Table 2. 0.02 M K_2CO_3 in 0.50 M $^+H_3NCH_2COO^-$/0.05 M $H_2NCH_2COO^-$.

	c_{metal} glycinate	c_{CO_2} total millimole	% carbonate in eq.	$k_{amate} + k_{onate}$	k_{amate}	k_{onate}
Zinc glycinate	0	21.3	33.5	0.0047	0.0016	0.0031
	0.005	18.3	39.3	0.0058	0.0023	0.0035
	0.01	18.0	34.9	0.0065	0.0023	0.0042
	0.02	18.2	36.7	0.0085	0.0031	0.0054
Cupric glycinate	0.01	18.0	35.6	0.0048	0.0017	0.0031

and cupric cyanide ion, are not titrated together with excess barium ion.

The details are as follows:

20.00 ml of the specimen are run into a flask containing 2.00 ml of a mixture that is 6.4 M in sodium hydroxide and 2 M in potassium cyanide. 8.00 ml of this mixture are precipitated with barium chloride and analysed as described by Jørgensen. It was found that a faint blue colour appeared before the equivalence point was reached, but at the end point a distinct jump in colour intensity was observed.

The complexometric titration gives results which are identical to those obtained by the method previously used.

The experiments are listed in Table 2. It is seen, that the catalytic action of zinc glycinate is significant but it can not be compared in order of magnitude with the catalytic action of zinc tetrammine.

A corresponding experiment with 0.01 M cupric glycinate gave no significant effect.

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Acid Soluble Cytidine Nuoleotide Linked Amino Acids in Extracts of Rabbit Liver

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In recent papers [1,2] it was reported that ^{32}P-labeled trichloroacetic acid (TCA) extracts of liver and kidneys of the rat and rabbit contained an ultra violet (UV) absorbing peak (X_I) which contained ninhydrin positive substances. After purification by ionophoresis and paperchromatography the material from the peak still gave a ninhydrin positive reaction. A labeled UV-peak in the same position of the elution curve could also be observed in TCA extracts of ^{32}P-labeled tissue homogenates of rat kidneys. The nucleotide-amino acid complex exhibited changes in UV-absorbtion spectrum characteristic of cytidine nucleotide (CMP). Since CMP-linked amino acids do not seem to have been observed in animal cells previously some properties of the fraction will be described briefly.

In Fig. 1 a section of the elution diagram of the TCA extract is presented consisting of the peaks immediately before and after AMP (adenylic acid). The TCA extract of rabbit livers was eluted according to Hurlbert *et al.*[3] The two first labeled UV-peaks (X_I) appearing with formic acid concentration between 0 and 0.23 M were taken to dryness. At paper electropherograms in

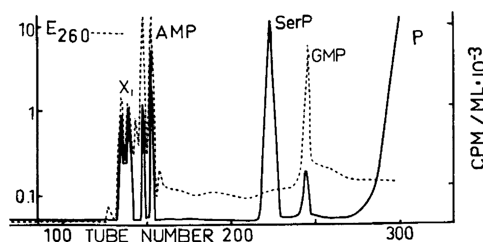

Fig. 1. Acid soluble nucleotides from 7 rabbit livers. The nucleotides were separated on a 6.5 × 45 cm Dowex 1 formate column by gradient elution with reservoir content changed at the tubes numbered as follows: *92,* 1 N formic acid; *250,* 4 N formic acid. The continuous line represents radioactivity and the broken line represents optical density values at 260 mμ.

1 M acetic acid most of the free amino acids were carried away leaving a labeled UV-spot which gave a positive ninhydrin reaction. In one dimensional paper chromatography with *iso*butyric acid and ammonia materials from both fractions behaved differently. Only with material from the second X_I peak one sharp spot with congruent UV, ^{32}P-labeling and positive ninhydrin reaction was obtained together with a ninhydrin negative UV-spot. All of the material from the second X_I peak was purified in this way. When this fraction was again run through a Dowex 1 formate column with a gradient 0 → 1 M formic acid concentration a sharp peak giving UV-absorbtion, activity and positive ninhydrin reaction was observed. Both compounds from X_I exhibited changes in their UV-absorbtion spectra characteristic of cytidine nucleotides; namely an absorbtion maximum in acid medium at 280 mμ with an absorption minimum at 240 mμ. The absorption maximum in neutral solution was located at 270 mμ and the minimum at 250 mμ [4]. Following hydrolysis in 0.1 N HCl for 3 min [5] the hydrolysate of the purified material from the second X_I fraction showed the following properties. In electropherograms in 1 N acetic acid a sharp, labeled ninhydrin positive spot with UV-absorbtion was still observed together with three ninhydrin positive spots slowly moving towards the anode and one spot towards the cathode. At pH 5 in 0.1 N pyridine acetate buffer a labeled, ninhydrin positive spot with UV-absorbtion could be observed together with one ninhydrin positive spot moving towards the anode and one to the cathode. After hydrolysis in sealed tubes for 20 h at 120°C the compound was completely hydrolyzed. Electropherograms in acetic acid showed at least seven ninhydrin positive spots all moving towards the cathode and one or two which did not move. Papers run at pH 5 showed two ninhydrin positive spots moving against the anode and several to the cathode. A two dimensional electrophero-chromatogram [5] showed spots corresponding to aspartic acid, glutamic acid, at least six neutral and one basic amino acid. Following hydrolysis in 1 N HCl for 7 min electropherograms in pyridine acetate showed four sharp, labeled spots giving UV-absorbtion and positive ninhydrin reaction. Two spots corresponding to one acid and one basic amino acid were also observed.

All these data taken together seem to prove that extracts from animal tissues contain amino acids linked to CMP-nucleotides probably in peptide combinations. The results with 7 min hydrolysis are rather complicated and do not exclude the possibility that the material may consist of a complex of CMP-nucleotides with adjacent amino acids linked to each other. The partial acid hydrolysis may produce free nucleotides, nucleotides with amino acids, peptides and free amino acids. So far we have not been able to isolate a substance with quite similar properties from *L. casei.*

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