

the bulb a few times. We claim no new principle,<sup>2</sup> but give this note for the benefit of those who may own an electrode of the Hildebrand type.

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF STANFORD UNIVERSITY]

## THE CARBAZYLIC ACIDS: THE AMMONIA ANALOGS OF THE CARBOXYLIC ACIDS<sup>1</sup>

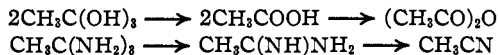
BY E. F. CORNELL

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**The Ammonia System.**—Liquid ammonia shows a striking similarity to water in many of its properties, particularly as an electrolytic solvent. This close correspondence purely as solvents has been extended so as to include similarities between derivatives of ammonia and the analogous water compounds. A system of acids, bases and salts related to ammonia as the aquo acids, bases and salts are related to water has been developed by Franklin.<sup>2</sup>

**The Carbazylic Acids.**—In this paper is given an account of the investigation of a class of ammono acids which are to be regarded as the ammonia or nitrogen analogs of the carboxylic acids. Just as aquo-acetic acid, for example, may be looked upon as being derived from a hypothetical ortho-acetic acid by the loss of a molecule of water, so a analogous ammono-acetic acid may be regarded as being derived from a hypothetical ortho-ammono-acetic acid by the loss of a molecule of ammonia, as represented by the parallel equations



The acid amidines and the nitriles have come to be known as carbazylic acids and acid anammonides inasmuch as they represent the nitrogen analogs, respectively, of the carboxylic acids and acid anhydrides. In the following discussion, it will be shown that the carbazylic acids and their derivatives are identical in several of their reactions with the carboxylic acids or their derivatives.<sup>3</sup>

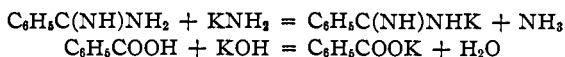
<sup>2</sup> See Michaelis and Rona, *Biochem. Z.*, **18**, 317 (1909).

<sup>1</sup> The material in this paper is from a thesis submitted to the Department of Chemistry and the Committee on Graduate Study of Stanford University in partial fulfillment of the requirement for the degree of Doctor of Philosophy, 1926.

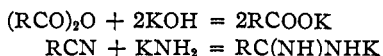
<sup>2</sup> Franklin, *Am. Chem. J.*, **47**, 285 (1912); Eighth Int. Cong. App. Chem., **6**, (1912); *THIS JOURNAL*, **46**, 2137 (1924).

<sup>3</sup> Franklin in his earlier papers [*THIS JOURNAL*, **27**, 800 (1905); *Am. Chem. J.*, **47**, 291 (1912)] mistakenly refers to acetamide and diacetamide as ammono-acetic acids,

**Salts of the Carbazylic Acids.**—Pinner<sup>4</sup> describes the formation of the silver salt of benzamidine by the action of silver nitrate on a water solution of benzamidine hydrochloride. The sodium and potassium salts cannot be prepared in such a manner since they are very readily hydrolyzed. Benedict<sup>5</sup> first prepared a salt of benzamidine in liquid ammonia solution by the action of potassium amide on benzamidine. The close analogy between this reaction and that involved in the formation of potassium benzoate by the action of potassium hydroxide on benzoic acid is clear from inspection of the equations



**Action of Ammono Bases on Acid Anammonides.**—The preparation of a number of salts of the carbazylic acids by the action of metallic amides on the nitriles will be described in the experimental part of this paper. Just as acid anhydrides react with bases to form salts, so should one expect the analogous reaction to take place when the acid anammonides are treated with ammono bases in liquid ammonia solution.



The carbazylic acids are formally tribasic acids and as such might be expected to form di- or possibly tri-metallic derivatives. It was found possible, however, to form only the monometallic derivative.

**Action of Hydrocyanic Acid on Potassium Amide.**—Hydrocyanic acid may be looked upon as the anammonide of formic acid and as such might be expected to react with metallic amides in liquid ammonia solution to form salts of ammonoformic acid. Hydrocyanic acid may also be considered as an ammonocarbonous acid,<sup>6</sup> and might be expected to react with an ammono base to form an ammonocarbonite and ammonia. As a matter of fact, this is what happens, for when a solution of hydrocyanic acid in liquid ammonia solution is treated with potassium amide, potassium cyanide is formed and not potassium formamidine.

