STUDIES ON THE "BROWNING REACTION" OF MILK AND MILK PRODUCTS. III. ON THE ACTION OF UREA IN THE HEATED UREA-LACTOSE SYSTEM WITH REFERENCE TO THE HEATED BROWNING MILK.

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By

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The high temperature sterilization procedure for milk is frequently used in milk processing, for instance, evaporated milk is heated at 243°F for 15 minutes and sterilized milk which is sold in the northern Continent is heated at 212°F for 25 minutes[1]. When the milk is subjected to prolonged heat treatment as described above, the color of this medium turns gradually to brown. This phenomenon, known as "browning reaction", is important in the dairy industry.

Although the browning reaction of heated milk, namely, evaporated milk consists of complex chemical changes, it has been believed[2-5] that the lactose-milk protein systems are the principal reactants among the numerous chemical factors affecting the reaction. In the browning reaction played with lactose-milk protein systems of heated milk, a brown pigment known as "mellanoidin" is formed as the result of the interaction between lactose and amino groups of milk proteins, and absorbed on the surface of milk proteins. Thereby the milk discolors to brown. Recently, it was reported that the absorption, here called "binding" of lactose by the protein is initiated prior to color development, and the lactose-protein complex appears to be heat labile according to Patton and Flips, who investigated the reaction of lactose with milk protein using lacotse-1-C[14] in their studies of heated milk.[6] In spite of this considerable discoloration in milk, the destruction of amino acid contained in the milk proteins is slight as shown by Hadson and Krueger, who have demonstrated that small amounts of lysine and histidine are lost during the sterilization process of evaporated milk.[7] To explain satisfactorily the promotion of this browning reaction, it seems that the theory of lactose-milk protein systems as the principal factors on this reaction is only partially demonstrated, and unknown factors exist. It was found that

urea as the one of the other factors to the browning reaction are predominant, and the urea-lactose system in milk is important.[8] The action of milk salts are not important as already shown by some workers.

The problems regarding the mechanisms of the action of urea in the browning reaction of heated milk remains unexplained.

The studies on the mechanisms of the action of amino compounds in amino sugar reaction have been almost limited to some amino acids.[9][10] In the interaction between amino acids and reducing sugars, the initial stage of condensation is succeeded by brown pigment "mellanoidin" formation. Thereafter decarboxylation, intermolecular loss of water and deamination generally occur for the browning reaction before mellanodiin is gradually formed. As the indispensable structure of amino acid for this reaction, it has been indicated that the presence of a free amino group and a hydrogen atom in the α-position are necessary, according to Wolfrom, Cavalieri and Cavalieri.[11] Gottschalk and Partridge showed that the browning reaction seems to be promoted by the presence of the group -CH NH_2 in amino acids, and its rate increases with the increasing distance of this group from the therminal carboxyl group and is greatly depressed by substituting the alkyl group for a carbon bond hydrogen.[12]

As urea has the most simplest and specific structure among the amino compounds causing browning reaction, investigation of the mechanism of urea action in this urea-lactose system is an interesting and important problem in the field of dairy industry, and organic chemistry.

In this report, the reactive structure of urea and the mechanism of the browning reaction in the urea-lactose system reaction with reference to heated milk have been studied.

Methods and Materials

Methods.

All of the samples were dissloved in a suitable Sörensen's phosphate buffer sealed in testing tubes of 20 cc., and then subjected to heat treatment at 120°C. in the oil bath. The pH of the reaction systems were determined potentiometricall by means of the potentiometer in Shimazu's manual polarograph using a quinhydron electrode before and after heat treatment of these systems. The color of these medium were measured photometrically by Shimazu's photoelectric colorimeter with No. 48 filter to obtain absorbance. The concentration of ammonia was determined quantitatively by the aeration procedure of Folin, ammonia being driven from the weakly alkaline solution at room temperature with a rapid stream of air.[13]

Materials.

As the browning reaction is very sensitive to impurities, it was necessary to subject the organic compounds employed to rigorous purification. Lactose was purified by the previously reported procedure. The urea derivatives used in these experiments were synthesized by the following methods, and subjected to purification with the optimum solvent for recrystallization.

Methylurea : Methyurea was synthesized from N, N'-acethylmethylurea by means of Hofmann's method using hydrochloric acid as the decomposition agent.[14] M. P. 102°C.

