kept in a desiccator until used. All the determinations were carried out in an atmosphere of dry nitrogen. The apparatus used was similar to that described by Zerewitinoff, and the magnesium methyl iodide was prepared in accordance with his directions.

No attempt was made to explain the results given in the above table, which differ so strikingly from those obtained by Zerewitinoff. It is certain, however, that the pyridine used was of a higher grade of purity than the technical pyridine which he used, and it was at least as dry. The fact that Oddo[1] obtained satisfactory results by its use may be due to the fact that he employed magnesium ethyl iodide.

In conclusion, attempts to utilize pyridine as a solvent in accordance with Zerewitinoff's directions have failed completely from a quantitative point of view, not only with the technical product but with the repurified Kahlbaum material. Even with added precautions, pyridine has not been found to be a suitable solvent for use in this method; and it would therefore seems advisable that this method be subjected to further investigation before results obtained by its use are accepted.

The author wishes to acknowledge his indebtedness to H. Hibbert, at whose suggestion this work was undertaken.

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THE ORIGIN OF PURINES IN PLANTS.[2]

BY TREAT B. JOHNSON.
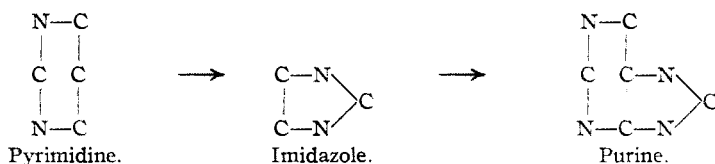
In the plant kingdom purines occur as normal constituents of the plant cell, linked to sugar in the nucleic acids, and also in a free condition as stable end products of the metabolism of nitrogen. They are found in certain plants associated with protein in all the organs, apparently, in which protein is synthesized or utilized in plant growth. Regarding the mechanism of the processes of formation, or the chemical nature of the simpler organic compounds, which participate in their syntheses, we have, however, practically no knowledge. The primary object of this paper is to present some interesting data, which are very suggestive of the mechanism of purine formation, and which have led us to undertake some new investigations in this fruitful field.

Every process, which has been applied successfully in the laboratory, for the synthesis of purine compounds, is based upon the general principle that the imidazole ring can be introduced into the pyrimidine nucleus.

[1] *Ber.*, **44**, 2040 (1911).
[2] Pyrimidine Paper LXV.

N—C $\quad\quad\quad$ C—N $\quad\quad\quad$ N—C
 | | $\longrightarrow$ | $>$C $\longrightarrow$ | |
C   C $\quad\quad\quad$ C—N $\quad\quad\quad$ C   C—N
 | | $\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad$ | $>$C
N—C $\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad$ N—C—N
Pyrimidine. $\quad\quad\quad$ Imidazole. $\quad\quad\quad$ Purine.

This has been effected in different well known ways and it is not necessary to discuss these methods here. The only exception to this general procedure is apparently the observation of Horbaczewski[1] that uric acid is formed in small amount by fusion of urea with aminoacetic acid or the amide of trichlorolactic acid. Gautier,[2] in 1884, claimed to have obtained the purines, xanthine and methylxanthine, by heating hydrocyanic acid with acetic acid and water and expressed the transformation according to the following equation:

$$11HCN + 4H_2O = C_5H_4O_2N_4 + C_6H_6O_2N_4.$$
$$\text{Xanthine.}\quad\text{Methylxanthine.}$$
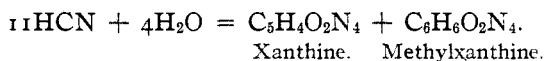
Gautier's products showed a long series of precipitation and color reactions also given by xanthine. This work of Gautier's was repeated, thirteen years later, by Emil Fischer,[3] who showed that the products obtained by Gautier did not give the murexide test and consequently he concluded that they could not be purine compounds. During the following year Gautier[4] replied to Fischer's paper and again discussed the several reactions of his products, which were characteristic of xanthine. He contributed, however, no new experimental evidence to support his conclusions, and, so far as the writer is aware, no further work of Gautier or other investigators, confirming these observations, has since been published.