**Action of Cyanogen on Potassium Amide.**—Cyanogen may be looked upon as the anammonide of oxalic acid. However, when a solution of cyano-urea as an ammonocarbonic acid and to calcium cyanamide as a salt of ammono-cyanic acid. Of course, we now speak of the first two compounds as aquo-ammono-acetic acids and of the latter two as an aquo-ammono-carbonic acid and a calcium ammono-carbonate, respectively. The term carboxazylic acid is now used to denote the mixed aquo-ammono-carboxylic acids of which acetamide is an example, since they represent compounds derived simultaneously from water and ammonia.

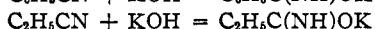
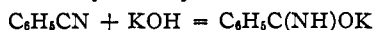
<sup>4</sup> Pinner, *Ber.*, 11, 6 (1878).

<sup>5</sup> Benedict, Master's "Thesis," Stanford University, 1924.

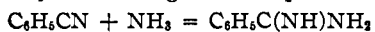
<sup>6</sup> Franklin, *J. Phys. Chem.*, 27, 168 (1923), has discussed in detail a number of reactions which have been interpreted in support of the view that hydrocyanic acid is a carbonous acid of the ammonia system.

anogen in liquid ammonia is treated with potassium amide, the potassium salt of ammono-oxalic acid is not formed. The products are potassium cyanide and potassium cyanamide, the potassium salts of ammonocarbonous and ammonocarbonic acids, respectively. Cyanogen thus behaves toward potassium amide as a mixed carbonous and carbonic anammonide,  $C=N-C\equiv N$ , rather than as an oxalic acid anammonide.

**Action of Aquo Bases on Acid Anammonides.**—We have seen that the nitriles react with metallic amides in liquid ammonia solution to give salts of carbazylic acids. It is reasonable to expect that they would also react with aquo bases in liquid ammonia solution to give salts of the carboxazylic acids. Potassium hydroxide does react, slowly at room temperature and fairly rapidly if heated to  $100^\circ$ , with benzonitrile and propionitrile in liquid ammonia solution to give potassium benzamide and potassium propionamide, respectively.



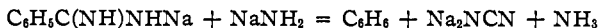
**Ammonation of the Acid Anammonides in the Presence of Acid.**—Benzonitrile and propionitrile, when heated in liquid ammonia solution containing ammonium chloride,<sup>7</sup> are ammonated to benzamidine and propionamidine, respectively, according to the equations



Attempts to ammonate hydrocyanic acid and cyanogen by heating them with ammonia and ammonium chloride failed, probably because of the polymerization which these substances undergo on heating.

**Pyrogenetic Decomposition of Salts of the Carbazylic Acids.**—It will be recalled that when an alkali salt of a carboxylic acid is heated with sodium hydroxide, there is produced a hydrocarbon containing one less carbon atom than the parent carboxylic acid and an alkali carbonate.

The analogous reaction has been found to take place with the salts of the carbazylic acids when heated with sodium or potassium amide, the reaction proceeding in the case of sodium ammonobenzoate, for example, as follows



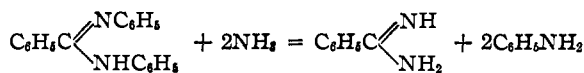
There are formed a hydrocarbon and sodium cyanamide, the salt of one of the ammonocarbonic acids. This again bears out the striking relationship between the carbazylic and carboxylic acids.

**Esters of the Carbazylic Acids.**—A large number of compounds are known which are related to the carbazylic acids just as ordinary esters are related to the carboxylic acids. These compounds are of the types  $RC(NR)NH_2$ ,  $RC(NR)NHR$ ,  $RC(NH)NR_2$  and  $RC(NR)NR_2$  and are

<sup>7</sup> Franklin and Kraus [*Am. Chem. J.*, **23**, 305 (1900)] have shown that this substance exhibits acidic properties in liquid ammonia solution.

to be regarded as esters of the carbazylic acids. These esters can be prepared by reactions similar to those used in the preparation of aquo esters. For example, ethylbenzamidine, the ethyl ester of ammonobenzoic acid, has been prepared<sup>8</sup> by the action of ethyl iodide on benzamidine, the ammonobenzoic acid. This is analogous to the preparation of an aquo ester by the action of an alkyl halide on a carboxylic acid.

