THE DISTILLATION OF CYANIC ACID FROM AQUEOUS SOLUTIONS OF CYANATE

by

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In a previous communication (DIRNHUBER AND SCHÜTZ, 1948) it was shown that the spontaneous formation of ammonium cyanate from urea by isomeric change in aqueous solutions, occurred to an appreciable degree at 38°. Thus it seemed possible that cyanate might be formed from urea in the body.

Both the spectroscopic and manometric methods for the detection and determination of cyanate previously described (DIRNHUBER AND SCHÜTZ, 1948) were suitable for pure aqueous solutions of urea, but considerable difficulties were encountered in trying to apply these methods to biologial material. An alternative method was therefore needed in order to investigate whether cyanate was present in the organism.

Distillation *in vacuo* of cyanic acid, set free from aqueous solutions of cyanate by acidification, has apparently never been carried out successfully. This has been ascribed to the rapid decomposition of cyanate into carbon dioxide and ammonia on acidification. In the foregoing communication it was shown, however, that decomposition of cyanate is rapid only below p_H 5, and that above this p_H, especially at lower temperatures, it decomposes very slowly. It seemed possible, therefore, that distillation had been hitherto unsuccessful, not because mild acidification *in vacuo* would necessarily cause complete decomposition of the liberated cyanic acid, but because cyanic acid was lost on condensation in an aqueous medium, through decomposition or polymerisation. On this assumption a special technique for the condensation of cyanic acid has been worked out. This paper describes a procedure by which a high vacuum distillation of cyanic acid, set free from cyanate in aqueous solution, can be achieved. Experiments with tissues will be reported in the following paper.

METHODS

DISTILLATION

Principle. An acid buffer is introduced into the aqueous cyanate solution after the establishment of a high vacuum. The water containing the distillation flask is then raised to 50–55°, and the vapour given off is led beneath the surface of sodium hydroxide solution at 0°. By avoiding condensation before the vapour reaches the alkali, the free acid is caught by the latter and stable sodium cyanate is formed. The latter is then oonverted into urea for determination.

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The apparatus used is shown in Fig. 1. The distillation flask (*A*) contained the solution of NaCNO or the biological material. Provision was made for an acid buffer (*B*) to be added when the vacuum was established. The splash head (*C*) was connected with a tube (*D*) which ended under the surface of NaOH in a distillation flask (*E*) cooled in a bath (*J*) containing ethanol and solid CO_2. The wide side arm of flask *E* was connected to a glass tube leading into a similar distillation flask, which also was placed in an ethanol — solid CO_2 bath (water trap). From this flask a connection was made to a vacuum gauge (Pirani type), two water traps (ethanol-solid CO_2 and P_2O_5), and finally to a two-stage high vacuum pump. The capillary (*H*) was made of stout glass, and was as narrow as possible over a length of 10–20 cm, in order to obtain the best possible vacuum. The thermo-regulated water bath (*I*) surrounding flask *A* was empty when the distillation was started, and could be filled at a certain moment with water of the required temperature. The side arms and connections (*P*) were sufficiently wide (8–12 mm) to maintain a good vacuum in flask *A*. The diameter of the tube leading to the first water trap was considerably greater inside the flask to prevent it from becoming occluded by frozen distillate.

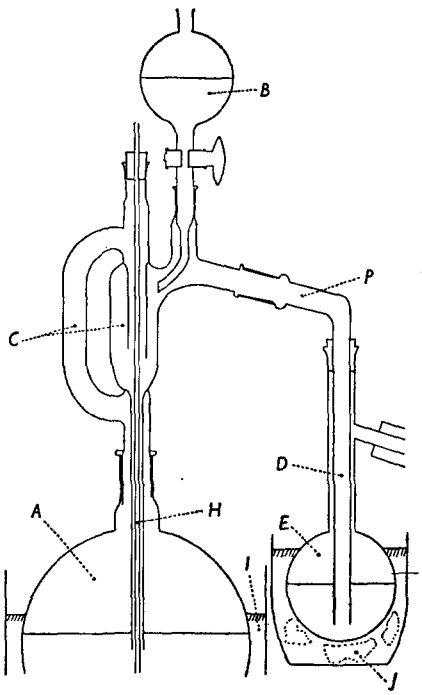

Fig. 1. Apparatus for distillation of cyanic acid from aqueous solutions of cyanate in a high vacuum. Condensation in 0.2 *N*-NaOH at 0°. *A* = distillation flask; *B* = acid buffer, added when vacuum was < 0.5 mm Hg; *C* = splash head; *D* = wide tube leading vapour into NaOH, contained in *E* = receiver flask, cooled at 0° by *J* = an ethanol bath containing CO_2; *H* = the capillary for the inlet of air, made of thermometer tubing, specially narrowed over a length of approximately 10–20 cm. *I* = water bath.