The reverse process of purine formation, namely, that involving their synthesis from imidazoles or hydantoins by incorporation of the pyrimidine urea nucleus has, so far as the writer is aware, never been accomplished. Attempts to effect such transformations, which have been made in this laboratory,[5] have so far proved unsuccessful. It is of interest to note in this connection that Latham's theory of the formation of uric acid in the animal body is based on the ground, that this purine results by synthesis through the stages of hydantoin and biuret. Uric acid, however, has never been detected in plants, and it has not been definitely established that hydantoin is a naturally occurring compound. The only evidence that hydantoin may occur in plants, which the writer has been able to find, is that given in a paper by Lippmann[6] who writes as follows: "doch

---

[1] Ber., 15, 2678; Monatsh., 6, 356; 8, 202.

[2] Compt. rend., 98, 1523.

[3] Ber., 30, 3131.

[4] Ibid., 31, 449.

[5] Unpublished work.

[6] Ber., 29, 2652.

sei bei dieser Gelegenheit erwähnt, dass Hydantoin einmal aus dem Safte der bleichen Schösslinge erhalten wurde, die bei feuchtwarmem Wetter in oft erstaunlicher Länge durch das sogenannte 'Auswachsen' der Rüben in den Mieten entstehen und reich an stickstoffhaltigen Verbindungen vershiedener Natur sind.[1] Es bildete kleine weisse Krystalle, deren Schmelzpunkt, der wegen vorherigen Sinterns schwer genau festzustellen ist, bei 214° lag, löste sich leicht in heissem Wasser, besass die Zusammensetzung $C_3H_4O_2N_2$ (gef. 35.85 p. ct. C, 4.17 p. ct. H, 27.88 p. ct. N, 32.10 p. ct. O; ber. 36.00 p. ct. C, 4.00 p. ct. H, 28.00 p. ct. N, 32.00 p. ct. O) und gab mit ammoniakalischem Silbernitrat eine mikrokrystallinische Silberverbindung $C_3H_3O_2N_2Ag + H_2O$." Whether this interesting compound results from the decomposition of allantoin, which has been shown to occur in plants, or is formed by direct synthesis from glycocoll, or is produced by the oxidation of purine compounds, we have no knowledge.
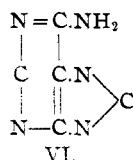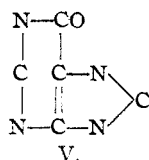
The important fact that purine can be so easily synthesized from pyrimidine compounds is a very strong argument, from a chemical standpoint, that representatives of the latter class of compounds are probably the precursors of purines in plant growth. Several investigators have called attention to this genetic relationship and have suggested that cytosine, I, and uracil, II, may be the two pyrimidines involved in these changes. The close structural relationship between these two pyrimidines and the purines, adenine, III, and xanthine, IV, respectively, for example,
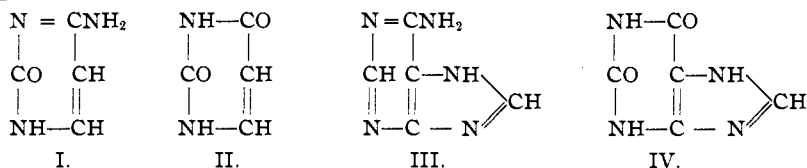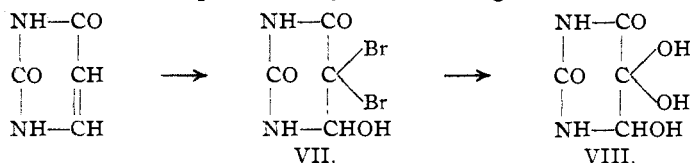

is surely very suggestive, but notwithstanding this fact, it seems very safe to assume that this relationship will never be of more than theoretical interest, until new methods of converting these two pyrimidines into purines have been developed. Admitting that it is possible to convert pyrimidines of this type into purines,[2] yet, no method has been developed by which purines can be obtained from uracil and cytosine, which still retain the original CO or $C-NH_2$ pyrimidine groupings in the 6-position of the purine nucleus. These two groupings are characteristic of all the naturally occurring purines. The 3-carbon chain is likewise a characteristic grouping in all naturally occurring purines, and also pyrimidines with the exception of thymine, which contains a methyl group in the

[1] Z. Rübenz. Ind., 35, 159.

