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The Formation of Nicotinamide from Nicotinic Acid by the Rat

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Part of the essential content of this paper has been communicated to the Biochemical Society (Ellinger, 1946*a*). The publication of details was postponed because the quantitative estimation of the nicotinamide formed, was carried out by an indirect biological method, which involved some uncontrollable factors. Confirmation of the results was desired using a more exact method which would permit the separate determination of nicotinamide and nicotinic acid in the same solution; but no suitable method has so far been found. No method based on the König (1904) principle is sensitive enough for the small amounts of nicotinamide and nicotinic acid to be measured in these experiments, and the errors involved in the estimation of nicotinamide by the differences found before and after alkaline hydrolysis are far too large. Scudi's (1946) method, based on the fluorimetric measurement of a pigment developed from nicotinamide with cyanogen bromide and alkali and extracted by *iso*butanol, permits the measurement of as little as 1 μg. of nicotinamide in the presence of nicotinic acid; the latter does not give a fluorescent product, but aneurin gives with the reagents a product of so high fluorescent efficiency that the method cannot be used for the determination of nicotinamide in the presence of aneurin. Microbiological methods using bacteria such as *Pasteurella* strains (Koser, Berkman & Dorfman, 1941) or *Leuconostoc mesenteroides* (Johnson, 1945; Krehl, de la Huerga, Elvehjem & Hant, 1946) including some strains of A.T.C.C. 9135 proved unsatisfactory, as all strains of both types of bacteria so far available responded to nicotinamide as well as nicotinic acid. It has been decided, therefore, to publish the results so far obtained.

Surviving rat liver, but not kidney or muscle, methylate nicotinamide to nicotinamide methochloride. None of the tissues mentioned converts nicotinic acid into either nicotinamide methochloride or trigonelline (Perlzweig, Bernheim & Bernheim, 1943). Ellinger & Coulson (1944) have

shown that in man two separate processes are involved in the formation of nicotinamide methochloride from nicotinic acid; these could be affected separately in certain diseases.

The present paper deals with the site and mechanism of the formation of nicotinamide from nicotinic acid by rat tissue. The only other known formation of a simple acid amide in the animal body is that of glutamine from glutamic acid (Krebs, 1935). The nicotinamide formed could only be determined by measuring the nicotinamide methochloride formed from it by liver slices, and the suitability of this method for quantitative measurement had first to be evaluated. Different rat tissues were then tested for their ability to form the amide with or without the addition of potential NH_3 donors such as glutamine or asparagine.

METHODS

Rats of both sexes, weighing 150–400 g., of black and white Lister stock or albinos of own stock, were fed after weaning on a mixed diet (Ellinger, 1946*b*); their daily urinary output of nicotinamide methochloride and their response to injected nicotinamide were studied by the method of Coulson, Ellinger & Holden (1944) in most cases. For experiment, the rats were stunned and killed by bleeding, the various organs to be examined being removed and kept in closed Petri dishes. The method of examination used was similar to that of Perlzweig *et al.* (1943). Pieces of the organs were quickly weighed, cut into slices 0·2–0·4 mm. thick, washed twice in modified Ringer's solution (Krebs & Henseleit, 1932; Krebs, 1933) and suspended in weighing bottles in 10 ml. of a similar solution containing 1 mg. of ammonium nicotinate. This solution which had previously been saturated with a mixture of O_2 (95 vols.) and CO_2 (5 vols.) was again aerated with the same gas mixture, the bottle closed securely and agitated for 3 hr. at 37°. In some experiments, slices of the organ to be examined were shaken together with liver slices, in others they were shaken separately with the ammonium nicotinate solution, the solution being decanted into another weighing bottle and liver slices added to it. After renewed aeration with the gas mixture, agitation was continued at 37° for 3 hr. In each instance the following controls were carried out. To measure the methylating efficiency of the liver used, 1 mg. nicotinamide was added instead of ammonium nicotinate to liver slices. Blanks without addition of nicotinate or nicotinamide were run, and the nicotinamide methochloride values obtained were subtracted from those of the nicotinate experiments. After shaking, the supernatant liquid was decanted into calibrated centrifuge tubes, the tissue was washed twice with 0·5 ml. of Ringer's solution, the washings being added to the supernatant liquid; 0·5 ml. of 20% (w/v) trichloroacetic acid was added, and after thorough mixing the tubes were kept in a water bath at 75–80° for 30 min. After cooling, the volume was adjusted to 12 ml. with Ringer's solution and the precipitated proteins were removed by centrifugation. In portions of the supernatant liquid nicotinamide methochloride was first estimated by the method of Perlzweig *et al.* (1943) without adsorption on Decalso and by the method of Coulson *et al.* (1944). The former frequently gave erroneously high values since the carbinols, the fluorescence of which was to be measured, were immediately oxidized into pyridones which possess much greater fluorescence efficiency than the carbinols. This method was therefore discontinued.