Procedure. Immediately after starting the evacuation, solid CO_2 was added to the ethanol bath *J*. Previous addition would have caused the contents of flask *E* to freeze, and even after starting the evacuation care was taken not to add excess of CO_2. With some experience it was easy to arrange that a small part of the solution in *E* was frozen when the distillation was started. When a vacuum of *c* 0.5–0.4 mm. Hg was attained, the acid buffer (*B*) was let in carefully, while keeping the pump going. Shortly before use the buffer solution was exposed to a vacuum in order to avoid undue splashing when it was let into flask *A*. The addition of the buffer did not diminish the vacuum to any great extent. If the addition was too long delayed the liquid in flask *A* froze, but with some experience the acidification could be timed so as to avoid this. After the buffer had been added hot water was poured into the water bath *I*, and considerably more solid CO_2 was simultaneously added to *J*. It now became essential to keep *E* maximally cooled by very frequent additions of solid CO_2 to *J*; a small part of the NaOH in flask *E* could be kept frozen throughout the procedure. The contents of flask *A* were much below room temperature even when the surrounding temperature was raised to 50°. The actual temperature depended on the vacuum, which varied mainly according to the leak through capillary *H*. The distillation was carried on for 0.5 h when the vacuum usually reached 0.1 mm Hg. The determination of any cyanate present in the distillate after conversion into urea is described below.

MATERIALS

Pure sodium cyanate was made from urea according to BADER, DUPRÉ AND SCHÜTZ (1948).

EXPERIMENTAL

A series of experiments was made in order to establish the optimal conditions. Sodium cyanate (2.4 mg) in 200–400 ml of water was distilled under different conditions, and the yields of urea found in the distillate after conversion were compared. All yields are expressed in urea equivalents of ammonium cyanate.

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p_H and temperature. Citrate buffers (2 *M*) of different p_H were added (20–40 ml to each 100 ml of solution in flask *A*). Citrate buffer was chosen because of its low volatility, and because high concentrations could be used. In other experiments water bath *I* was raised to different temperatures after the addition of the buffer. The yields obtained are shown in Table I. It was concluded that p_H 5.0–5.3 was optimal, with a water bath temperature of 50–55°. When in one control experiment the usual amount of sodium cyanate was left standing under ordinary pressure without distillation, but at approximately the temperature usually obtaining during distillation (4–8°) and with the buffer solution at p_H 5.3, 80% of the cyanate originally present was destroyed in 45 min. For this reason the higher p_H of the two (5.0 and 5.3), which gave approximately equal results (Table I), was thought to be safer. For the same reason 50 or 55° was preferred to 60° for the water bath *I*.

TABLE I

HIGH VACUUM DISTILLATION OF CYANIC ACID FROM PURE SODIUM CYANATE IN AQUEOUS SOLUTION (p_H IS THAT OF 2 *M* CITRATE BUFFER ADDED WHEN VACUUM 0.5 mm Hg. ° = TEMP. OF WATER BATH CONTAINING THE DISTILLATION FLASK. CONDENSATION OF VAPOUR IN 0.2 *N*-NaOH AT 0°. THE CYANATE IN THE DISTILLATE WAS CONVERTED TO UREA)

No.	p_H	Temp.	NaCNO (mg)	Yield of urea (% of theoretical)	Remarks
1	5.0	50	2.4	34	
2	5.0	50	2.4	37	
3	5.3	55	2.4	35	
4	5.3	62	2.4	30	
5	5.3	60	2.4	18	
6	5.8	60	2.4	3	
7	6.0	50	2.4	6	
8	6.0	50	2.4	4	
9	4.8	50	2.4	18	
10	5.3	30	2.4	7	
11	5.3	40	2.4	11	Condensation on filter paper soaked in *N*-NaOH
12	5.3	50	2.4	11	
13	5.3	50	2.4	18	
14	5.3	50	2.4	14	Imperfect cooling
15	5.3	50	2.4	20	NaCNO distilled in presence of Na_2CO_3 and NaCl
16	5.3	50	50.0	12	
17	5.3	55	50.0	13	
18	5.3	55	0.24	14	
19	5.3	50	2.4	± 0	Receiver contained water only
20	5.3	55	2.4	31	NaCNO distilled in presence of an equivalent of NH_4Cl

