this spectrograph with those in the standard series,[1] it is seen that the alfalfa chlorophyllan contains 66% neo- and 34% allochlorophyllan. It is of course evident that this only means that the particular lot of alfalfa with which I was working gave these results, and that the same species of *Medicago*, grown under different conditions, would in all probability give different values. Later on I shall try to investigate the influence of the conditions of growth upon the production of one or the other of these chlorophyll constituents.

Chlorophyll in leaves is accompanied to a larger or smaller extent by carotin and xanthophylls. These are known as the yellow coloring matters, although the former is a red crystallin substance.

To determin the amount of yellow coloring matters in the chlorophyll residue from alfalfa, 51.981 grams of the latter were treated with about 2.5 grams potassium hydroxide and some alcohol. After stirring for a while the solution was evaporated on a water bath to a thick sirup, after which about 150 cc. water was added, and the whole transferred to a separatory funnel and extracted with ether. The extractions were continued until the ether separated colorless. The ether portions were added together and the solvent evaporated, leaving a residue weighing 0.8638 gram.

Besides carotin and xanthophylls, there may have been other substances removed by the ether, but the color of the latter and of the residue indicated that no chlorophyll product had been removed. The amount of the yellow coloring matters present in the chlorophyll residues from alfalfa is therefore 1.66%, or 0.28% of the weight of the dry alfalfa hay.

To summarize the foregoing it may be said that the chlorophyll from alfalfa closely resembles that from nettle leaves. The lot with which I was working contained 66% neochlorophyll and 34% allochlorophyll. However, this ratio may vary in other lots, depending upon the conditions of growth. In air-dried alfalfa hay there is 0.68% of chlorophyll and 0.28% yellow coloring matters. The latter do not influence the chlorophyll absorption bands in the concentration most suitable for photographing, but when the concentration is five times as strong, they show two regions of absorption in the ultraviolet.

[CONTRIBUTION FROM THE NEVADA AGRICULTURAL EXPERIMENT STATION.]

A DELICATE METHOD FOR DETERMINING MINUTE QUANTITIES OF CHLOROPHYLL.[2]

BY C. A. JACOBSON.

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After finishing the work on the two methods for determining the ratio

[1] *Am. Chem. J.*, **47**, Aug. (1912).

[2] The experimental work involved in the present paper was also carried out in Prof. Marchlewski's laboratory at Cracow.

of neo- and allochlorophyll in the presence of one another,[1] Professor Marchlewski suggested a modification of the photographic method so as to make it applicable for determining very small quantities of chlorophyll in a given sample It would of course be very desirable to be able to determin the quantity of chlorophyll in one leaf whose green weight is less than a gram. This has now been accomplished.

The method is simply a comparison of the intensity of the chlorophyllan absorption bands of the solution to be examined with the intensity of the corresponding bands produced by solutions of known concentrations. The absorption bands of known but varying concentrations of chlorophyllan were photographed in the ultraviolet with the copper spectrum added above for reference. The chlorophyllan solutions were prepared from equal quantities of neo- and allochlorophyllan in order that all the bands should come out to the full extent. Chloroform was used as the solvent. The thickness of the layers, counting from the copper lines, was 2, 4, 6, 8, 10 and 12 mm. Plate I represents a solution of chlorophyllan containing 0.00004 gram per cc., Plate II, a concentration of 0.00002 gram per cc., Plate III, a concentration of 0.000013 gram per cc., and Plate IV, a concentration of 0.00001 gram per cc. A fifth photograph was taken whose chlorophyllan solution had a concentration of 0.000005 gram per cc., and whose bands were visible on both the negative and print, but since they would probably be lost in the reproduction this photograph has been omitted.

Twenty cc. of solution was required in the absorption vessel I used, and therefore it is seen that the actual amount of chlorophyll that can be determined by this method need be no more than 0.1 mg. An absorption vessel could easily be constructed, however, where only 4 or 5 cc. of solution would be necessary, and thus reduce the amount of chlorophyll necessary for a determination to 0.02 mg.