RESULTS

To test the suitability of the method for the quantitative estimation of nicotinamide, the following experiments were carried out. Results are described in terms of Q_{NM} defined as mg. nicotinamide methochloride formed/g. of tissue/hr.

Table 1 shows that the methylating efficiency of livers of various rats differs considerably, and that this efficiency parallels the height of nicotinamide methochloride elimination of the rat while alive. Different pieces of liver of the same rat, however, show a fairly consistent methylating efficiency, the maximal deviation being less than 10% when pieces of similar size are used. From this it is evident that the amount of nicotinamide present in a solution can be assayed when the methylating efficiency of the same liver is determined with known amounts of nicotinamide.

Table 1. *Methylating efficiency (Q_{NM}) of different pieces of the same rat liver and relation of such efficiency to the previous nicotinamide methochloride* (NM) *elimination of the rat when alive*

(Incubation 3 hr. in 5 ml. Ringer's solution with 1 mg. nicotinamide. Each mean is the result of eight experiments with liver slices weighing between 300 and 600 mg.)

No. of rat	One week's average daily NM output (μg./day)	Mean wet wt. of liver slices used (mg.)	Mean NM formed (μg.)	Mean Q_{NM}
1	57	452	5·84	4·4
		S.D. ±34·9	S.D. ±0·264	S.D. ±0·47
2	138	459	15·55	11·4
		S.D. ±36·9	S.D. ±0·902	S.D. ±0·71
3	417	438	35·6	27·0
		S.D. ±32·9	S.D. ±2·368	S.D. ±0·72
4	937	437	68·6	52·5
		S.D. ±34·8	S.D. ±4·634	S.D. ±1·31

Table 2 demonstrates that if the amount of Ringer's solution, of nicotinamide and the time of incubation are kept constant, the Q_{NM} depends on the amount of liver tissue used in the experiment, being greater with small amounts of liver and smaller with larger amounts. Between about 250 and 550 mg., the Q_{NM} is sufficiently constant to obtain comparable results.

Table 2. *Dependence of Q_{NM} of liver slices on the amount of liver used in the experiment*

(Incubation 3 hr. in 10 ml. Ringer's solution with 1 mg. nicotinamide.)

No. of rat	One week's average daily NM output (μg./day)	Wet wt. of liver used (mg.)	NM formed in 3 hr. (μg.)	Q_{NM}
5	228	53	3·6	20·4
		108	6·1	18·7
		158	8·7	18·3
		217	11·2	17·2
		263	13·3	16·8
		302	14·7	16·2
		357	17·9	16·7
		417	20·9	15·9
		480	23·5	16·3
		550	25·5	15·4
		627	29·0	15·2
		754	31·9	14·1
		877	33·6	12·8
		1012	36·9	12·2

Two more similar experiments yielded essentially identical results.

The speed of methylation was examined by incubation of pieces of the same liver (300–400 mg.) with 1 mg. nicotinamide for 0·5, 1, 2, 3 and 4 hr. and measuring the methylated product formed. The speed was fairly constant for the first 3 hr. and subsequently slowed down. An incubation of 3 hr. was therefore adopted. The influence of nicotinamide concentration on the Q_{NM} was measured by varying the amounts of nicotinamide added between 50 μg. and 5 mg. Under the conditions of the experiment, amounts increasing from 50 to 200 μg. produced linearly increasing amounts of the methylated product, but between 200 and 500 μg. a maximum of methylation was reached. The effect of pH on the methylating mechanism was examined by varying the pH (measured by glass electrode before incubation) of the Ringer's solution. The pH was varied by changing the bicarbonate concentration. Table 3 shows that the methylation of nicotinamide by liver depends on the pH and has a maximum between pH 7·35 and 7·18.