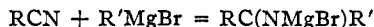
**Ammonolysis of Esters of Carbazylic Acids.**—Just as esters of the carboxylic acids can be hydrolyzed, so can the analogous process of ammonolysis be carried out with esters of the carbazylic acids. Niemann<sup>9</sup> has ammonolyzed several of these esters. He has shown that diphenylbenzamidine, for instance, undergoes ammonolysis when heated in liquid ammonia solution in the presence of an acid, the products being the carbazylic acid, benzamidine and the ammono alcohol, aniline.



This reaction is exactly analogous to the hydrolysis of aquo esters.

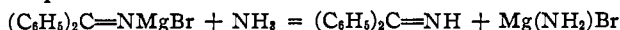
**Action of Ammonia on the Grignard Addition Product to a Nitrile.**—

The nitriles form addition products with the Grignard reagent, according to the equation



Moureu and Mignonac<sup>10</sup> have shown that hydrolysis of the addition products at low temperatures gives rise to the ketimines,  $\text{R}_2\text{C}=\text{NH}$ . These compounds, generally speaking, are ammonia analogs of the ketones. However, they are either acids or alcohols at the same time, by virtue of the presence of a hydrogen atom in the imide group.

The writer has shown that ammonolysis of the addition products gives the same compounds.



The reaction is analogous to the formation of ketones by the hydrolysis of the addition product of the Grignard reagent to the acid anhydrides.

### Experimental

The preparation of nineteen salts of the carbazylic acids was carried out in Faraday tubes, following the manipulation developed in this Laboratory.<sup>11</sup> The reactions generally consisted in the action of a liquid ammonia solution of a metallic amide on a liquid ammonia solution of a nitrile, according to the equation



<sup>8</sup> Pinner and Klein, *Ber.*, **11**, 7 (1878).

<sup>9</sup> Niemann, Master's "Thesis," Stanford University, 1926.

<sup>10</sup> Moureu and Mignonac, *Compt. rend.*, **156**, 1790 (1900).

<sup>11</sup> Franklin, *THIS JOURNAL*, **27**, 831 (1905); **35**, 1460 (1913); *J. Phys. Chem.*, **15**, 509 (1911); **16**, 694 (1912); Fitzgerald, *ibid.*, **29**, 1694 (1907).

The salts described in the table, with four exceptions, were prepared according to this general equation. In the notes following the table is given a description of the methods used in the preparation of these four salts, as well as observations of interest on several of the other salts. The metallic amide was prepared in every case by the reaction of the metal with ammonia, a spiral of iron wire being used as a catalyst. The salt in each case was washed several times by distilling fresh solvent onto it and decanting the wash liquor. Final drying was accomplished at room temperature in a vacuum. All the soluble salts were obtained beautifully crystallized and, in general, of sharply definite composition.