N, N'-Acethylmethylurea : N, N'-Acethylmethylurea was prepared from acetamide and soduim hypobromide by the method of Hofmann.[15] M. P. 180°C.

Acethylurea : Acethylurea was prepared from the mixture of urea and acethylchloride by reflux heating according to the procedure of Moldenhauer.[16] M. P. 212°C.

N, N'-Dimethylurea[17] : N, N'-Dimethylurea was synthesized from the equivalent mixture of potassium isocyanate and dimethylaminsulfate by heating on the boiling water bath. N, N'-dimethylurea was then extracted from this dried material by ethylalcohol. M. P. 180°C.

N, N'-Dimethylurea : N, N'-dimethylurea was obtained by the method of blowing dried ammonia gas into the benzol solution of dimethylurea chlorid which was synthesized from the benzol solution of phosgen dissolving dried dimethylamin gas.[18] M. P. 102°C.

Trimethylurea : Trimethylurea was prepared from the benzol solution of dimethylurea chlorid by blowing into the dried monomethylamin gas as described above. M. P. 75.5°C.

Phenylurea, N, N'-diphenylurea : Phenylurea and N, N'-diphenylurea were obtained from the mixture of urea and anilin hydrochloride by boiling under a reflux condenser.[19][20] M. P. : Phenylurea, 147°, Diphenylurea 235°C.

Urethan : Urethan was prepared from the 50 per cent alcohol solution of potassium isocyanate by the addition of the hydrochloride saturated alcohol solution.[21] M. P. 54°C.

Methylurethan : Methylurethan was separated from the methylamin solution by the addition of chlorocarbonic acid ethylester solution.[22] B. P. 170°C.

Semicarbazid, thiourea, acetamid and the other ammonium compounds were commercial products that were subjected to recrystallization.

Results

1. *Comparison of the browning action of urea with ammonium compounds in lactose solution.*

It has been known that urea gradually decomposes to ammonium carbonate

by prolonged super heat treatment. To determine whether urea reacted with lactose with its own structure or with verified decomposed structure, i.e. ammonium compound in the browning reaction of urea-lactose system, the equimolecular lactose solution of urea and ammonium carbonate were heated to 120°C. for 120 minutes. Before heating 20 cc. of N/10 lactose solution were mixed with 120 mg of urea and 228 mg of ammonium carbonate respectively. The degree of decomposition of urea was indiceted by the value of as follows : b/a×100, or the weight of urea (b) calculated from the ammonia before heat treatment divided by the original weight of urea(a). The experimental evidence obtained in these systems are shown in Table 1.

Table 1. Reactivity of urea and ammonium carbonate in the lactose solution heated to 120°C for 2 hours.

Reactant	Urea	Ammonium carbonate
Absorbance	0.19	0.22
pH before heating	6.91	6.79
pH after heating	6.73	6.62
Decomposition of urea	5.68	—

The results obtained indicate that ammonium carbonate is more reactive than urea in these systems. However, it is probable that the bulk of urea reacts with lactose in this own structure, non-decomposed type, though the ammonium carbonate formed from urea by the heat treatment considerably participates in the browning reaction of the urea-lactose system. The decomposition of urea is too slight to react with lactose in ammonium carbanate form. It is necessary to note that the decomposition degree of urea may actually give higher values, because the ammonia produced there-from is consumed in the mellanoidin formation of this system,[23] and the ammonium carbonate used is not pure. It contains considerable amounts of the mixture of ammonium carbamate and ammonium bicarbonate owing to the unstability of ammonium carbonate.

The results of comparison of the action of urea with ammonium compounds in the browning lactose systems are shown in Table 2. 20 cc of M/20 lactose solution were mixed with a equimolecular weight of inorganic and organic ammonium compounds, and amines before heating to 120°C. for 180 minutes.

The results show that the reactivity of ammonium compounds are not pronounced in comparison with urea, and the dibasic ammonium compounds show more reactivity than monobasic compounds in brown discoloration. Ammonium acetate and dimethylamin hydrochloride, however, have no reactivity to discolor the solution. It will be worthy to note that the action of negative ions in these compounds should not be neglected in comparison of the reactivity in

Table 2. Reactivity of urea, ammonium compounds, and amines in
the lactose solution heated to 120°C for 3 hours.