The effect of anaerobiosis on the methylating mechanism of the liver was examined either by saturating Ringer's solution with a mixture of N_2 (95 vol.) and CO_2 (5 vol.) or by adding KCN (10^{-5}, 10^{-4} or 10^{-3} M) to the Ringer's solution. Table 4 demonstrates that the methylating process is strictly aerobic and inhibited by cyanide. To examine the influence on the Q_{NM} of the thickness of the slices, these were cut 0·2–0·4 and 1–2 mm. thick from pieces of 500–550 mg. of the same liver and incubated under identical conditions. The approximate thickness was determined by cutting cubes from liver, measuring their length, and dividing by the number of slices obtained. No appreciable difference could be seen when slices of 0·2, 0·3 and 0·4 mm. were used, but slices of 1 or 2 mm. were 10–20 % less efficient.

Table 3. *Influence of* pH *on methylation of nicotinamide by liver slices*

(Incubation 3 hr., 1 mg. nicotinamide.)

No. of rat	Concentration of bicarbonate (M)	pH of Ringer before incubation	Wet wt. of liver used (mg.)	NM formed (μg.)	Q_{NM}	Percentage of maximum
14	0·130	8·23	371	9·4	8·4	48·1
	0·100	8·10	358	11·5	10·7	61·0
	0·070	7·87	367	14·6	13·2	75·1
	0·040	7·69	382	19·0	16·6	94·9
	0·020	7·35	353	18·3	17·3	98·8
	0·010	7·18	388	20·4	17·5	100·0
	0·005	6·76	367	15·7	14·3	81·5
	0·002	6·41	359	11·6	10·8	61·6

One other experiment yielded essentially identical results.

Table 4. *Influence of anaerobiosis and cyanide on the methylation of nicotinamide by liver slices*

(Incubation 3 hr., 1 mg. nicotinamide.)

No. of rat	Gas mixture	Concentration of KCN (M)	Wet wt. of liver used (mg.)	NM formed (μg.)	Q_{NM}
16	O_2/CO_2	0	396	29·4	24·7
	O_2/CO_2	0	363	27·4	25·1
	N_2/CO_2	0	372	2·2	0·2
	N_2/CO_2	0	388	0	0
	O_2/CO_2	10^{-5}	352	20·9	19·8
	O_2/CO_2	10^{-4}	393	11·9	10·1
	O_2/CO_2	10^{-3}	376	3·1	2·7

The livers used in the methionine experiment (Table 5) were chosen from various rats, whose individual spontaneous nicotinamide methochloride

elimination and whose response to injected nicotinamide had been found to differ markedly. The addition of methionine to the system nicotinamide, liver, Ringer's solution, had in many cases no effect on the methylating efficiency. However, in some instances, particularly when the initial methylating efficiency was low, methionine increased the Q_{NM} appreciably.

Table 5. *Influence of added methionine on the methylation of nicotinamide by liver slices*

(Incubation 3 hr., 1 mg. nicotinamide.)

No. of rat	One week's average daily NM output (mg./day)	Methionine (mg.)	Wet wt. of liver used (mg.)	NM formed (μg.)	Q_{NM}
1	57	0	443	5·6	4·2
		0	491	6·6	4·5
		1	455	10·7	7·8
		1	477	11·5	8·0
2	138	0	437	14·8	11·3
		0	485	16·8	11·5
		1	422	16·6	13·1
		1	447	17·3	12·9
3	417	0	412	34·0	26·8
		0	453	36·9	27·1
		1	423	33·3	26·2
		1	437	34·8	26·6
4	937	0	402	64·6	53·4
		0	449	71·0	52·8
		1	438	68·9	52·5
		1	453	72·8	53·6

Three more experiments yielded essentially identical results.

Liver alone synthesized nicotinamide methochloride from nicotinamide (Tables 6–8), but not from nicotinic acid (Tables 6, 7). If, however, glutamine was added to the system liver, ammonium nicotinate, Ringer's solution, some formation of nicotinamide methochloride could be observed in about half of the experiments, the addition of asparagine having no effect (Table 6). The glutamine used was tested before use with Nessler's reagent (Archibald, 1944).

Table 6. *Influence of added glutamine* (Gl) *or asparagine* (As) *on the formation of nicotinamide methochloride* (NM) *from* 1 *mg. of ammonium nicotinate* (Am.nic.) *by liver slices*

(Incubation 3 hr.)

No. of rat	Added Gl or As (mg.)	Substrate	Wet wt. of liver slice (mg.)	NM formed (μg.)	Q_{NM}
22	0	Nicotinamide	433	24·5	18·8
	0	Nicotinamide	448	25·9	19·3
	0	Am.nic.	471	0·3	0·2
	0	Am.nic.	406	0	0
	10 Gl	Am.nic.	423	0	0
	10 Gl	Am.nic.	434	3·0	2·3
	10 Gl	Am.nic.	444	11·1	8·3
	10 Gl	Am.nic.	412	11·9	9·6
	10 As	Am.nic.	433	0·1	0·1
	10 As	Am.nic.	428	0·4	0·3

Four more experiments yielded essentially identical results.

