibit oxidation decrease in milk when the fodder is deficient in vitamin C. According to the latest American research, the substances which cause the tallowy taste reduce the utilization of vitamin A and are detrimental to health.

Table 5. Vitamin A effect of butterfat. (Expressed in international units* per 100 g of butterfat.)

		Stable feeding				*Pasturage feeding*	
		Without AIV fodder		*With plentiful AIV fodder*			
Farm number	*Breed of cow*	*Total vitamin A effect (I.U.)*	*Proportion of carotene in vitamin A effect (%)*	*Total vitamin A effect (I.U.)*	*Proportion of carotene in vitamin A effect (%)*	*Total vitamin A effect (I.U.)*	*Proportion of carotene in vitamin A effect (%)*
1–2	Ay	1,833	20	—	—	3,783	21
14–16	Ay	—	—	4,000	22	4,450	26
3–6	LSK	2,083	20	—	—	4,100	24
18–19	LSK	—	—	3,583	28	—	—
7–8	ISK	2,000	20	—	—	4,234	25
17	ISK	—	—	4,050	26	5,099	26
9–13	PSK	2,817	15	—	—	4,249	23

* 1 vitamin A international unit = 0.6 γ carotene = 0.3 γ vitamin A.

The above differences between milk produced with different feeds show the many-sided influence of cattle feed on the biocatalysts and nutritive value of milk.

Research to increase the quality of milk products

The use of milk for human nutrition is also essentially associated with the processing of milk to form various products. It is important that these products are of the highest possible quality both in respect of taste and nutritive physiological properties, and that they keep well. As already mentioned, the

oxidation of fat in milk and milk products also results in deterioration of their nutritive physiological properties.

In this connection I will just briefly deal with one aspect of our research which has been of great practical importance, namely work in connection with the keeping-quality of butter, which clearly shows the decisive influence of the hydrogen-ion concentration on the oxidation phenomena in butter. Butter with the best flavour is prepared from sour milk. During the souring process a pleasant aroma develops, which originates mainly from diacetyl. Although such high-quality butter is prepared with the maximum care, all possible infection being avoided, it cannot be stored for any appreciable length of time. At a temperature above 0° it frequently acquires an oily or fishy taste or even a tallowy taste after a few weeks, and in cold storage after 1-3 months. These defects reduce the value of the stored butter considerably and it is difficult normally to market such butter. In 1925 I was successful in finding a way of preventing these defects. It was in fact found that the oily or fishy taste which is probably due to oxidation products of lecithin, occurs in butter if the pH of the latter drops below 6. The lower the pH of the butter, the more quickly this defect occurs. Heavy metals, more particularly copper, catalyze this reaction strongly, but the butter still contains so much heavy metals that the oily taste occurs sooner or later. At a pH above 6 the oily or fishy taste never occurs, so that defects associated with this

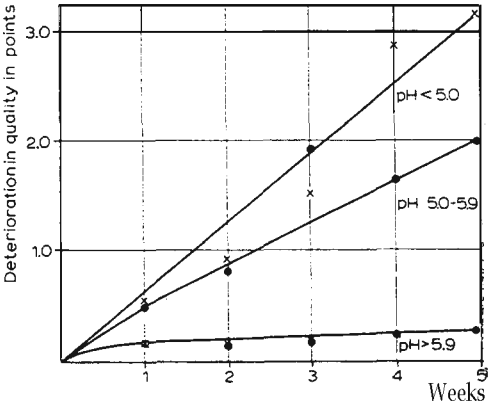


Fig. 10. Deterioration of quality in butter samples whose pH was below 5, between 5.0 and 5.9, and above 5.9. In the latter the pH in the butter was increased by the addition of disodium phosphate. The deterioration of the butter quality is expressed in points counting from the number of points the butter had when fresh. (Storage time is expressed in weeks.)

group can be prevented by adjusting the pH of the butter to above 6 (Fig. 10). For this reason I proposed the use of a salt in the preparation of butter, in which salt was mixed a suitable quantity of basic substances, for example disodium phosphate and anhydrous soda. In this way it was possible to adjust the butter acidity to pH 6-7, which guaranteed its storability. This simple method was of decisive importance in the storage of butter and the quality thereof. The discovery also made it possible to separate the bacteriological butter defects from the chemical and it has therefore resulted in ready controllability of butter quality.

Ladies and gentlemen. I have now come to the end of my lecture. We have followed the fixation of atmospheric nitrogen and the formation of amino acids in the root nodules of legumes, the production of protein-rich legume crops, the preservation of them to form a high-grade animal feed, and the production of physiologically high-grade milk adapted to the improvement of human nutrition. These questions represent widely different problems within biochemical research but are bound up with one another organically, and form a whole from the practical aspect.

I consider myself fortunate in having had the opportunity of working in this interesting field and in having advanced development somewhat. The Nobel Prize will both directly and indirectly increase our research facilities, and the Swedish Academy of Sciences will therefore be promoting biochemical research in my country, and for this I should like to express my deeply felt gratitude.