When a determination of the chlorophyll in a green leaf is made in this way, the question naturally arises if the yellow coloring matters will influence the chlorophyllan bands. This was answered in the negative by Jacobson and Marchlewski.[1] In this connection it was interesting however, to learn what sort of ultraviolet absorption the yellow coloring matters actually produce when the concentration is sufficiently strong and freed from all chlorophyll. This question is answered by Plate V, which represents a photograph of the ultraviolet absorption of a chloroform solution of the yellow coloring matters prepared according to the method given in the paper to which reference has just been made. The concentration was 0.0002 gram per cc., or five times as strong as the strongest chlorophyllan solutions photographed. Two regions of absorption are seen, which extend in the 6 mm. layer, from $\gamma = 398$ to the

[1] *Am. Chem. J.*, **47**, Aug. (1912).

less refrangible end of the plate, and from $\gamma = 350$ to the more refrangible end. Two ill-defined bands are seen in the 2 and 4 mm. layers, the more pronounced being approximately located between $\gamma = 452$ and $\gamma = 460$ in the 2 mm. layer. The other is a short distance from this in the direction of the less refrangible end.

The two following examples are given to illustrate the method: The first of these is a photograph, Plate VI, originally intended for showing the relative amount of neo- and allochlorophyll in alfalfa, as outlined in my previous paper published in this issue, but since the bands appeared so weak, a photograph of the pure alfalfa chlorophyllan, in the right concentration, was substituted. The solution whose spectrograph is represented by Plate VI was prepared by dissolving 0.1279 gram of the alfalfa "chlorophyll residue' described in the paper just mentioned, in 319.7 cc. chloroform, which makes the concentration 0.0004 gram per cc. This solution was photographed without diluting further, since its color indicated less chlorophyllan than the photographs of the standard series.[1] A comparison of this plate with the foregoing reveals the fact that the intensity of its absorption bands is very nearly a mean between the intensities of the bands on Plates II and III. Consequently, the chlorophyllan concentration was approximately 0.000017 gram per cc. This solution, however, contained 0.0004 gram solid matter per cc., and therefore the chlorophyllan present was only a little over 4%, which is in perfect accord with the results of the chlorophyllan found in the "chlorophyll residues" by the extinction method.

The second example is an experiment which was carried out primarily for illustrating the method. A small leaf of *Aspidistra elatior* was cut into two equal parts. One of these, weighing 1.9355 grams in the green state, was macerated in a mortar with cold 92% alcohol, the extract poured through a filter and the residue in the mortar again ground with alcohol until all the green color was removed. The filtered alcoholic solution, thus obtained, was treated with 2 cc. of a 10% alcoholic oxalic acid solution and allowed to stand 2 or 3 hours. The solution was then treated with calcium carbonate in excess of the oxalic acid, and evaporated to dryness on the water bath. The residue was extracted with chloroform until the green color was removed, and the chloroform solution filtered. This solution, measuring 65 cc., contained the chlorophyllan of all the chlorophyll in the half leaf that was taken for the experiment. The solution was apparently too strong for photographing, so that it was diluted with an equal volume of chloroform. The photograph of the solution, thus obtained, is represented on Plate VII.

It is seen that the intensity of the bands is still a little greater than that shown by the bands on Plate I, where the solution contained 0.00004

[1] *Am. Chem. J.*, 47, Aug. (1912).

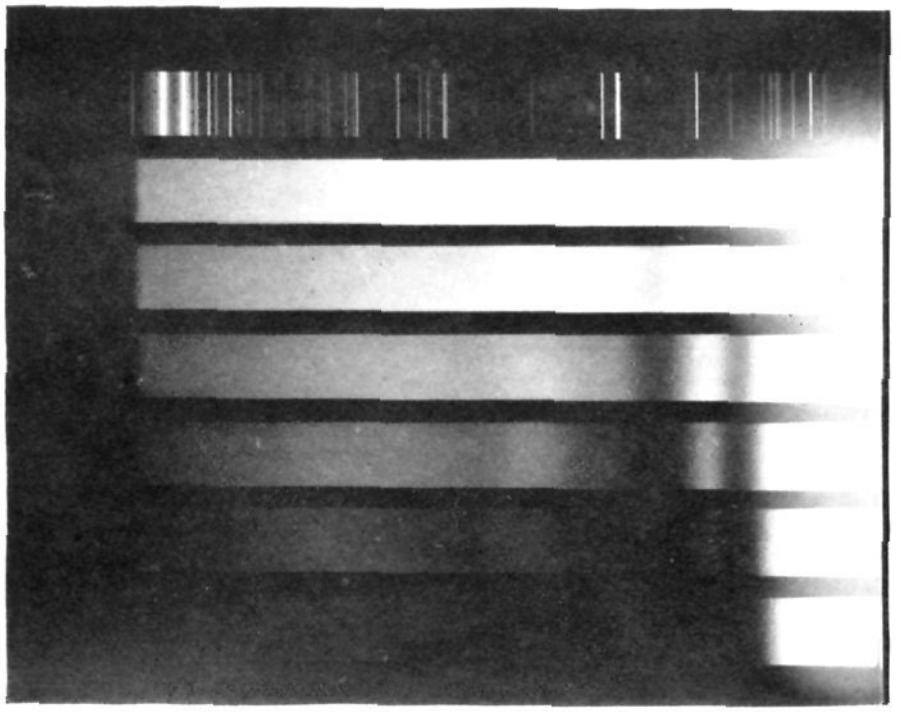

Plate I.