The following organs were tested for amidation of nicotinic acid: kidney cortex, brain cortex, spleen,

Table 7. *Synthesis of nicotinamide methochloride* (NM) *from ammonium nicotinate* (Am.nic.) *and nicotinamide* (NA) *by slices of various organs*

(Incubation 3 hr.)

Tissue	No. of experiments	Limits of wt. of liver (mg.)	Substrate	Limits of wt. of tissue other than liver (mg.)	NM formed/g. liver/hr. (μg.) (average in brackets)
Liver	43	230–610	NA, 1 mg.	—	3·6–73·5 (27·3)
Liver	22	290–580	Am.nic., 1 mg.	—	0–2·9 (0·5)
Kidney	7	—	NA, 1 mg.	230–320	0–2·1 (0·5)
Kidney	7	—	Am.nic., 1 mg.	220–340	0–1·2 (0·3)
Brain	8	—	NA, 1 mg.	180–280	0–1·9 (0·3)
Brain	8	—	Am.nic., 1 mg.	170–300	0–0·8 (0·2)
Liver	7	354–560	Kidney + NA, 1 mg.	140–590	4·3–58·2 (26·4)
Liver	30	340–590	Kidney + Am.nic., 1 mg.	255–585	4·1–62·3 (22·1)
Liver	8	360–580	Kidney + Am.nic., 1 mg. (anaerobically)	330–470	0–1·3 (0·3)
Liver	7	360–440	Brain + NA, 1 mg.	130–190	7·2–59·2 (26·1)
Liver	20	380–520	Brain + Am.nic., 1 mg.	120–210	7·6–51·3 (21·7)
Liver	4	365–490	Brain + Am.nic., 1 mg. (anaerobically)	140–190	0–1·1 (0·3)
Liver	4	330–430	Spleen + Am.nic., 1 mg.	165–225	0
Liver	4	320–480	Skelet. muscle + Am.nic., 1 mg.	530–620	0
Liver	4	330–450	Heart muscle + Am.nic., 1 mg.	260–300	0
Liver	4	350–450	Pancreas + Am.nic., 1 mg.	50–70	0–0·9 (0·3)
Liver	4	380–510	Intestin. mucous membrane + Am.nic., 1 mg.	110–130	0–0·8 (0·2)

skeletal muscle, heart muscle, pancreas and intestinal mucosa. In each case slices were incubated with 1 mg. of ammonium nicotinate and liver slices, and parallel experiments were made with nicotinamide instead of nicotinate. In addition, the formation of nicotinamide methochloride from ammonium nicotinate or nicotinamide by kidney or brain slices without liver was tested. Table 7 shows that, of all tissues tested, only kidney and brain cortex were able to form nicotinamide from ammonium nicotinate. In some of the experiments, the pyridine carbinols were immediately oxidized to pyridones when estimated by the method of Coulson *et al.* (1944) when kidney was agitated together with liver. No measurement was possible owing to the different fluorescence efficiencies of the

Table 8. *The influence of disintegration of liver tissue on the methylation of nicotinamide and of kidney and brain on the amidation of ammonium nicotinate* (Am.nic.)

(Incubation 3 hr.)

No. of rat	Tissue	Wet wt. of liver used (mg.)	Substrate	NM formed (μg.)	NM formed/g. liver/hr. (μg.)
27	Liver slices	354	1 mg. NA	24·8	23·3
	Liver slices	377	1 mg. NA	26·8	23·7
	Liver broken up cells	348	1 mg. NA	0·2	0·2
	Liver broken up cells	386	1 mg. NA	0	0
	Liver slices	349	1 mg. Am.nic.—302 mg. kidney slices	18·6	17·8
	Liver slices	362	1 mg. Am.nic.—312 mg. kidney slices	19·8	18·2
	Liver slices	380	1 mg. Am.nic.—331 mg. kidney broken up cells	0·4	0·3
	Liver slices	337	1 mg. Am.nic.—347 mg. kidney broken up cells	0·1	0·1
	Liver slices	367	1 mg. Am.nic.—173 mg. brain slices	17·8	16·2
	Liver slices	358	1 mg. Am.nic.—194 mg. brain slices	18·8	17·4
	Liver slices	371	1 mg. Am.nic.—167 mg. brain broken up cells	0·1	0·1
	Liver slices	349	1 mg. Am.nic.—203 mg. brain broken up cells	0	0

Table 9. *Influence of* pH *on amide formation from ammonium nicotinate by kidney and brain slices*

(Incubation 3 hr., 1 mg. ammonium nicotinate.)