Compounds	Addition weight	Color	pH Before	pH After
Ammonium chloride	53	0.15	6.43	5.84
Ammonium nitrate	80	0.14	6.01	5.68
Ammonium sulphate	132	0.18	6.18	5.72
Ammonium phosphate dibasic	132	0.18	6.06	5.60
Ammonium acetate	65	0.05	5.75	5.55
Ammonium tartarate	184	0.15	5.88	5.49
Monomethylamine hydrochloride	67	0.16	6.02	5.94
Dimethylamine hydrochloride	81	0.04	6.03	5.75
Urea	60	0.23	6.05	6.21
Control	—	0.01	6.18	5.83

ammonium ion series. If the all molecules of urea were compeletely decomposed to ammonium carbonate the specific reactivity of urea could not be explained with the action of the ammonium ions. Though the pH of the urea-lactose system which may be partially decomposed by the heat treatment are moved to the alkaline side, the depression of pH in these compounds may be due to the loss of ammonium ions resulting from the mellanoidin formation.

II. *The browning reaction of some urea derivatives in lactose solution.*

To understand the indispensable structure of urea in the browning reaction of the urea-lactose system, the relation of the chemical structure of some urea derivatives to brown color production has been examined. The urea derivatives that substituted the reactive groups of urea for the other groups were limited to compounds which are soluble and have little polarizable methyl, phenyl and acethyl groups. These urea derivatives were mixed equimolecularly with 20 cc. of M/10 lactose solution, and then heated to 100°C. for 4 hours in the two phosphate buffers, 5.5 and 6.0. The results obtained are given in Table 3.

The brown color production series of these compounds are as follows :

Urea > Thiourea, Phenylurea, N, N'-Dimethylurea, Methylurea > Acethylurea > N, N'-Dimethylurea, N, N'-Diphenylurea, N, N'-Methylacethylurea, Trimethylurea > Urethane, Semicarbazid, Acetamide, Methylurethane

that is to say, urea is most remarkable in the brown color formation. N, N'-dimethylurea, N, N'-diphenylurea, N, N'-methylacethylurea and trimethylurea are hardly recognized to be the promoters of the color formation. Urethane, semicarbazid, acetamide and methylurethane belong rather to the negative catalyser than to the accelator in color formation and may react with lactose as

Table 3. The browning reaction of urea derivatives in lactose solution heated to 120 C for 4 hours.

Urea derivatives	Structural formula	Addition weight	pH 6.0		pH 5.5		Color	
			Before	After	Before	After	pH 6.0	pH 5.5
Urea	NH_2CONH_2	12	6.02	6.03	5.46	5.88	0.60	0.26
Thiourea	CH_2CSNH_2	17	6.07	6.14	5.52	5.55	0.27	0.14
Methylurea	$CH_3NHCONH_2$	15	6.08	5.95	5.40	4.86	0.38	0.18
N,N-Dimethylurea	$(CH_3)_2NCONH_2$	18	6.04	5.77	5.46	5.45	0.23	0.07
N, N'-Dimethylurea	$CH_3NHCONHCH_3$	18	6.24	6.02	5.49	5.84	0.37	0.13
Trimethylurea	$CH_3NHCON(CH_3)_2$	20	5.80	5.99	4.78	5.32	0.18	0.05
Phenylurea	$C_6H_5NHCONH_2$	27	2.08	6.06	5.13	5.72	0.41	0.13
N, N'-Diphenylurea	$C_6H_5NHCONHC_6H_5$	42	6.31	5.98	5.45	5.39	0.18	0.06
Acethylurea	$CH_3COHNCONH_2$	20	6.04	5.86	5.54	5.38	0.26	0.14
N, N'-Acethylmethyl-urea	$CH_3CONHCONHCH_3$	23	6.14	5.65	5.59	5.04	0.13	0.05
Urethane	$NH_2COOC_2H_5$	17	5.93	5.97	5.51	5.30	0.12	0.05
Methylurethane	$CH_3NHCOOC_2H_5$	20	5.96	6.09	5.30	5.43	0.08	0.04
Semicarbazide	NH_2CNHNH_2	22	3.83	3.25	3.18	3.16	0.09	0.06
Acetamide	CH_3CONH_2	12	6.04	5.98	5.56	5.98	0.06	0.04
Control	—	—	6.05	6.05	5.49	5.35	0.12	0.05