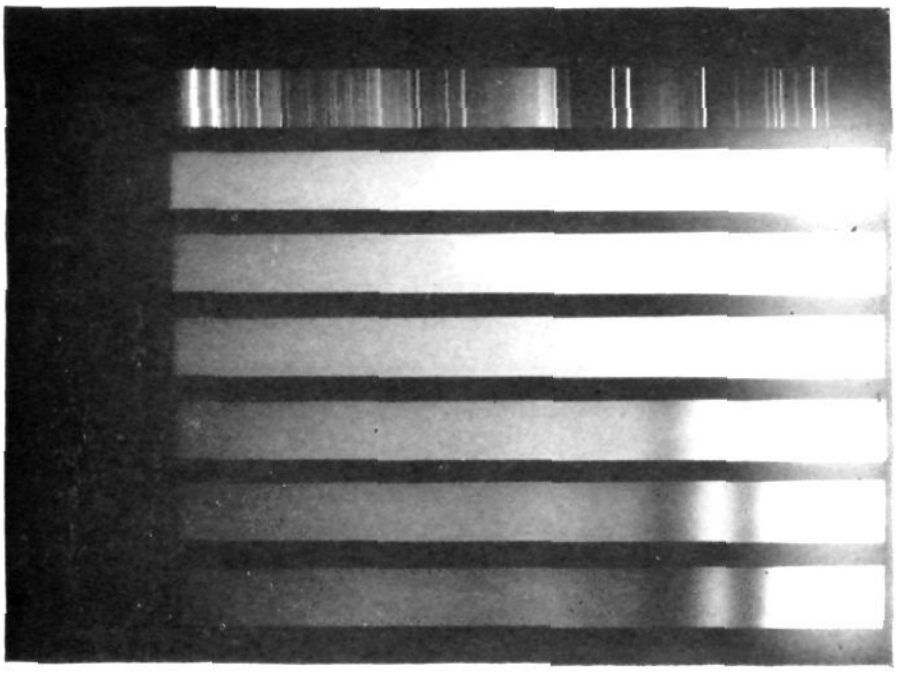

Plate II.

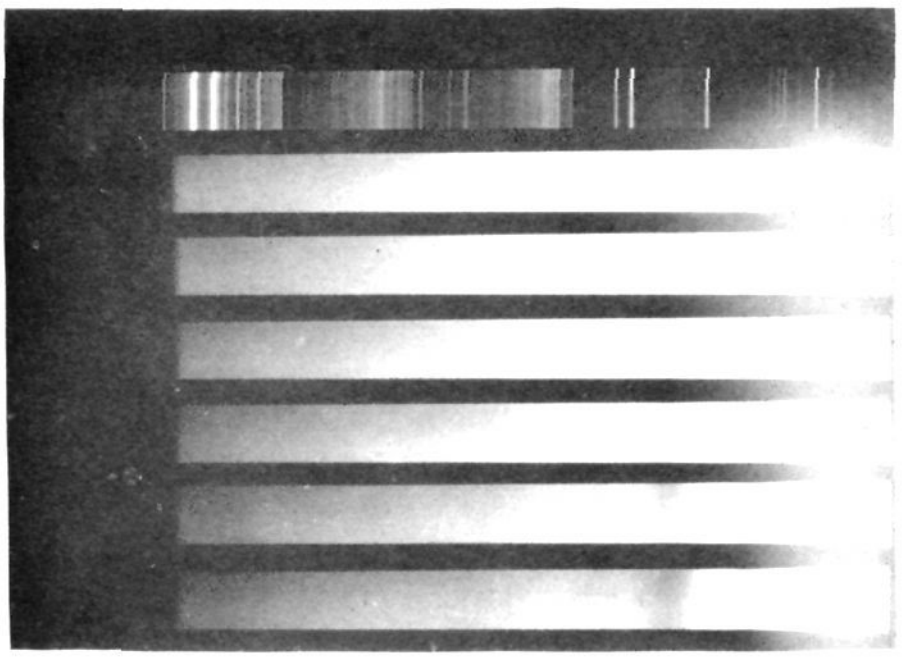

Plate III.