[2] Johns, Am. Chem. Jour., 41, 59; 45, 79; J. Biol. Chem., 9, 161; 11, 67, 73, 393; 12, 91; 14, 1, 299; 15, 119, 515; 16, 135.

$$
\begin{array}{ccc}
N\!-\!CO & & N\!=\!C.NH_2 \\
| \quad | & & | \quad | \\
C \quad C\!-\!N & & C \quad C.N \\
| \quad \| \quad \rangle C & & | \quad \| \quad \rangle C \\
N\!-\!C\!-\!N & & N\!-\!C.N \\
V. & & VI.
\end{array}
$$

5-position of the pyrimidine ring. This pyrimidine, therefore, occupies an anomalous position. It is possible that plant enzymes may be found, which are capable of oxidizing uracil and cytosine to isodialuric and aminoisodialuric acids, respectively, but no such transformation has yet been effected in the laboratory by direct oxidation. Wheeler and Johnson[1] have shown that both uracil and cytosine can be converted into isodialuric acid, VIII, but the change involves, first, the formation of the dibromopyrimidine VII, by the action of hypobromous acid and secondly a treatment with alkali to remove the two halogen atoms. The transformation of uracil is represented by the following formulas:

$$
\begin{array}{ccccc}
NH\!-\!CO & & NH\!-\!CO & & NH\!-\!CO \\
| \quad | & & | \quad | \,/Br & & | \quad | \,/OH \\
CO \quad CH & \longrightarrow & CO \quad C\langle & \longrightarrow & CO \quad C\langle \\
| \quad \| & & | \quad | \,\backslash Br & & | \quad | \,\backslash OH \\
NH\!-\!CH & & NH\!-\!CHOH & & NH\!-\!CHOH \\
& & VII. & & VIII.
\end{array}
$$

It is important to note, furthermore, that the pyrimidines—uracil and cytosine have never been found in a free condition in plants, but always in combination linked as neucleosides.

If now uracil and cytosine are not intermediate stages in the natural syntheses of purines in plants, then what is the mechanism of their formation. In other words, what further evidence have we that pyrimidines may be the precursors of these substances?
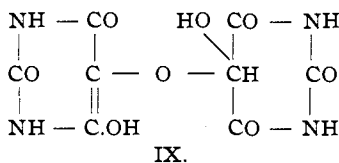
It seems to the writer that the interesting results obtained by Ritthausen and coworkers,[2] in his investigations of the extracts of certain plant seeds, are worthy of careful study here, because of the possibility that a correct interpretation of his observations may lead to a definite conception of the mechanism of pyrimidine and purine formation. It is not improbable that we may be able to draw the conclusion from Ritthausen's work, that the changes involved in the natural formation of purines, are analogous to those which take place in the synthetical processes applied in the laboratory.

This investigator isolated from extracts of vetch seeds (*Vicia sativa*) and beans (*Vicia faba* and *Vicia fabaminor*), by precipitation with mercuric chloride, two characteristic nitrogenous substances, *vicine* and *convicine*, which apparently are closely related to the plant purines.

[1] *J. Biol. Chem.*, **3**, 183.

[2] Ritthausen and Kreusler, *J. prakt. Chem.*, **2**, 333; Ritthausen, *Ibid.*, **7**, 334; [2] **24**, 218; *Ber.*, **9**, 301; **29**, 894, 2106; Ritthausen and Preusse, *J. prakt. Chem.*, **59**, 487.