inhibitors in these systems. The amino groups of these inhibitors seem to be inactive. In general, the color production of the systems in the more acidic buffer are not accompanied with the more alkaline systems. The shifting in pH of these systems, having important questions for the interpretation of the mechanism of these browning reactions, are different. Namely, pH of urea and thiourea systems are moved to the alkaline side and acethyl, methyl and phenyl derivatives of urea are moved to the acidic side. The fact that urea is more reactive than thiourea substituted sulfer for oxygen in urea indicates the importance of carbonyl radical in urea for this reaction. The structure of these urea derivatives, phenylurea, N, N'-dimethylurea, methylurea, whith belong to the predominant reactants for this browning reaction are as follows :

$$\begin{array}{c} R \cdot NH \\ \diagdown \\ C = O \\ \diagup \\ R' \cdot NH \end{array}$$
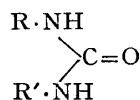
R, R' indicate the alkyl or phenyl radicals or hydrogen atom.

III. *Interaction of urea with lactose.*

To ascertain the mechanisms of the interaction of urea with lactose, studies
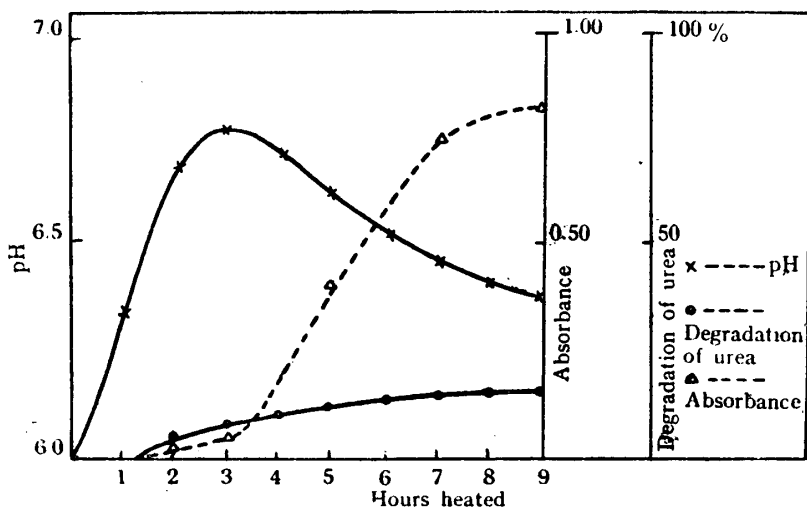

Fig. 1. Relation of pH shifting, color formation, and
degradation of urea in the reaction of urea-lactose
system heated to 100°C for nine hours.

were made under different experimental conditions. Some years ago, the kinetics were investigated by Frankel and Katchalsky,[24][25][26][27][28][29] and Shiga with the calculation of the shifting of pH in the mixture of amino acid and sugars. In the browning reaction of urea-lactose system the relations of pH shifting, brown discoloration and degradation of urea were repeatedly examined to obtain the interpretation for the mechanism of this reaction. Sörensen's

phosphate buffer was used in the experiments. 0.02 M solution of the mixture of lactose and urea was heated to 100°C. for 9 hours, and the results are given in figure 1.

The rapid rising of pH observed in this medium in initial heating stage gradually decreased with the increase in brown color formation. As the color formation commences slowly after heating for some time in this system, it appears that the inductive period is necessary to form the brown color. Though the degradation of urea is slight during the course of this reaction, its degree increases with prolonged heating to 15 per cent at the end of this reaction. From the existence of the inductive period in color formation and of two stages in pH shifting, it is probable that this browning reaction consists of at least two reaction stages. The rising of pH in the initial stage is due to the inductive period, and the striking color formation is accompanied with a depression of pH in the medium.