No. of rat	Concentration of bicarbonate (M)	pH of solution before incubation	Wet wt. of liver (mg.)	Wet wt. of kidney (K.) or brain (B.) (mg.)		NM formed (μg.)	Q_{NM}	Percentage of maximum
28	0·130	8·23	356	259	K.	8·4	7·9	37·2
	0·100	8·10	349	254		11·3	10·8	51·0
	0·070	7·87	377	263		16·1	14·3	67·4
	0·040	7·69	383	258		20·8	18·3	86·0
	0·020	7·35	354	255		22·0	20·7	97·3
	0·010	7·18	392	260		25·1	21·3	100·0
	0·005	6·76	351	257		22·7	19·8	93·2
	0·002	6·41	366	256		15·1	13·7	64·5
	0·130	8·23	348	202	B.	7·1	6·8	30·1
	0·100	8·10	352	207		10·0	9·5	49·4
	0·070	7·87	361	203		13·8	12·7	66·0
	0·040	7·69	354	208		17·0	16·1	83·7
	0·020	7·35	357	204		20·2	18·9	98·0
	0·010	7·18	349	203		20·2	19·3	100·0
	0·005	6·76	360	207		19·5	17·7	92·8
	0·002	6·41	352	202		13·6	12·9	67·0

two compounds and these experiments had to be discarded. The mechanism of amidation by kidney and brain was studied under anaerobic conditions by using a mixture of N_2 (95 vol.) and CO_2 (5 vol.) for saturating and aerating Ringer's solution in which the kidney or brain were incubated. Before adding the supernatant liquid to the liver slices, it was thoroughly aerated with the O_2-CO_2 mixture for 15 min. The methylating process was strictly aerobic.

Broken up cells (*brei*) of liver, kidney and brain were also tested for their amide-forming and methylating activities. They were prepared by grinding the finely cut tissue in an agate mortar. Table 8 shows that the methylating effect of the liver as well as the amidation by kidney or brain is bound up with the intact structure of the cell.

To investigate the dependence on pH of the amidation by kidney or brain, slices of these organs were incubated in Ringer's solutions of various pH (glass electrode) prepared by using varying amounts of bicarbonate. In order, however, to avoid an effect of altered pH on the methylation by liver, the supernatant liquid after incubation was adjusted to pH 7·2 by addition of bicarbonate or hydrochloric acid solutions (external indicators) before it was incubated with the liver slices. The amounts of kidney or brain used in this experiment differed from each other by less than 10%. Table 9 shows that the amide formation by kidney and brain depends on the pH. The maximum in both cases lies between pH 7·35 and 6·76.

DISCUSSION

Examination of the conditions under which liver slices methylated nicotinamide showed the reaction to be suitable as a qualitative test for nicotinamide and, under strictly controlled experimental conditions, also to provide a quantitative measure of small amounts of nicotinamide in the presence of nicotinic acid. The findings of Perlzweig *et al.* (1943) were confirmed that liver, but neither kidney nor muscle, methylated nicotinamide, that methionine increased the methylation in some cases, that methylation proceeded at a fairly constant speed for about 3 hr., that a maximum effect was reached by amounts of 250 μg. nicotinamide/350–400 mg. of liver, that the process is aerobic and requires intact cells; but, contrary to the findings of Perlzweig *et al.* (1943), it was found that different pieces of liver of the same rat were of the same methylating efficiency. Perhaps their 'somewhat erratic results' are due to their method of assay without adsorption and elution. Some information could be obtained concerning properties of the enzymic system responsible for nicotinamide methylation such as dependence on pH, inhibition by anaerobic conditions and HCN, and dependence on intact cell structure. The specificity of the enzyme for nicotinamide is demonstrated by the observation of Perlzweig *et al.* (1943) that after incubation of nicotinic acid with liver slices no trigonelline formation was observed with the method of Sarett (1943), and by Ellinger, Fraenkel & Abdel Kader's findings (1947) that none of a large number of nicotinamide derivatives was methylated by the enzyme.