Vicine has also been found in beet juice by Lippmann[1] and likewise in vetch seeds and peas (*Pisum sativum*) by Schulze.[2] The latter writes as follows regarding vicine: "Dieser, an seinen Reactionen sehr leicht erkennbare Stoff, findet sich in den ungekeimten Wicksamen in ziemlich beträchtlicher Menge vor." Ritthausen concluded that vicine and convicine are glucosides and assigned to them the formulas $C_{28}H_{47}O_{19}N_{11}$.-$2H_2O$ and $C_{10}H_{15}O_8N_3.H_2O$, respectively. Both compounds are characterized by their behavior on hydrolysis with sulfuric and hydrochloric acids. Vicine is transformed, by heating with 20% sulfuric acid, into *divicine* having the formula $C_4H_7O_2N_4$ (according to Ritthausen),[3] while convicine, on the other hand, is converted, under practically the same conditions, into alloxantine, IX. Ritthausen established the identity

$$
\begin{array}{ccccc}
\text{NH} - \text{CO} & & \text{HO} & \text{CO} - \text{NH} & \\
| & | & & \diagdown| & | \\
\text{CO} & \text{C} - \text{O} - & & \text{CH} & \text{CO} \\
| & || & & | & | \\
\text{NH} - \text{C.OH} & & & \text{CO} - \text{NH} &
\end{array}
$$
$$\text{IX.}$$

of this substance and the alloxantine formed by oxidation of uric acid and states that convicine yields about 36–37% of its weight in this pyrimidine. So far as the writer is aware, this is the only pyrimidine except the three compounds, uracil, thymine and cytosine, which has been found to occur in nature.

If the formula assigned to convicine by Ritthausen is correct, it is apparent by inspection of the equation below that the change involved in its transformation into alloxantine, by hydrolysis with acids, is not a simple one. The only conclusion that one can draw is that alloxantine
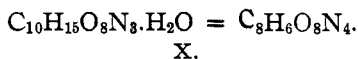
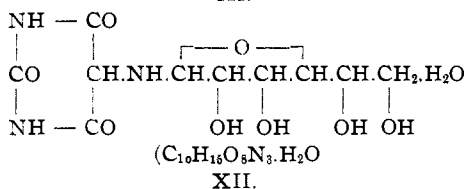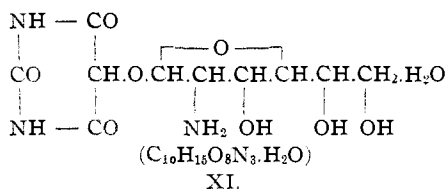$$C_{10}H_{15}O_8N_3.H_2O = C_8H_6O_8N_4.$$
$$\text{X.}$$

is not a primary product of the change but is formed by oxidation of a simpler pyrimidine, which is formed as a product of the hydrolysis. If, on the other hand, the molecule of convicine is double that assigned to it by Ritthausen, *viz.*, $C_{20}H_{30}O_{16}N_6.2H_2O$, then alloxantine might be considered a primary product of decomposition, but two of the nitrogen atoms would still be unaccounted for. Ritthausen apparently obtained no evidence of the nature of the secondary, nitrogenous hydrolytic products.

It is a very interesting fact that Ritthausen's formula for convicine corresponds exactly to that for an aminoglucoside of dialuric acid or a glucoside of uramil and its constitution may be provisionally represented by formulas XI or XII.

[1] *Ber.*, 29, 2653 (1896).
[2] *Z. physiol. Chem.*, 15, 140; 17, 215.
[3] *J. prakt. Chem.*, 59, 482.

NH — CO
| | ┌— O —┐
CO   CH.O.CH.CH.CH.CH.CH.CH₂.H₂O
| | | | | |
NH — CO   NH₂ OH   OH OH
                 ($C_{10}H_{15}O_8N_3.H_2O$)
                     XI.