Condensation of distillate. Sodium hydroxide (100 ml, 0.2 *N*) was placed in flask *E*. Higher concentrations were tried, but these made the conversion of cyanate into urea and the following determination of urea more difficult (see below). Other liquids in which cyanic acid is known to be more stable than in water were unsuitable because of their volatility.

The optimal diameter of tube *D* was found to be 8–12 mm. With narrower tubes smaller yields were obtained. A narrow tube is kept empty by the flow of air during distillation, and since it is effectively cooled, the surrounding sodium hydroxide being

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at 0°, some of the cyanic acid probably condenses inside the tube before coming into contact with the alkali; it is then likely to be lost at once through decomposition and polymerisation. With wider tubes the alkali rises occasionally and the film left on the sides of the tube effectively traps the cyanic acid. For the same reason, and to maintain an optimal vacuum in flask *A*, no attempt was made to use sintered glass at the end of the tube *D*.

Experiments were carried out with two condensation flasks *E* in series. The further yield, recovered from the second flask, was found to be of the order of 0.5%, while the first flask contained 30%. Only one condensation flask was therefore used.

Before adopting the above procedure another method of condensation was tried (DIRNHUBER AND SCHÜTZ, 1947). A roll of filter paper (WHATMAN No. 541), soaked in *N*-sodium hydroxide was placed inside a condenser cooled with ethanol and solid CO_2. The filter paper, together with the distillate, was then treated for conversion of cyanate into urea. The yields were always significant, but smaller and more irregular than those obtained with the method of condensation described above.

Duration of distillation. When using lower temperatures in water bath *I* the distillation was carried on for much longer periods. With the procedure finally adopted (50°, p_H 5.3, and other points as described above) the following experiment was made. A solution of sodium cyanate (50 mg in 200 ml of water) was distilled with a final vacuum of 0.1 mm Hg. After 30 min the receiver was exchanged for another one containing the usual amount of alkali. The distillation was then continued for a further 30 min. Whilst a copious yield (24%) was obtained from the first receiver, none was obtained from the second.

Another experiment was carried out to determine whether any cyanate was left behind in flask *A* after distilling for 30 min at p_H 5.3 and 55°. For the determination of the amount of cyanate left behind, sodium hydroxide was added in amount calculated to bring the citrate buffer added during the distillation to p_H 7. The residue was then treated, similarly to the distillate, with ammonium sulphate for conversion of cyanate into urea, and the latter was determined as described later. The alkali was added before the vacuum was reduced, ammonium sulphate was added, and only then was air let into the assembly. Thus the contents of flask *A* were not warmed up until the ammonium sulphate had been added. Only 0.4% of the amount of sodium cyanate originally present was found in flask *A* after distilling for 30 min while a 26% yield was obtained from the receiver flask *E*. These experiments show that distillation for more than 30 min would not improve the yield.

Conversion of cyanate into urea in the distillate. When the distillation was stopped, and with it the flow of air through flask *E*, the cooling of the latter had to be interrupted at once, in order to prevent the contents from freezing completely; part of the solution, however, was always frozen. Ammonium sulphate was added as soon as possible, while the solution was still at 0°, since cyanate or cyanic ions are known to be converted quickly into urea in presence of an excess of ammonium ions. Since some of the ammonium sulphate added to the distillate was used up by the sodium hydroxide present in the solution, a fairly large amount (4–5 g) was added. This brought the p_H down to 6.0–6.5, as desired for the conversion of cyanate into urea.

After thawing slowly, with continuous agitations, the solution was incubated at 65° for 6 h, in the presence of octanol as bacteriostatic. Incubation at higher temperatures gave smaller yields, perhaps because a greater proportion of cyanate was decom-

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posed before undergoing isomerisation. Incubations at lower temperatures were abandoned, because they had to be prolonged, and because of the great error which could then be caused by slight bacterial contamination with urease-containing organisms.