The experimental evidence obtained from the interaction of lactose with the increasing amounts of urea are shown in figure 2. 5, 10, 20, 30, 45, and 60 mg of urea were dissolved in 20 cc. of M/20 lactose solution before heating to 120°C. for 3 hours.
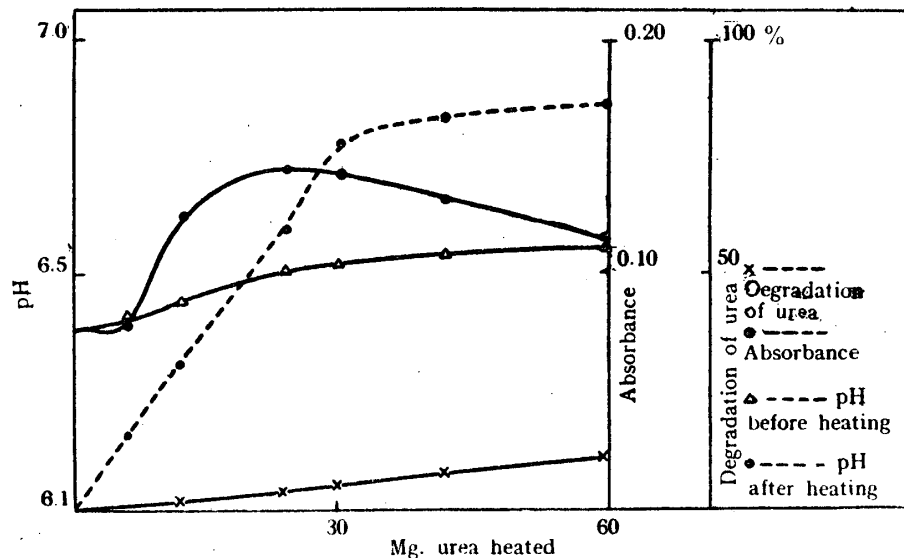

Fig. 2. Relation between the browning reaction in urea-lactose system and the amount of urea heated to 120°C for 3 hours.

The color production and the decomposition of urea in these medium increased with the increasing amounts of urea, though its degree of decomposition is slight. The shifting in pH of these media after heating are specific and pH which rises once again becomes depressed with the boundary of being due to the addition of

20 mg. urea. The color formation accompanied with the increasing urea, also increases in response to the pH shifting similarly as in the above described experiment. The depression in pH commences with the formation of distinct color. It appears that the relation of the deviation in pH and the color production suggests the existence of the two main stages in this browning reaction.

The variation of the interaction and of the medium pH are given in figures 3 and 4. The 0,02 M lactose-urea solution was prepared from the Sörensen's phosphate buffers with the range of pH from 4.3 to 7.5 as shown herein. These solutions were heated to 100°C. for 9 hours at 3 hours intervals for to permit sampling.
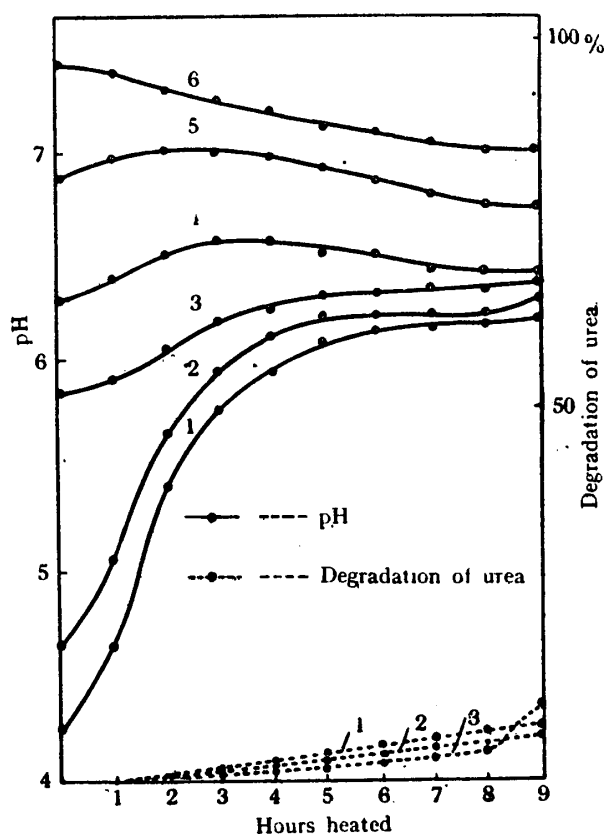
Fig. 3. Rate of shifting of pH and degra dation of urea in the reaction of urealactose system at the different initial pH medium heated to 100°C. fornine. hours.