NH — CO
| | ┌— O —┐
CO   CH.NH.CH.CH.CH.CH.CH.CH₂.H₂O
| | | | | |
NH — CO   OH OH   OH OH
                 ($C_{10}H_{16}O_8N_3.H_2O$
                     XII.

The evidence is in favor of formula XI. Theoretically such a combination would be expected to undergo hydrolysis easily with acids giving glucosamine and dialuric acid. When one considers the instability of dialuric acid and the ease with which this pyrimidine undergoes spontaneous oxidation in the air, forming alloxantine, it is at once apparent how the latter compound might be obtained as a final product of the hydrolysis of convicine.

Speculation leads to still more interesting possible relationships, when one comes to study Ritthausen's work on *vicine*. This appears to be a definite compound and Ritthausen's description of its properties is very complete. It dissolved in alkali without decomposition and was precipitated unaltered from such solutions by neutralization with acids. The compound was very stable in the presence of cold alkali, and could even be heated with dilute barium hydroxide solution without evolution of ammonia. On the other hand, when heated intensely with strong alkali the substance underwent complete decomposition with evolution of ammonia and formation of potassium cyanide. Vicine also dissolved in cold acids without decomposition. Ritthausen wrote as follows regarding these properties:[1]

"Das Studium dieser Zersetzung, und der dabei sich bildenden Produkte, lässt werthvolle Aufschlüse über die Constitution des Vicins erhoffen; leider war es bisher nicht möglich, mich eingehender mit demselben zu befassen." Ritthausen concluded from his early analyses that vicine is to be represented by the formula $C_{28}H_{51}O_{21}N_4$, but decided later[2] that the formula $C_8H_{16}O_6N_3$ is more probably correct. He writes however, as follows: "dass das Molekül grösser als das aus dieser Formel berechnete ist; eine Molekulargewichtsbestimmung konnte aber nicht ausgeführt werden." His final conclusions regarding the constitution of vicine are all expressed in the following statement from his paper:[3] "Bei den

[1] *J. prakt. Chem.*, **24**, 202.

[2] *Ibid.*, **59**, 480.

[3] *Ibid.*, **59**, 481.

Studien über die Spaltungsprodukte durch Erhitzen mit Schwefelsäure liess sich mit sehr grosser Sicherheit feststellen, dass Zucker in reichlicher Menge entsteht.[1] Vicin demnach als Glucosid zn betrachten ist."

The most characteristic property of vicine is its unique behavior on hydrolysis with 20–30% sulfuric acid. According to Ritthausen it undergoes hydrolysis, when heated with this reagent, giving about 29–30% of its weight in divicine sulfate. This salt was characterized by its insolubility in water, and gave, on analysis, values, from which Ritthausen calculated the formula $2(C_{22}H_{38}O_9N_{20}).5SO_3$. It is very doubtful, however, whether this formula is the correct one.

Divicine was obtained by careful decomposition of its sulfate with the required amount of alkali. It separated in a crystalline condition, but according to Ritthausen, it is not a very stable compound, and gradually undergoes decomposition on exposure to the air, becoming colored dark brown. The base even underwent partial decomposition when crystallized from water. It is a strong reducing agent in aqueous solution and gave, on analysis, results agreeing with the calculated values for a compound having the composition $C_4H_7O_2N_4$. Ritthausen did not suggest a structural formula for this substance, but believed it to be related to allantoin. Attempts to obtain allantoin by oxidation with nitric acid were unsuccessful.