TABLE I  
SALTS OF CARBAZYLIC ACIDS

Name	Formula	Solubility <sup>a</sup>	Metal, %		Nitrogen, %	
			Calcd.	Found	Calcd.	Found
Potassium ammonoacetate <sup>b</sup>	CH <sub>3</sub> C(NH)NHK	ex. sol.	40.7	42.2	29.1	25.8
				42.0		24.8
Sodium ammonoacetate	CH <sub>3</sub> C(NH)NHNa	ex. sol.	28.8	28.7	35.0	33.7
Potas. ammonopropionate	C <sub>2</sub> H <sub>5</sub> C(NH)NHK	m. sol.	35.5	35.4	25.4	24.9
Sodium ammonopropionate	C <sub>2</sub> H <sub>5</sub> C(NH)NHNa	m. sol.	24.5	24.3	29.7	27.9
Cal. ammonopropionate <sup>c</sup>	[C <sub>2</sub> H <sub>5</sub> C(NH)NH] <sub>2</sub> Ca	insol.	21.9	25.9	..	..
Potas. ammono- <i>n</i> -butyrate	C <sub>3</sub> H <sub>7</sub> C(NH)NHK	m. sol.	31.5	31.7	22.6	21.9
Sodium ammono- <i>n</i> -butyrate	C <sub>3</sub> H <sub>7</sub> C(NH)NHNa	m. sol.	21.3	21.2	25.9	25.3
Potas. ammono- <i>n</i> -valerate	C <sub>4</sub> H <sub>9</sub> C(NH)NHK	m. sol.	28.3	28.5	20.2	19.9
Potas. ammono- <i>iso</i> -caproate	C <sub>5</sub> H <sub>11</sub> C(NH)NHK	m. sol.	25.7	26.0	18.4	18.2
Potas. ammonopalmitate	C <sub>14</sub> H <sub>29</sub> C(NH)NHK	insol.	13.4	12.9	9.6	7.9
Potas. ammonosuccinate	(CH <sub>2</sub> C(NH)NHK) <sub>2</sub>	insol.	41.1	41.7	..	..
Potas. ammonobenzoate <sup>d</sup>	C <sub>6</sub> H <sub>5</sub> C(NH)NHK	m. sol.	24.7	25.0	17.7	17.8
				24.5		17.6
Sodium ammonobenzoate	C <sub>6</sub> H <sub>5</sub> C(NH)NHNa	m. sol.	16.2	16.7	19.8	20.0
Silver ammonobenzoate <sup>e</sup>	C <sub>6</sub> H <sub>5</sub> C(NH)NHAg	insol.	47.6	47.0	12.3	15.5
Calcium ammonobenzoate <sup>e</sup>	[C <sub>6</sub> H <sub>5</sub> C(NH)NH] <sub>2</sub> Ca	insol.	14.4	17.4	..	..
Cupric ammonobenzoate <sup>f</sup>	[C <sub>6</sub> H <sub>5</sub> C(NH)NH] <sub>2</sub> Cu	insol.	21.1	23.5	18.6	15.2
Cuprous ammonobenzoate <sup>g</sup>	C <sub>6</sub> H <sub>5</sub> C(NH)NHCu	insol.	34.9	34.5	15.3	13.4
Potas. ammono- <i>p</i> -toluate	CH <sub>3</sub> C <sub>6</sub> H <sub>4</sub> C(NH)NHK	m. sol.	22.7	22.0	16.3	16.2
Sod. dibenzoylimidoimide <sup>h</sup>	(C <sub>6</sub> H <sub>5</sub> C=NH) <sub>2</sub> NNa	m. sol.	9.4	9.2	17.1	17.0

<sup>a</sup> Rough estimates of solubilities at room temperature are indicated: ex. sol. = extremely soluble; v. sol. = very soluble; m. sol. = moderately soluble; insol. = insoluble or very slightly soluble.

<sup>b</sup> This salt is extremely soluble and could be washed but once. The high analytical values are due to the presence of potassium amide in the sample.

<sup>c</sup> The high values for calcium on this salt and on calcium ammonobenzoate are explained on the basis of the presence of some unchanged calcium amide in the samples. Since both the calcium amide and the salt are insoluble, the reaction is slow and incomplete, even though the nitrile solutions are allowed to stand in contact with the calcium amide for some time.

<sup>d</sup> Benedict, Master's "Thesis," Stanford University, 1924, first prepared this salt by the action of potassium amide on benzamidine and also upon benzonitrile. Found: K, 23.9; N, 18.1. An attempt was made to prepare the dipotassium salt

by using an excess of two equivalents of potassium amide. The salt was found on analysis to be the monopotassium derivative. Found: K, 24.5; N, 17.6.

\* Silver amide does not dissolve in either benzamidine or benzonitrile solution. This salt was prepared by the action of silver nitrate on potassium ammonobenzoate, according to the equation  $C_6H_5C(NH)NHK + AgNO_3 = C_6H_5C(NH)NHAg + KNO_3$ .

† The cupric salt was prepared by the action of a solution of  $Cu(NO_3)_2 \cdot 4NH_3$  on potassium ammonobenzoate,  $2C_6H_5C(NH)NHK + Cu(NO_3)_2 = [C_6H_5C(NH)NH]_2Cu + 2KNO_3$ . The analysis of this salt indicates that it is contaminated with some of the cuprous salt.

‡ A quantity of the cupric salt contained in the wash leg of the tube was found to change gradually from a green to a white color. The analysis of this white salt showed it to be cuprous ammonobenzoate. The change from cupric to cuprous condition has also been noted in the case of cupric amide, Fitzgerald, *THIS JOURNAL*, 29, 657 (1907); Franklin, *ibid.*, 34, 1501 (1912).