After incubation, the volume of the solution was reduced *in vacuo* (outside temperature 65°) to approximately 10–15 ml. It was found convenient to keep the contents of flask *E* in the same flask throughout the procedure so far described. The material was then removed, and the flask washed three times with minimal amounts of water. The urea content was then determined as described below.

Direct formation of urea cyanic acid. During experiments with tissues, to be reported separately, the question arose whether the amounts of urea found in the distillate were indeed due to converted cyanate. To investigate this point, one half only of the distillate was treated with an ammonium salt for conversion of cyanate into urea, and thereafter the urea content of both halves was separately determined. Although significantly more urea was found in the sample previously incubated with ammonium ions, an appreciable yield of urea was also obtained from the other half. It is known that formation of urea occurs when solutions of sodium cyanate are acidified (WERNER AND FEARON, 1920). Cyanic acid, on hydrolising into ammonia and carbon dioxide, provides one molecule of ammonia which will react with another molecule of cyanic acid, yielding urea, thus:

$$HCNO + H_2O = CO_2 + NH_3 \quad (1)$$

$$HCNO + NH_3 = (NH_2)_2CO \quad (2)$$

In order to establish how much urea was formed without any special addition of ammonium ion during the incubation of the distillate and, possibly, during the distillation, the following experiments were made. Pure sodium cyanate, in doubly glass-distilled water, was acidified *in vacuo* and distilled as described above. One half of the distillate was brought to p_H 8 by addition of acetic acid, and then an excess of ammonium sulphate (Analar) was added, which brought the p_H to *c.* 6. The other half of the distillate was brought to the same p_H by addition of acetic acid. Both samples were incubated for 3 h at 65°, and reduced to a volume of approximately 10 ml by distillation *in vacuo* (air for leak through *N*-sulphuric acid). Urea was then determined.

Both samples always contained urea; *c.* 25% of the usual equivalent yield was found to be converted into urea without previous incubation with ammonium ion. Since any urea found in the distillate could only have come from the distillation of cyanic acid, the amount of urea found without incubation with ammonium ion represents the yield obtained according to equations (1) and (2); that obtained after incubation with ammonium ions minus that obtained without incubation with ammonium ions represents the yield from sodium cyanate, the latter having been formed by conversion of the free acid into sodium cyanate:

$$HCNO + NaOH = NaCNO + H_2O \quad (3)$$

$$2\,NaCNO + (NH_4)_2SO_4 = Na_2SO_4 + 2\,(NH_2)_2CO \quad (4)$$

Whilst two molecules of cyanate yield one molecule of urea according to (1) and (2), only one molecule of cyanate is needed to yield one molecule of urea according to (3) and (4).

In two experiments with 2.5 and 5 mg of sodium cyanate distilled in 200–300 ml of water, 15–17% (mean 16%) of the urea equivalent of the cyanate were found in

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one half of the distillate on incubation with ammonium ions. Only 4% were found in the half incubated without ammonium ions. Thus *c.* 16% was converted into urea according to (1) and (2), providing a yield of half this amount (8%), and *c.* 24% of the cyanate originally present was transformed according to (3) and (4). The total amount of cyanate which could be accounted for was thus of the order of 40%, giving a yield of only *c.* 32.5%. Approximately 60% must be assumed to have been lost during the procedure, since after the distillation practically no residue of cyanate (< 0.5%) was left behind in the distillation flask *A*.

Determination of urea in the distillate. The urea content was determined by the procedure of ENGEL AND ENGEL (1947), a valuable development of the xanthydrol method (FOSSE, ROBIN AND FRANÇOIS, 1914), depending upon a colour reaction of dixanthylurea with sulphuric acid. The presence of the large excess of ammonium salts, added for the conversion of cyanate into urea, excluded the use of those methods for the determination of urea which involve Nesslerisation, aeration, etc.

The specificity of the xanthydrol method was checked by incubating a sample of the same material for 1 h at 50° with urease (aqueous jack bean extract). The latter was removed by acidification to p_H 5 with acetic acid, immersion in boiling water, and completion of the precipitation with ethanol (4 vol). Ethanol was removed from the centrifuged and filtered solution by distillation *in vacuo*. Urea was then determined according to ENGEL AND ENGEL (1947).