After a critical examination of the analytical values obtained by Ritthausen in his analyses of divicine and its sulfate, and a study of Ritthausen's descriptions of the unique, chemical properties of this base, the writer is forced to the conclusion, that it is not improbable that divicine may be a diaminopyrimidine and that in vicine we may be dealing with a *pyrimidine-nucleoside* combination. The solubility of divicine in alkali and the low percentages of oxygen and hydrogen suggest a cyclic structure and the presence of —CO—NH— groupings in the molecule. Are we dealing here with the precursors of purine nucleosides?
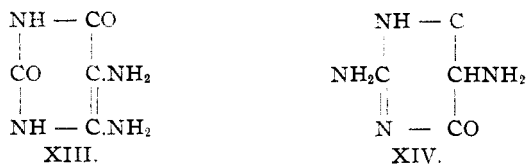
I find that the analytical data obtained by Ritthausen in his analyses of divicine sulfate agree with the calculated values for the sulfate of Traube's[2] 2,6-dioxy-4,5-diaminopyrimidine XIII. In fact, the agreement is perfect for every element except carbon where there is a difference of 4%. Whether there is an error here in Ritthausen's work must be decided by further investigation. The values found and the calculated values for Traube's salt are tabulated below:

Values obtained by Ritthausen.		Calculated for Traube's salt $(C_4H_4O_2N_4)_2H_2SO_4.1\frac{1}{2}H_2O.$	
C	27.3	C	23.47
H	3.9	H	4.16
N	27.42	N	27.38
H_2SO_4	24.00	H_2SO_4	23.96

[1] *Ber.*, **29**, 2108.

[2] *Ibid.*, **33**, 1382.

Still more interesting is the remarkable fact that the formula for Traube's 2,6-dioxy-4,5-diaminopyrimidine XIII, differs from that, which

$$
\begin{array}{cc}
\text{NH — CO} & \text{NH — C} \\
| \quad\quad | & | \quad\quad\quad | \\
\text{CO} \quad \text{C.NH}_2 & \text{NH}_2\text{C} \quad \text{CHNH}_2 \\
| \quad\quad \| & \| \quad\quad | \\
\text{NH — C.NH}_2 & \text{N — CO} \\
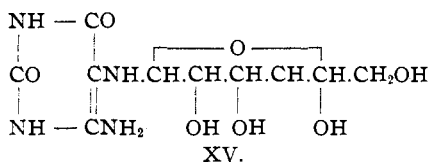\text{XIII.} & \text{XIV.}
\end{array}
$$

Ritthausen assigned to divicine by only one hydrogen atom, consequently the theoretical percentages of the elements are so close that it would be extremely difficult to distinguish by analyses alone between the two compounds. These values are tabulated below:

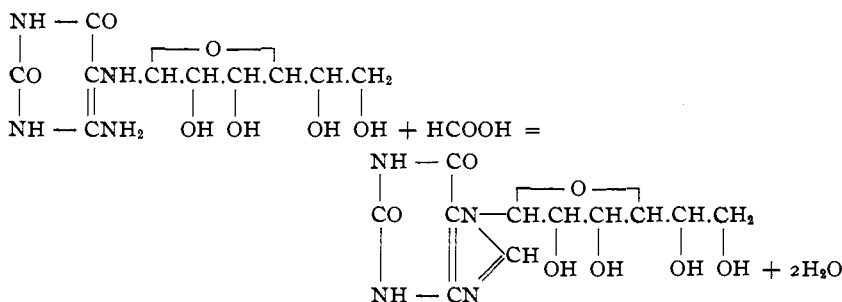| Ritthausen's divicine, $C_4H_7O_2N_4$.		2,6-Dioxy-4,5-diamino-pyrimidine $C_4H_6O_2N_4$.	
C	33.5	C	33.80
H	4.8	H	4.22
N	39.16	N	39.4

2,5-diamino-4,6-dioxypyrimidine XIV, is also to be considered as a possibility.