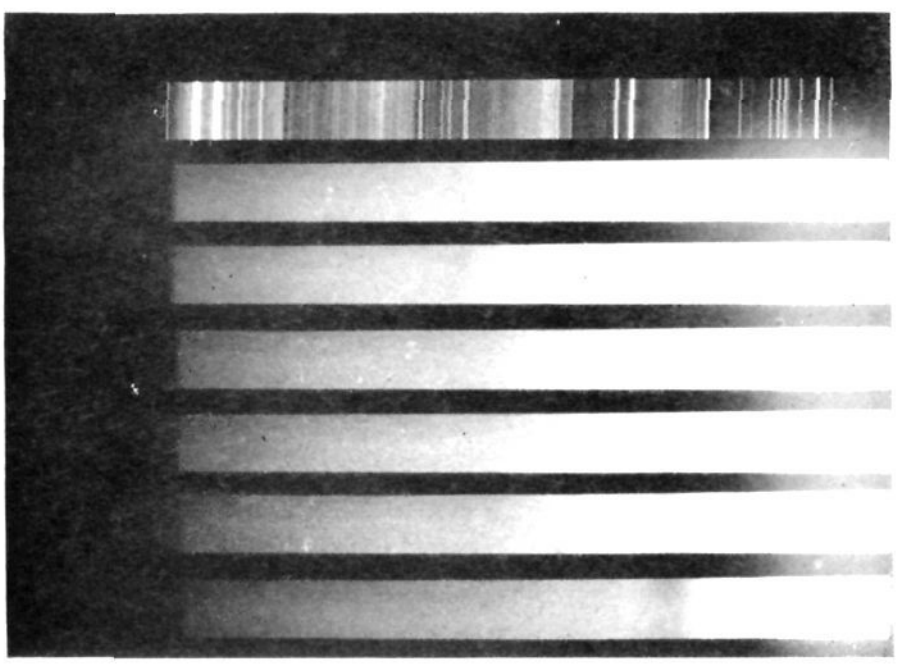

Plate IV.

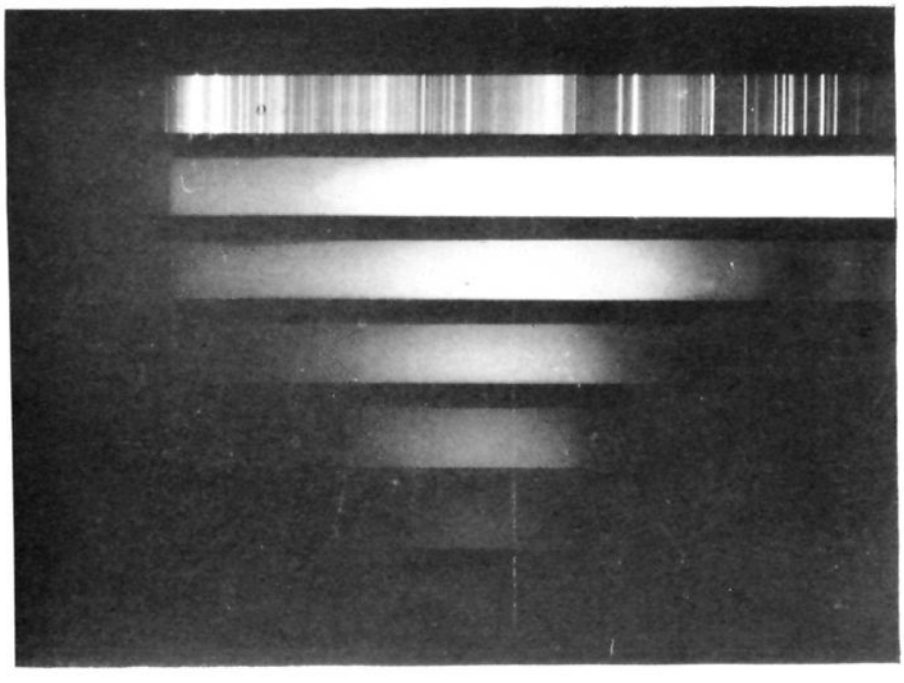

Plate V.