§ Benzonitrile is formed by the loss of a molecule of ammonia from benzamidine. It is possible for two molecules of benzamidine to lose a molecule of ammonia between them. The resulting compound would still be an ammonobenzoic acid. Pinner, *Ber.*, 11, 8 (1878), describes such a compound, dibenzoylimido-imide,  $[C_6H_5C(NH)]_2NH$ . Sodium amide reacts with this acid to give a salt. An attempt was made to prepare the potassium salt. Because of its high solubility, however, a sample sufficiently pure for analysis was not obtained.

**Action of Potassium Amide on Hydrocyanic Acid.**—A quantity of hydrocyanic acid, 0.52 g., was obtained in the following manner. A solution of equimolecular quantities of ammonium chloride and sodium cyanide contained in one leg of a reaction tube was evaporated to dryness. The resulting residue was heated to  $40^\circ$  while keeping the empty leg in a bath of liquid ammonia. Ammonium cyanide sublimed over, leaving the sodium chloride as a residue. The leg containing the residue was opened, the residue removed and 0.72 g. of potassium introduced. Addition of potassium amide solution to the hydrocyanic acid solution caused the immediate formation of a crop of white crystals of potassium cyanide. Calculated for KCN: K, 60.0. Found: K, 60.8.

**Action of Potassium Amide on Cyanogen.**—A quantity of cyanogen was liquefied in one leg of a reaction tube. The addition of the potassium amide from 0.80 g. of potassium contained in the other leg produced a heavy white amorphous precipitate of potassium cyanide and potassium cyanamide. The presence of cyanide and cyanamide was demonstrated by the usual qualitative tests. The equation is  $C_2N_2 + 3KNH_2 = K_2NCN + KCN + 2NH_3$ .

**Action of Potassium Hydroxide on Benzonitrile and Propionitrile.**—In a straight tube, sealed at one end, were placed 1.31 g. of benzonitrile and 0.71 g. of potassium hydroxide which had been freshly fused and heated to redness to remove traces of water. Ammonia was distilled into the tube until it was about one-third full and the open end sealed off. The tube was heated for sixteen hours at  $175^\circ$ , following the procedure as described by Blair.<sup>12</sup> The solution, after cooling, deposited white flaky crystals of potassium benzamide. Calcd. for  $C_6H_5CONHK$ : K, 24.5. Found: K, 21.6.

In a similar manner, 1.39 g. of propionitrile and 1.40 g. of potassium hydroxide were heated for twenty-four hours at  $200^\circ$ . The solution, upon cooling, contained a quantity of light, flaky crystals of potassium propionamide. Calcd. for  $C_2H_5CONHK$ : K, 35.2. Found: K, 35.8.

In both cases, the major portion of the hydroxide and nitrile remained unchanged after heating.

<sup>12</sup> Blair, *THIS JOURNAL*, 48, 91 (1926).

**Ammonation of Benzonitrile and Propionitrile.**—A solution of 2.45 g. of benzonitrile and 1.32 g. of ammonium chloride was heated for sixteen hours at 200° in the same manner as stated above. The residue, after evaporation of the ammonia and drying in vacuum, was extracted with absolute alcohol. The alcohol solution contained a small quantity of benzamidine, which was identified by forming its insoluble picric acid salt, melting at 225°.

A solution of 1.89 g. of propionitrile and 1.85 g. of ammonium chloride was heated for twenty-four hours at 200°. The residue after evaporation of the ammonia was dried in a vacuum and extracted with absolute alcohol. The alcohol solution was found to contain a small quantity of propionamidine, which was identified through the formation of a double salt with platinic chloride.

**Pyrogenetic Decomposition of Salts of Carbazylic Acids.**—The potassium salts of ammonobenzoic acid, ammono-*isocaproic* acid, ammono-*n*-valeric acid, ammono-*n*-butyric acid, ammonopropionic acid and ammono-acetic acid were heated with potassium amide. In the case of the first three, a quantity of a volatile liquid was obtained upon which the molecular weights were determined by the Victor Meyer method. Values of 80.3, 73.3 and 63.4, respectively, were obtained, which correspond fairly closely to benzene, pentane and butane. The hydrocarbons produced by the decomposition of the last three salts, propane, ethane and methane, respectively, had such low boiling points that they could not be condensed readily. It is significant that a combustible gas was produced in each case.

