CXLIV. THE PHOTOSYNTHESIS OF UREA FROM AMMONIUM CARBONATE.

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THE formation of urea from ammonium carbonate in aqueous solutions at temperatures below 50° is a reaction of considerable biochemical interest in connection with the obscure mechanism of the formation of urea in the animal body.

Under these conditions urea has been obtained from ammonium carbonate or bicarbonate by a variety of apparently unrelated methods, which may be summarised as follows.

1. Electrolysis, using an alternating current. Drechsel [1880].
2. Electrolysis, using a direct current. Millot [1885]; Fichter, Steiger and Stanisch [1918].
3. Zymolysis, using concentrated preparations of urease. Kay [1923]; Mack and Villars [1923].
4. Incubation with various charcoals. Fichter and Kern [1925].
5. Incubation in a sealed tube for 12 months. Werner [Fearon, 1926].

As a result of the present investigation it has been found possible to detect urea in concentrated solutions of ammonium carbonate after ultra-violet irradiation for periods of 2–10 hours. This is in agreement with the conclusions of Bailey, who detected traces of urea after the exposure of gaseous mixtures of ammonia and carbon dioxide to radium emanation [1925] and to ultra-violet irradiation [1927].

EXPERIMENTAL.

Reagents and apparatus.

1. The ammonium carbonate employed was the purest form supplied by Kahlbaum. It was not analysed for carbamate or bicarbonate.
2. The xanthhydrol was obtained from Poulenc. It was used in 10 % concentration in methyl alcohol.
3. The urease used was the Dunning preparation.
4. The source of irradiation was a Hanovia "Alpine Sun" A.C. mercury vapour lamp. To compensate for any decrease in the efficiency of the lamp the quartz arc tube was replaced by a new one early in the investigation.

Method.

The solutions were irradiated in a flat porcelain dish, 21 cm. in diameter, on account of the observation made by Fichter and Kern [1925] that the prolonged action of warm, concentrated ammoniacal solutions is liable to extract silicic matter from glass-ware, and thus interfere with the specificity of the xanthhydrol test. The distances of the solutions from the lamp were such that the temperature of the liquid did not rise above 45°. In most of the experiments the temperature range was 30°–40°.

After irradiation, the solutions were concentrated to about 5 cc. on a water-bath, and either tested directly by means of urease, or treated with four volumes of glacial acetic acid and filtered free from dust particles. 2 cc. of the 10 % xanthhydrol solution were then added drop by drop and the mixture having been shaken was allowed to stand for 12 hours. Where a precipitate was obtained its identity as dioxanthylurea was established by the following tests:

- (1) the characteristic sheen of the precipitate, and its microcrystalline nature;
- (2) the rosy colour of the warm residue when dried on the filter paper;
- (3) the melting-point (260°) as determined in the vapour of diphenyl ether.

Results.

It was found easy to demonstrate the formation of urea in 10 % and 20 % solutions of ammonium carbonate after irradiation of 200 cc. for periods of 2 to 6 hours. The urease test was positive, and the precipitates of dioxanthylurea were definite although very small, being of the order of 1–5 mg. per 200 cc.

The control experiments of non-irradiated ammonium carbonate solutions gave no trace of urea after concentration.

250 cc. of a 10 % solution of ammonium carbonate which had been kept in a glass flask exposed to daylight for one year was found to be free from urea.

The effect of various accessory reagents on the reaction was then examined. Glucose, hydroxylamine and formaldehyde were selected as reducing agents; methylene blue, ammonium nitrate and hydrogen peroxide as oxidising agents; copper, iron, and uranium as possible metallic catalysts; methyl orange, malachite green, and eosin as possible photo-catalysts.

The principal results are summarised in Table I.

Of all the accessory reagents examined, malachite green had the most definite effect in increasing the yield of dioxanthylurea. During the irradiation the solutions effervesced in a manner not observed with any of the other dye-stuffs examined. Accordingly, these experiments were repeated with controls, and the weight of dioxanthylurea determined. After irradiation, the unchanged dye-stuff was removed by acidifying the concentrated solution with acetic acid and extracting with chloroform.

Exps. 3 and 4 are controls, and indicate that the urea did not originate from the action of the ammonium carbonate on the dye-stuff in the absence of the irradiation, or from the irradiation of the dye-stuff in the absence of the ammonium carbonate.

Table I. *Irradiation of ammonium carbonate.*

Time, 2 hours. Temperature, 10°—40°. Distance from lamp, d cm.					
Expt.	(NH ₄) ₂ CO ₃ g.	H ₂ O cc.	d cm.	Urea	Accessory reagent
1	10	100	25	+	None
2	20	200	15	+	None
3	36	120	15	++	None
4	8	100	25	+	Glucose, 2 g.
5	20	100	15	—	NH ₂ OH·HCl, 1 g.
6	40	200	10	—	NH ₄ NO ₃ , 1 g.
7	40	200	20	++	(Copper stirrer)
8	40	200	10	+	Uranium acetate, 0.2 g.
9	20	100	10	+	Eosin, 0.001 g.
10	40	200	10	+++	Malachite green, 0.04 g.
11	40	200	10	+	Methyl orange, 0.03 g.
12	10	100	25	—	Methylene blue, 0.01 g.

Table II. *Photocatalytic action of malachite green.*

Time, 10 hours. Temperature, 10°—40°. Distance from lamp, d cm. Vol. of solution, 200 cc.

Expt.	(NH ₄) ₂ CO ₃ g.	Dixanthylurea g.	Malachite green g.	d
1	40	0.061	0.02	10
2	40	0.046	0.01	8
3	40	0.000	0.01	infinity
4	0	0.000	0.01	10

DISCUSSION.

In the absence of more adequate data it appears premature to discuss the possible mechanisms concerned in these low temperature syntheses of urea. A general conclusion is that urea is a stable end-product which may be formed in various ways in systems containing ammonia and carbonic acid. Two such ways are (1) alternate reduction and oxidation of carbamic acid, amounting to indirect dehydration; and (2) direct dehydration of carbamic acid. The former process offers an explanation of the production of urea during the electrolysis of solutions of ammonium carbonate, and also, it is believed, is concerned in the photosynthesis of urea.

The latter process, direct dehydration of carbamic acid, is probably responsible for the synthesis of urea by the action of urease, active charcoal, or incubation in a sealed tube.

No attempt was made in the present investigation to determine the range of the ultra-violet spectrum concerned in the synthesis of urea. It was observed, however, that the efficiency of the quartz lamps decreased notably during a lengthy series of experiments, and eventually the photosynthetic properties became negligible¹.

¹ Note added Sept. 18th, 1927. By means of the ultra-violet spectroscope it was found that the effective radiations were between 250 μ and 200 μ . When a glass screen was interposed, or when the lamp had aged sufficiently, the radiations did not extend into the ultra-violet beyond 250 μ . Under these conditions no urea could be obtained photosynthetically.

SUMMARY.

1. Urea has been produced by the ultra-violet irradiation of solutions of ammonium carbonate at temperatures not exceeding 45°.
2. The yield of urea was increased by the presence of malachite green, but not by that of methylene blue, methyl orange, or eosin.
3. The effective radiations for the synthesis of urea lie between 250 $\mu\mu$ and 200 $\mu\mu$.

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