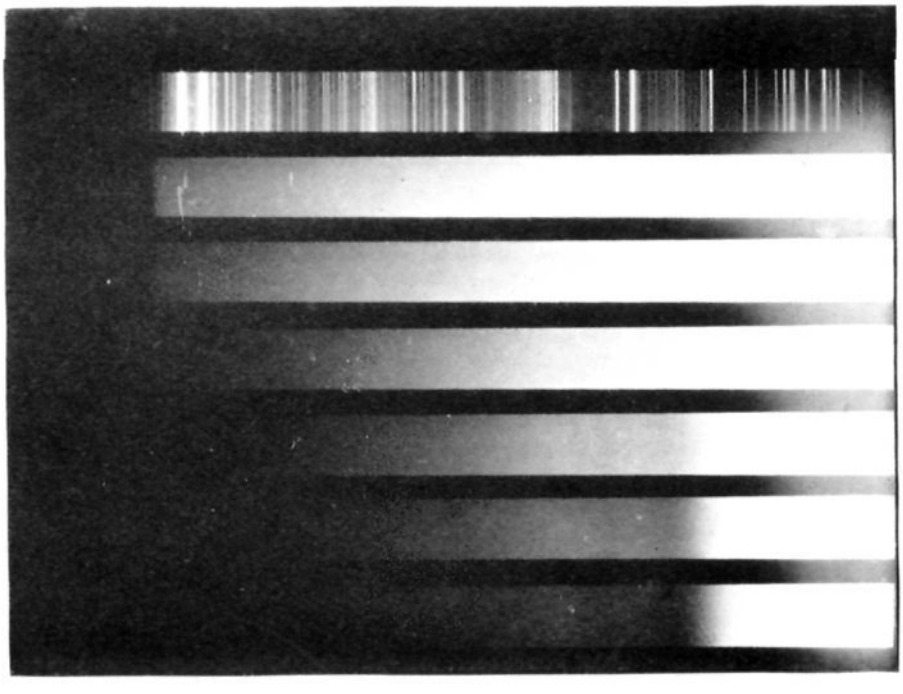

Plate VI.

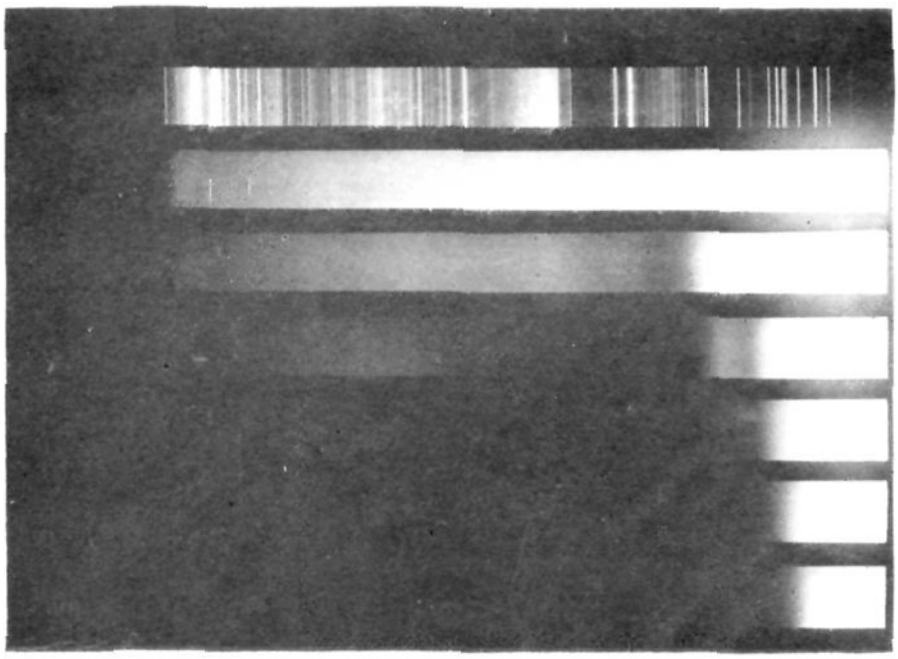

Plate VII.