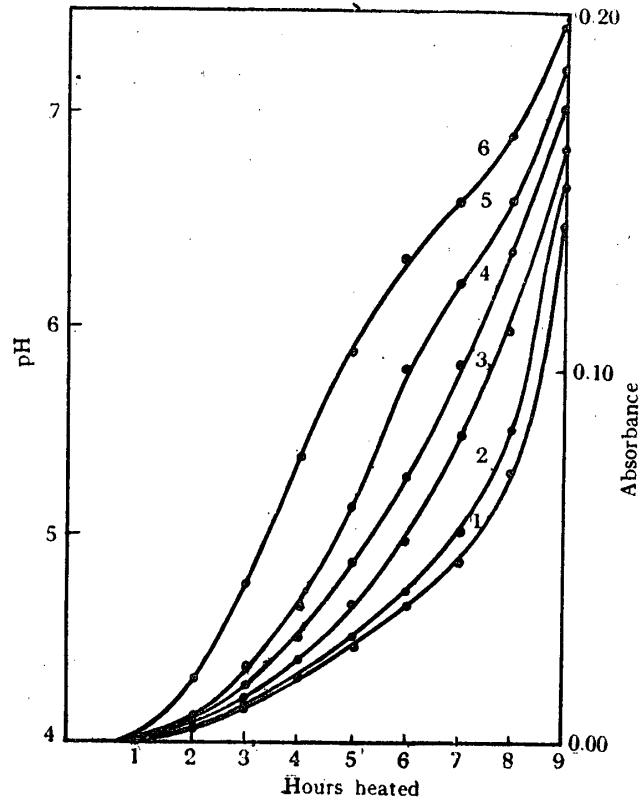| Sample number	Theoretical pH	Actual pH
1	4.53	4.29
2	5.59	4.68
3	6.47	5.90
4	6.98	6.43
5	7.38	6.93
6	8.30	7.52

Fig. 4. Rate of color formation in the reaction
of urea-lactose system at the different intial
pH medium beated to 100°C. for nine hours.

The data obtained indicate that the reaction in these systems change with the initial pH of the media, that is to say, the difference accompanying the medium pH depends upon the length of the inductive period for color formation and the range of variance in the pH before and after the heat treatment. In the acidic medium, the variance of pH in the course of the reactions decrease with the increase of initial pH in the medium, and the length of the inductive period and the decomposition of urea also decrease at the same time. In the alkaline side, however, the variance in pH was not observed but there was a gradual decrease with the increase of heating time and the inductive period is so reduced that the color formation commences immediately after the beginning of heat treatment. The available data shows that the degradation of urea in the alkaline side is sligat though the degree of degradation increased with the increase of heating time. The experimental evidence also show the existence of two stages in this browning reaction. On the basis of these data it is probable that the mechanism of browning reaction in the urea-lactose system differs according to whether the medium pH is acidic or alkaline.
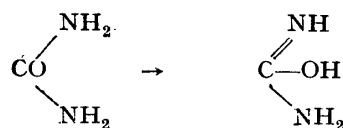
Discussion

To determine the effect of urea in the browning reaction in heated milk, the mechanisms of urea action was studied during browning reaction in the urea-lactose system with reference to milk. It is thought that there are three courses for the reaction of urea in the urea-lactose system as follows : (1) urea reacts with sugar by its own original structure, (2) urea decompose to ammonium carbonate during the heat treatment, then the ammonium carbonate takes part in the browning reaction reacting with sugars, (3) urea reacts with sugar in the intermediate type of the above described procedure, namely, the reaction in cooperation with urea by its own structure and ammonium carbonate as decomposed products from it all belong to this reaction.
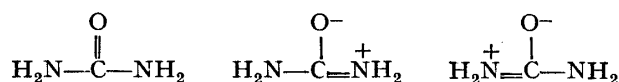
The following discussion refers to the course and mechanism for the action of urea in the browning reaction of the haeted urea-lactose system.