It is a very significant fact, from a chemical standpoint, that the carbon and nitrogen ratio in divicine is 1 : 1, and this important evidence alone leads to the conclusion that vicine may be a glucoside of 2,6-dioxy-4,5-diaminopyrimidine, which may provisionally be represented by formula XV. Such a constitution would harmonize with the observation of the

$$
\begin{array}{l}
\text{NH — CO} \\
| \quad\quad | \quad\quad \lceil\text{——— O ———}\rceil \\
\text{CO} \quad \text{CNH.CH.CH.CH.CH.CH.CH}_2\text{OH} \\
| \quad\quad \| \quad\quad\quad | \quad | \quad\quad | \\
\text{NH — CNH}_2 \quad \text{OH OH} \quad \text{OH} \\
\quad\quad\quad\quad \text{XV.}
\end{array}
$$

stability towards alkali and the instability when heated with acids. It would be expected to undergo hydrolysis easily with acid reagents giving 2,6-dioxy-4,5-diaminopyrimidine, XIII, and glucose.

The compound represented by structure XV, has the empirical formula $C_{10}H_{16}O_7N_4$. Ritthausen assigned to vicine the formula $C_{28}H_{47}O_{19}N_{11}.2H_2O$ and states that the water of crystallization is lost by heating to 160°. If now we take Ritthausen's formula for his anhydrous substance, which is very probably incorrect, and divide by the common factor 2.8, we obtain the expression $C_{10}H_{16.7}O_{6.7}N_4$, surely a remarkable agreement with the formula of the nucleoside of 2,6-dioxy-4,5-diaminopyrimidine $C_{10}H_{16}O_7N_4$. When one considers the fact, that the pyrimidine nucleoside, XV, by condensation with formic acid could undergo transformation into a nucleoside or xanthine hexoside, XVI, it will at once be apparent why the writer is interested in acquiring a better knowledge of Ritthausen's interesting compounds.

NH — CO
| | ┌— O —┐
CO   CNH.CH.CH.CH.CH.CH.CH₂
| ‖ | | | | |
NH — CNH₂   OH OH   OH OH + HCOOH =

$$\text{NH — CO}$$

NH — CO
| | ┌— O —┐
CO   CN—CH.CH.CH.CH.CH.CH₂
| ‖ ＼CH | | | | |
| ‖ ／   OH OH   OH OH + 2H₂O
NH — CN

XVI.

New investigations are now in progress and we hope to obtain new and important data bearing on these interesting problems.

NEW HAVEN, CONN.

RESEARCHES ON PYRIMIDINES. LXVI. THE FORMATION OF PYRIMIDINES FROM DIETHYL AMINOMALONATE AND AMINO MALONICNITRILE.

BY TREAT B. JOHNSON AND BEN H. NICOLET.

1. Introduction.   2. Diethyl Aminomalonate and its Derivatives.   3. Experimental Part.

1. Introduction.

In the previous paper[1] the assumption has been expressed that pyrimidines of the barbituric acid series may be the precursors of purines in plant growth, and we discussed new evidence that dialuric acid and uramil may be the representatives of this series, which are involved in these changes.   It is easily conceivable how both of these pyrimidines, if formed, might undergo condensations with urea or formamide[2] and be transformed into purine compounds, consequently it is of especial interest to consider how cyclic compounds of this type might be formed in growth from simpler substances.

The question of the relation of hydrocyanic acid to the mechanism of the natural synthesis of purines is an interesting problem, concerning

[1] Johnson, THIS JOURNAL, 36, 337 (1914).

[2] It is of especial interest to note here that W. Löb (Ber., 46, 684) has recently reported a synthesis of aminoacetic acid from formamide and water vapor, under the influence of the silent electrical discharge.   Oxamide was also formed under the same conditions.   The synthesis was therefore a complete one and the apparent course of the reaction was as follows:

$$2\,HCONH_2 \xrightarrow{-H_2} NH_2CO.CONH_2 \xrightarrow{+H_2O} NH_2COCOONH_4 \xrightarrow{+2H_2} NH_2CH_2COONH_4.$$
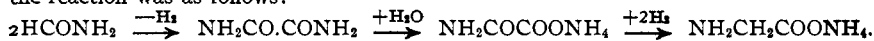
Though the yield of glycocoll was small, Löb considers this the nearest approach yet made to the actual conditions of plant synthesis.   It is not improbable that formamide may play an important part in other natural syntheses (T. B. J.).