A blank containing the amount of sodium hydroxide usually placed in the receiver, together with acetic acid and ammonium sulphate in the amounts usually added to the distillate, was incubated with and without urease, and the urease removed as described above. No precipitate or colour was obtained. If, on the other hand, urea was added to a similar blank, after the elimination of urease, the recovery was quantitative. Thus, by treating a part of the distillate with urease, the significance of the results obtained by the method of ENGEL AND ENGEL was greatly increased.

Positive results were also obtained on many occasions by means of the manometric method of KREBS AND HENSELEIT (1932). The volume of the distillate had to be reduced to *c.* 2–3 ml for use in a WARBURG-BARCROFT manometric vessel. The distillate, after incubation with ammonium ions, was therefore reduced *in vacuo* to a volume of *c.* 10 ml, and then dried in a desiccator over phosphorus pentoxide. The residue was finely powdered, dried thoroughly *in vacuo*, and finally extracted with absolute ethanol under anhydrous conditions. Practically all the sodium sulphate and ammonium sulphate was left behind; this was essential since, in such high concentrations, these substances interfere with the action of urease. The alcoholic extract was evaporated on a boiling water bath, taken up in a small amount of water and the urea determined according to KREBS AND HENSELEIT (1932).

When dealing with the distillate obtained from tissues, great difficulties were experienced after concentrating to 2 ml, because some substances which had distilled over were also extracted together with urea by ethanol and were found to be potent inhibitors of urease. By using an excess of urease and treating the distillate with hydrogen sulphide, however, evidence for the presence of urea in the distillate was obtained on several occasions in this way, but the yields were usually a small percentage of those obtained with the method of ENGEL AND ENGEL (1947). With the latter method the urea could be precipitated from a larger volume. Moreover, when using the method of ENGEL AND ENGEL there was no need to dry the sample and to extract it with ethanol,

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since excess of ammonium and sodium sulphate do not interfere with the xanthydrol precipitation.

The procedure finally adopted was, therefore, as follows: Glacial acetic acid (1–1.2 vol) was added to the cooled sample (usually 10–15 ml), then 1 ml of 5% (w/v) xanthydrol in methanol. The solution was kept 0.5 h at room temp. being frequently agitated with a glass rod, and 24 h at 4°. Since the concentration of salts was high, the solution was then diluted with an approximately equal vol. of freshly filtered methanol saturated with dixanthylurea (washing solution *A*). This precipitated any suspended crystals of dixanthylura, and some of the $(NH_4)_2SO_4$. The latter was quantitatively eliminated during the following 3–5 washings of the precipitate on the centrifuge. 50% (w/v) H_2SO_4 was then added to the thoroughly drained crystals. The amount of H_2SO_4 added, which varied according to the amount of crystals, was noted. Care was taken that all crystals dissolved, by frequently mixing the contents with a glass rod for 1 h after addition of the acid. The colorimetric readings were made with a KING photoelectric colorimeter (KING, 1942), and the amount of urea obtained from a standard curve. On rare occasions the solutions were left at 4° overnight before the readings were made. According to ENGEL AND ENGEL (1947), the colour is stable at this temperature.

When known amounts of sodium cyanate were converted into urea, in presence of large amounts of sodium sulphate, sodium carbonate, and ammonium sulphate, and determined as described above, the recoveries were between 95 and 98% in three experiments, showing that practically no loss occurs during this part of the procedure adopted.

Control experiments. To check the reagents, and some possible sources of error, the following experiment was made. The usual quantity of water containing 1 g of sodium carbonate, 1 g sodium bicarbonate was distilled, treated for conversion, and the urea content determined as described. Even though there was no crystalline precipitate after the addition of xanthydrol, some precipitate of ammonium and sodium sulphates etc., was brought down, as usual, on addition of washing solution *A*. This precipitate disappeared during the subsequent washing procedure, and the final result was nil. It seemed possible that traces of cyanate might have been carried over in very small droplets (entrained). Although this seemed unlikely, the following experiments were thought to yield some information on the point. An aqueous solution of 800 mg of pure urea, freshly prepared, was placed in flask *A* and distilled as usual. The distillate gave a blank, showing that no urea had been carried over by direct transference, by splashing, or as small droplets. It is hardly possible that as much as 30–37%, the usual yield, could have been carried over in this way.