However, in every case the presence of cyanamide in relatively large quantity was demonstrated by the formation of the characteristic yellow precipitate of silver cyanamide on addition of silver nitrate to an ammoniacal solution of the residue. The same reaction takes place when benzonitrile and sodium amide are heated together, benzene and sodium cyanamide being produced according to the equation  $C_6H_5CN + 2NaNH_2 = C_6H_6 + Na_2NCN + NH_3$ . Experimentally this was realized by sealing up equimolecular quantities of benzonitrile and sodium amide in straight tubes and heating for two hours at 250°.

**Preparation of Benzamidine from Potassium Ammonobenzoate.**—Benzamidine has been prepared from its potassium salt by treatment in alcohol solution with hydrochloric chloride,  $C_6H_5C(NH)NHK + 2HCl = C_6H_5C(NH)NH_2 \cdot HCl + KCl$ .

Potassium ammonobenzoate was prepared from 1.00 g. of benzonitrile and 0.30 g. of potassium in the usual way. The ammonia was allowed to evaporate, the salt was dried in a vacuum at room temperature and the dry salt then treated with absolute alcohol saturated with dry hydrogen chloride. The precipitate of potassium chloride was filtered off. Evaporation of the alcohol yielded 1.35 g. of a white solid. This was shown to be benzamidine hydrochloride by its melting point. The yield obtained is 89% of the theoretical.

It was possible also to obtain benzamidine from potassium ammonobenzoate by carefully hydrolyzing this salt. However, the yield was always poor since the hydrolysis proceeds further than with the formation of benzamidine; potassium benzamide and ultimately potassium benzoate are formed.

**Action of Ammonia on the Grignard Reagent Addition Product to Benzonitrile.**—A quantity of the addition product of phenylmagnesium bromide to benzonitrile was placed in a reaction tube and, after sealing, ammonia was distilled in. The solid was extracted repeatedly with ammonia and the ammonia was then allowed to evaporate completely. There remained in the leg containing the washings a light brown oil. This oil was dissolved in ether and the solution saturated with dry hydrogen chloride. The white crystalline precipitate which formed was filtered off, washed once with ether and recrystallized from chloroform and ether. The analysis showed the compound to

be diphenyl ketimine hydrochloride. Calcd. for  $C_{13}H_{12}NCl$ : N, 6.4; Cl, 16.3. Found: N, 6.6; Cl, 18.2.

In conclusion, the writer wishes to express his appreciation to Dr. Franklin, under whose kind guidance the work presented here has been carried out.

### Summary

1. It is shown in this paper that the amidines are to be regarded as the ammonia analogs of the carboxylic acids. The term carbazylic acid has been used to denote these acids.

2. The alkyl and aryl cyanides have been shown to be the anammonides of the carbazylic acids.

3. The preparation of a number of salts of the different carbazylic acids has been described.

4. The ammonation of the acid anammonides in the presence of an acid has been demonstrated.

5. The decomposition of the salts of the carbazylic acids, when heated with a metallic amide, proceeds with the formation of a hydrocarbon and an alkali salt of cyanamide.

6. The difference in behavior of hydrocyanic acid and cyanogen from that of other nitriles toward potassium amide in liquid ammonia solution has been noted and an explanation offered.

STANFORD UNIVERSITY, CALIFORNIA

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[CONTRIBUTION FROM THE LABORATORY OF PHYSICAL CHEMISTRY OF THE UNIVERSITY OF UPSALA]

## THE MOLECULAR WEIGHTS OF SERUM ALBUMIN AND OF SERUM GLOBULIN

BY THE SVEDBERG AND BERTIL SJÖGREN

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The two chief protein constituents of blood serum are serum albumin and serum globulin. Crystallized serum albumin was obtained as early as 1894,<sup>1</sup> while serum globulin crystals have not as yet been observed. The fact that serum albumin is easily crystallized has been taken as an indication of its homogeneity. Recently, however, the validity of this conclusion has been questioned by Sørensen,<sup>2</sup> who found that by fractional crystallization a series of protein samples with different solubilities could be obtained from serum albumin. The osmotic pressures of the various fractions were, however, the same. The question as to whether serum globulin is a simple protein or a mixture has been the object of much experi-

<sup>1</sup> A. Gürber, *Würzburger Physiol.-med. Ges.*, 1894, 113; 1895, 26.

<sup>2</sup> S. P. L. Sørensen, "Proteins," The Fleischmann Company, New York, 1925, p. 40.