Of all organs tested for their ability to convert nicotinic acid into nicotinamide, only kidney and brain were found active. These are the same organs which were shown by Krebs (1935) to form glutamine from ammonia and glutamic acid; retina was not examined in the present experiments. A few properties of the nicotinic acid amidase were investigated, such as pH activity curve and dependence on the presence of oxygen and intact cell structure. The specificity could not be tested; this can only be done when an exact method for separate estimation of nicotinamide and nicotinic acid becomes available.

Certain difficulty attended the construction of a pH activity curve for the nicotinic acid amidase, since the nicotinamide formed could be measured only indirectly by means of two separate biological processes with all their attendant inaccuracies. Unnecessary corrections were eliminated as far as possible by using constant amounts of kidney and brain.

No explanation can be given at present for the peculiar effect of glutamine on the conversion of nicotinic acid into nicotinamide methochloride by liver tissue, or for the finding that this effect occurs only on some occasions.

SUMMARY

1. The methylation of nicotinamide by liver tissue *in vitro* has been studied and found to be suitable as a qualitative, and with certain restrictions as a quantitative assay method for nicotinamide in the presence of nicotinic acid. The conditions of this process have been examined and some properties described of the enzyme system involved.

2. Kidney and brain have been found to convert nicotinic acid into nicotinamide *in vitro*. Some of the properties of the enzyme system involved in this amide formation have been studied.

3. Liver did not convert nicotinic acid into nicotinamide methochloride *in vitro*, but when glutamine was added formation of nicotinamide methochloride from nicotinic acid occurred in some instances.

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Electrophoretic Studies on Human Serum

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The purpose of the present work was to collect information of the extent to which the interpretation of electrophoretic patterns of serum proteins in phosphate buffer can account for 'boundary anomalies', an expression used by Tiselius (1930) and others to refer to small changes in field intensity and mobility across a boundary.

Although the nature of the changes in conductivity and ionic mobility across a protein boundary has been recognized for some time (Tiselius, 1930; Longsworth & MacInnes, 1940*a*; Abramson, Moyer & Gorin, 1942), it is only recently that these changes have been given a detailed theoretical consideration (Svensson, 1943; Dole, 1945; Svensson, 1946). A theoretical treatment is here given which incorporates Svensson's conclusions on boundary anomalies and has been used to study in particular (*a*) the relative migration velocities of the α_2 and A boundaries in human serum, and (*b*) the conditions during electrophoresis of solutions containing protein components of nearly the same mobilities.

THEORETICAL

Notation

The symbols used are defined as follows:

V_i = migration of protein i (numbered from the fastest component) at infinite dilution in the buffer of concentration equal to that immediately below the ϵ boundary;

V_{ai} = actual migration velocity of protein i below its boundary in descending limb;

r = ratio of velocities of the protein ions at infinite dilution in the bulk buffer to that in buffer below ϵ boundary;

C_i = apparent concentration of protein i on descending side $= \dfrac{\text{area under peak } i}{\text{total area desc. } -\epsilon} \times$ concentration of protein in starting solution;

C_1' = apparent concentration of protein i on ascending side $= \dfrac{\text{area under peak } i}{\text{total area asc. } -\delta} \times \rho \times$ concentration of protein in starting solution;

C_i and C_1' are expressed in g./100 ml.;

$\rho = \dfrac{\text{mean of asc. and desc. total areas } -\delta}{\text{mean of asc. and desc. total areas } -\epsilon}$;

a_i, a_i' = boundary displacements on the descending and ascending sides during an arbitrary time;

K_i = coefficients as defined in the text;

κ = conductivity;

I = current per unit area of cross-section.

Notation for Svensson's (1946) *equation* (23)

c_{i1} = ionic concentrations in electrochemical equiv./ml. above colloid boundary, with the signs of the charges;

u_{i1} = ionic mobilities in cm.^{2} V.$^{-1}$ sec.$^{-1}$ above colloid boundary, with the signs of the charges;

u_2/u_1 = mobility ratio across the colloid boundary;

C = concentration of a leading ion in equiv./ml. with the sign of the charge;

$\mathbf{U}$ = mobility of colloid ion above the boundary, defined by equation (22) of Svensson (1946); $\mathbf{U} = \overline{U}$ of Svensson;

κ_2/κ_1 = ratio of conductivities of solutions above and below the colloid boundary.

The displacement of the centroidal ordinate of the gradient curve of a boundary gives the migration velocity of the protein ion in the environment below its boundary (Longsworth, 1943). If the relative change in migration velocity of a protein ion, brought about by a change in the