Weast and Mackinney,[30] reported that little or no effect accurs on the browning observed with urea in glucose solution heated from 0.5 to 8 hours under refluxed boiling. In these experiments it is shown that the reaction of urea for lactose solution is excellent being almost similar to ammonium carbonate. Ammonium carbonate and the decomposition product from urea, are the most effective reactant in the browning reaction of lactose solution as shown in Tables 1 and 2. The fact that carbonate ion has an excellent oxidativity for reducing sugars among some negative ions bave been reported by Ashida and Sugio.[31] It may appear that the effectivity of ammonium carbonate is partially due to the activity of carbonate ion, because the browning reaction belongs to the oxidation reaction. Weast and Mackinney, and Rice, Kertesy and Stolz[32] have found that ammonium oxalate and ammonium chloride accelerates the browning reaction in sugar solution, and Englis and Hanahan[33] reported that ammonium acetate is less effective. In Table 2 it has been shown that the inorganic and organic ammonium compounds, are accelerators besides ammonium acetate and dimethylamin hydrochrolide. It is probable, however, that the difference in the reactivity for the browning reaction in the lactose solution depends upon the activity of the negative ions in themselves, though amonium ion is involved as an accelerator. From a consideration of the above reported data it seems reasonable to conclude that the excellent activity of ammonium carbonate for this reaction is due to the cooperation of two constructed ions which accelerate the color formation. As shown in Table 1 urea does not give a little value for the apparent decomposition rate from urea to ammonium carbonate. If urea is subjected to the partial degradation by heat treatment in the urea-lactose system, ammonium carbonate prepared from urea will further accelerate the browning reaction. From avaliable data it is suggested
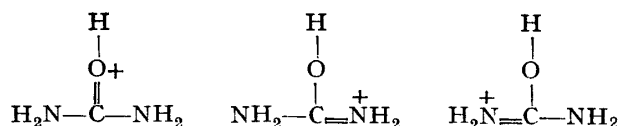
that urea principally reacted with lactose Ly its own structure accompanied with the decomposed product, ammonium carbonate. It appears that the experiments of some urea derivatives with lactose solution partially eliminated the possibility of the formation of ammonium carbonate as the initial stage reactant in urea-lactose system reaction. As shown in Table 3 the through structure of excellent reactants in some urea derivatives are found in the other less effective substances, as N, N'-acethylmethylurea, N, N'-diphenylurea. However, it seems that the less reactivity of these compounds depend upon the steric hindrance of substituted groups in amino radicals of urea to the approach of active groups in lactose. Though semicarbazide does not give the same medium pH of the other reactants and its reactivity could not be compared directly with the others, the depression of reactivity of thiourea substituted sulfur for oxygen in urea may reasonably indicate the striking reactivity of carbonyl radical in urea. The less effectiveness of the dimethyl substituted compound of one side of the amino groups in urea are partially due to the less reactivity of dimethylamine formed from their mother substances, even if these substances are completely decomposed by heat treatment. The mode of reaction of urea in the condensation process has been proved as follows :[34][35][36][37][38]

$$
\underset{\underset{NH_2}{\diagdown}}{\overset{\overset{NH_2}{\diagup}}{C O}} \quad \rightarrow \quad \underset{\underset{NH_2}{\diagdown}}{\overset{\overset{NH}{\diagup\!\!\diagup}}{C-OH}}
$$

that is to say, urea exhibits amide-imidol type of tautomerism. But Pauling, Brockway and Beach have shown that urea consists of a resonance hydrid of the following structure from the result of electron difraction.[39]
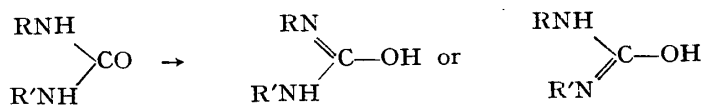
$$
\underset{H_2N-\overset{\overset{O}{\parallel}}{C}-NH_2}{} \qquad \underset{H_2N-\overset{\overset{O^-}{\mid}}{C}\!\!=\!\!\overset{+}{N}H_2}{} \qquad \underset{\overset{+}{H_2N}\!\!=\!\!\overset{\overset{O^-}{\mid}}{C}-NH_2}{}
$$

According to the work of Wheland, it has been found that the structure of urea ion is as follows,[40]

$$
\underset{H_2N-\overset{\overset{\overset{H}{\mid}}{\overset{O+}{\mid}}{\parallel}}{C}-NH_2}{} \qquad \underset{NH_2-\overset{\overset{\overset{H}{\mid}}{\overset{O}{\mid}}{\mid}}{C}\!\!=\!\!\overset{+}{N}H_2}{} \qquad \underset{\overset{+}{H_2N}\!\!=\!\!\overset{\overset{\overset{H}{\mid}}{\overset{O}{\mid}}{\mid}}{C}-NH_2}{}
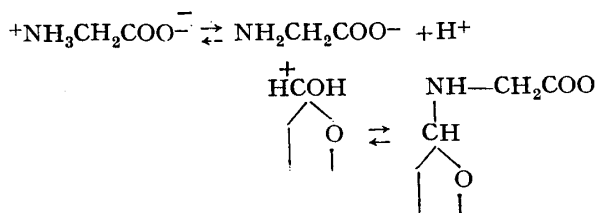$$