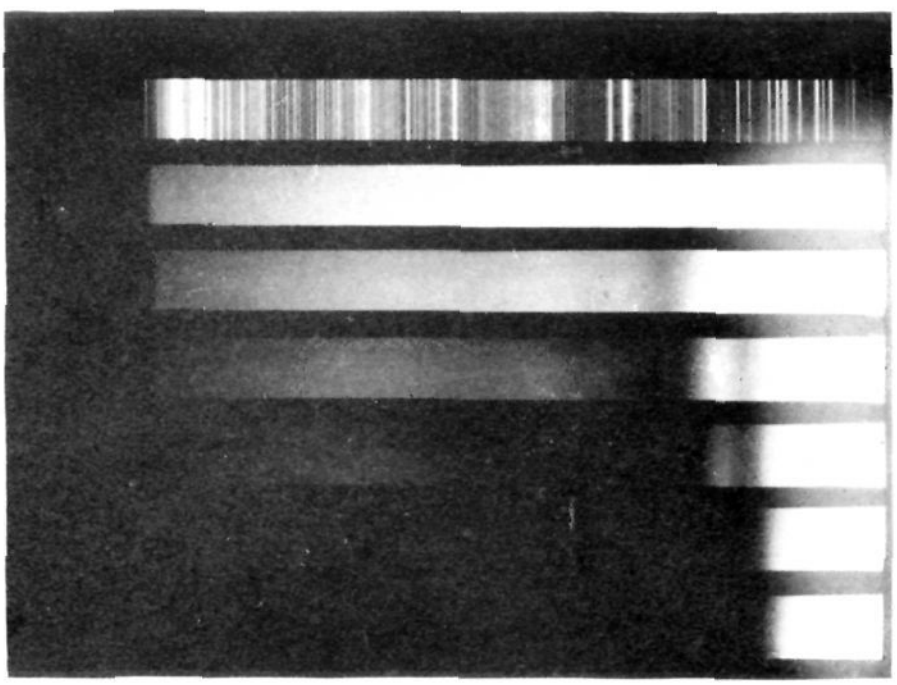

Plate VIII.—Spectrograph of alfalfa chlorophyllan.

gram per cc. A study of the gradation of intensities represented on the different plates allows us to place the concentration of the solution in question at very nearly 0.000045 gram per cc. Furthermore, on comparing Plate VII with the standard series, already referred to, it will be seen that the solution contained approximately 64% neo- and 36% allochlorophyllan.

The chloroform solution obtained from the half leaf, used for this experiment, measured 130 cc., which is seven times larger than necessary for making the determination. In other words, a leaf whose green weight is only 0.2 gram suffices for determining the quantity and character of the chlorophyll it contains.

NOTE.

Note on the Transformation of Ammonium Cyanate into Urea.—Chattaway[1] says, "The course of the reaction which takes place when ammonium cyanate is transformed into carbamide has never been satisfactorily explained. Up to a few years ago it was universally regarded as a peculiar case of isomeric change and no consideration was given to the process by which the conversion was effected." He then states that various specified reactions of carbamide, cyanic acid, isocyanic acid and their esters may be simply explained "by regarding them as instances of the well known tendency of the carbonyl group to add groups such as R_2NH and ROH, followed by a subsequent atomic rearrangement involving only the transference of a hydrogen atom from an oxygen atom to a nitrogen atom connected with it through the doubly linked carbon atom, thus:

$$N{:}C{:}O \longrightarrow \cdot N{:}C\begin{matrix}\diagup OH\\ \diagdown N{:}\end{matrix} \longrightarrow \cdot NH{\cdot}CO{\cdot}N{:}$$

The conversion of ammonium cyanate into carbamide should therefore be formulated as follows:"

$$NH_4{\cdot}N{:}CO \rightleftarrows H{\cdot}N{:}C{:}O + NH_3 \rightleftarrows H{\cdot}N{:}C\begin{matrix}\diagup OH\\ \diagdown NH_2\end{matrix} \rightleftarrows H_2N{\cdot}CO{\cdot}NH_2$$

The three stages, then, in the transformation are (1) the breaking up of ammonium cyanate into cyanic acid and ammonia, (2) the formation of an addition compound, and (3) a rearrangement of this compound.

A simpler explanation eliminates this addition compound and its rearrangement. I find in my note book on the lectures in Organic Chemistry by Professor H. B. Hill at Harvard University in 1896, this statement: Ammonium cyanate breaks up with heat into HNCO and NH_3 and then the NH_3 adds itself as follows:

$$\begin{matrix}H.N:C:O\\ \uparrow\ \ \uparrow\\ H\ \ NH_2\end{matrix} = H_2N{\cdot}CO{\cdot}NH_2.$$

[1] Chattaway, *J. Chem. Soc.*, **101**, 170 (1912).