RESULTS

Representative results are shown in Table I. It can be seen that consistent yields were always obtained when optimal conditions were maintained. When large as well as very small quantities of cyanate were distilled (Exps. 16–18), the losses seem to have been greater. Nevertheless, so small an amount as 240 μg of sodium cyanate in water still gave a yield (37 μg) which was quite easily detectable. The presence of other salts (carbonate, etc.,) seems also to lower the yield (Exp. 15). The loss of *c.* 60% under optimal conditions is probably due to decomposition of the free acid into carbon dioxide and ammonia, and to polymerisation.

We are indebted to Prof. A. C. FRAZER for his help in the presentation of the results contained in this paper, to the Medical Research Council for a grant to one of us (F.S.) in aid of equipment, and to the Board of Mental Dissease Research for financial assistance. We also acknowledge technical assistance by Messrs. J. DAVIS AND W. DUNN.

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SUMMARY

1. Cyanic acid can be distilled from aqueous solutions of pure sodium cyanate, when brought to p_H 5.0–5.3 in a high vacuum.

2. Without special arrangements for condensation, the acid undergoes rapid and complete decomposition in a neutral aqueous medium, even at 0°.

3. If the free acid was condensed in 0.1–0.2 *N*-sodium hydroxide at 0°, varying amounts were transformed into the more stable sodium cyanate.

4. Sodium cyanate present in the distillate was converted into urea by incubating with an excess of ammonium ions. Yields of 30–37% of the equivalent urea were regularly obtained from distillations under optimal conditions.

5. A part of the cyanic acid was hydrolysed during the procedure, yielding one molecule of ammonia, which, with another molecule of cyanic acid gave one molecule of urea. Thus some urea was found, in the distillate without previous incubation with ammonium ions.

6. Optimal conditions for the procedure are described.

RÉSUMÉ

1. L'acide cyanique peut être distillé à partir de solution aqueuse de cyanate de sodium pur après acidification de celle-ci à p_H 5.0–5.3, et sous un vide poussé.

2. Si l'on ne prend pas de précautions spéciales pour sa condensation, l'acide subit une décomposition rapide et complète en milieu aqueux neutre, même à 0°.

3. Si l'acide libre est condensé dans une solution de soude 0.1–0.2 N à 0°, des quantités variables en sont transformées en cyanate de sodium plus stable.

4. Le cyanate de sodium présent dans le distillat se transforme en urée par incubation avec un excès d'ions NH_4. Dans des conditions optimales, on obtient ainsi des rendements de 30–37% de l'urée théorique.

5. Une fraction de l'acide cyanique est hydrolysée au cours de l'opération, fournissant une molécule de NH_4, laquelle, réagissant avec une autre molécule d'acide cyanique, donne une molécule d'urée. On trouve ainsi un peu d'urée dans le distillat, même sans incubation préalable avec des ions NH_4.

6. Les conditions les meilleures pour la méthode sont décrites.

ZUSAMMENFASSUNG

1. Zyansäure kann aus wässrigen Lösungen reinen Natriumzyanats destilliert werden, wenn diese im Hochvakuum auf p_H 5.0–5.3 gebracht werden.

2. Ohne besondere Kondensationsvorrichtungen wird die Säure in neutraler wässriger Lösung sogar bei 0° schnell und vollständig aufgespalten.

3. Wenn die freie Säure in 0.1–0.2 n Natriumhydroxyd kondensiert wurde, wurden variierende Mengen in das stabilere Natriumzyanat umgesetzt.

4. Natriumzyanat, das im Destillat anwesend war, wurde durch Inkubation mit Überschuss an Ammoniumionen in Harnstoff übergeführt. Ausbeuten von 30–37% der aequivalenten Harnstoffmenge wurden regelmässig aus Destillationen unter optimalen Bedingungen erhalten.

5. Ein Teil der Zyansäure wurde während des Verfahrens hydrolysiert, wobei ein Molekül Ammoniak entstand, das mit einem anderen Molekül Zyansäure ein Molekül Harnstoff ergab. Dadurch wurde auch ohne vorhergehende Inkubation mit Ammoniumionen etwas Harnstoff im Destillat angetroffen.

6. Optimale Bedingungen für das Verfahren werden beschrieben.

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Received June 9th, 1948