In consequence, it seems that the existence of the amide-imidol type of tautomerism for urea can be explained from the results of resonance hidrid as indicated in the above described structures. Therefore, it may be concluded from the result of this consideration that the indispensable structure for urea derivatives causing the browning reaction with lactose change to the imidol type

structure as follows by reaction,[41]

$$
\begin{array}{ccc}
\text{RNH} & \text{RN} & \text{RNH} \\
\diagdown\text{CO} \rightarrow & \diagdown\text{C—OH or} & \diagdown\text{C—OH} \\
\diagup & \diagup & \diagup \\
\text{R'NH} & \text{R'NH} & \text{R'N}
\end{array}
$$

and should involve at least a free hydrogen atom in the amino group of urea. On the basis of these data it seems reasonable to assume that urea reacts with lactose in the imidol type structure as described above.

From the results of kinetic measurements using the determination of pH depression, Katchalsky and his coworkers reported that the reaction occurs with undissociated amino groups and aldoses, and the extent of its reaction will depend largely on the pH and they also stated that the overall reaction is represented by the following scheme.

$$^+NH_3CH_2COO^- \rightleftarrows NH_2CH_2COO^- + H^+$$

$$
\begin{array}{cc}
\overset{+}{\text{HCOH}} & \text{NH—CH}_2\text{COO} \\
\diagup\diagdown & | \\
\left(\;\text{O} \quad \rightleftarrows \quad \right. & \text{CH} \\
| \quad | & \diagup\diagdown \\
& \left(\;\text{O}\right. \\
& | \quad |
\end{array}
$$

It has been shown that lactose has a dissociation constant as dibasic acid according to Shaffer.[43] Consequently it is considered that the distinct rising of pH in medium observed in the urea-lactose system reaction, as shown in figures 1 and 2, may indicate the result of interaction urea with lactose, that is to say, the rising of pH is due to the closing of the acidic group in lactose owing to the addition or combination of urea, urea-lactose complex. As the color formation does not occur unitl the striking rising of pH is completed, the period of pH rising is better to be called "inductive period" for the color formation. It is found that the production of urea-lactose complex are necessary for the brown color formation in this system. However, the reaction observed only in the acidic medium is varied compared with other type reaction in alkaline medium as shown in figures 3 and 4. In alkaline medium, the presence of the inductive period for the color formation has hardly been observed and the pH decreases with the increase of heating time. The color formation accompanied with the heat treatment commences immediately. In the nearly neutral zone between the acidic and alkaline medium, the intermediate type of both typical reaction types observed in the alkaline and acidic medium are presented. Thus it seems that the reaction in the alkaline medium is separated from the acidic medium reaction regardless of whether the reaction of the inductive period is completed immediately prion to that of color formation or the color formation commances in dependently with the inductive effect. Shiga reported that the reaction of

amino-sugar complex can be classified into two types by the medium pH.[42]
In view of the above facts and from avialable data it is coucluded that the
mechanism of the reaction in the alkaline medium belongs to the former type,
and the browning reaction of urea-lactose system principally consists of a two
stage reaction having on relation with the medium pH.

Summary

In the browning reaction of the heated urea-lactose system with special
reference to the heated milk the following results are confirmed.

1.   Urea principally reacts with lacotse in it own structure accompanied with
ammonium carbonate as the decomposed product of urea by heat treatment.
Ammonium carbonate produced are the most excellent accelerator for the color
formation among some organic and inorganic ammoinum compounds.

2.   The indispensable structure of some urea derivatives for the color
formation in lactose solution is as follows ;

$$\begin{array}{c} RNH \\ \diagdown \\ \diagup {\Large\diagdown}CO \\ R'NH \end{array}$$
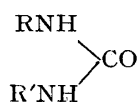
R, R' indicate the alkyl or phenyl radical and hydrogen atom.

3.   The fact that The reaction structure of urea is not of the amide type but
of imidol type of tautomerism is probatle, but not certain.

4.   The browning reaction principally consists of the two stages reaction, the
one is the inductive period reaction which may depend upon the formation of
urea-lactose complex, the other is the color formation.   In acidic medium, the
color formation commences after the inductive period indicating that the
striking rising of pH in the medium has been completed.   In the alkaline medium
however, the distinction of these reactions has not been observed.

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