In the papers by DROZDOV-TIKHOMIROV and KIKOIN[6] and by TU and FRIEDERICH[5], the IR- spectra of copper(II) complexes with Guo (and Ino) were studied in the solid state (nujol emulsion and KBr or KCl pellet). They found diminuition of the keto stretching band intensity at 1690 cm^{-1} by the formation of the copper(II) complex. We repeated their IR measurements; our results are in full agreement with both previous works about the copper(II) complexes of Guo and Ino, so that enolization of Guo, dGuo and Ino by the complex formation is possible.

But in our opinion it is not justified to transfer the results obtained with complexes in solid state to the different properties of an aqueous solution, as has been done by the authors cited above. The infrared spectra of the D_2O solutions of dGuo and Ino (Figures 1 and 2) are influenced by the copper(II) complex formation, but neither a diminuition of the keto stretching band nor an increase of an enolic band at wave-number smaller than 1630 cm^{-1} are observed. Only an unspecific broadening of the keto band accompanies the copper(II) complex formation. According to MILES[12,13] the enolic bands of both Guo and Ino are to be expected near 1615 cm^{-1}. The IR-spectra of Guo in dimethyl sulfoxide in the absence and in the presence of copper(II) ions also demonstrate the existence of the keto form in the complex, for the 1690 cm^{-1} band remains unchanged by the addition of copper (II) (measurements of DROZDOV-TIKHOMIROV and KIKOIN[6]). These results yield strong evidence against the enolization of Guo, dGuo and Ino by the formation of the copper(II) complexes in solution. Therefore, in our model of the DNA-copper(II) complex the guanine residues are in the keto form[9].

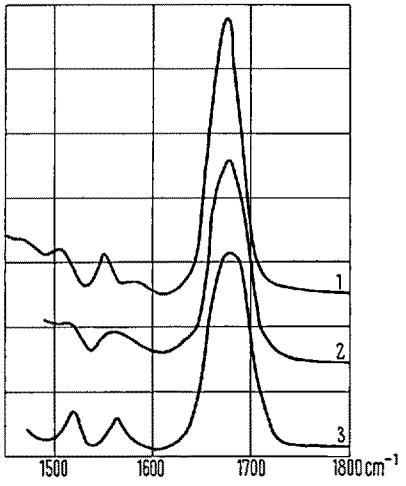

Fig. 2. IR-spectra of inosine (15 mM) in D_2O. Curve 1: Without addition of Cu(II). Curve 2: 75 mM copper sulphate added. Curve 3: 300 mM copper sulphate added.

Zusammenfassung. Durch infrarotspektroskopische Messungen wird bewiesen, dass Guanosin, Desoxyguanosin und Inosin bei der Bildung von Kupfer(II)-Komplexen entgegen früheren Darstellungen in wäßriger Lösung in der Ketoform vorliegen.

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Effect of Essential Amino Acids on Absorption of Phenylalanine by Rat Intestine

It has been known for some time that leucine and methionine can stimulate the uptake of lysine and arginine by the intestinal mucosa[1-4]. These are some of the first of the amino acids to be released during the process of digestion of proteins in the gastrointestinal tract. It was also noted that the essential amino acids were the first to be found in a free form during digestion[5]. It was therefore decided to study the effect of these amino acids on the absorption of phenylalanine by the instestine. It is possible that these amino acids may release some mechanism in the mucosal cells, thus stimulating the absorption of the amino acids released later in digestion.

Materials and methods. Male Wistar rats weighing 200 to 250 g were used in all experiments. In experiments in vitro segments of rat intestine were prepared according to the method of AGAR, HIRD and SIDHU[6]. A length of intestine was cut from the rat under anaesthesia, it was washed with physiological saline solution and then divided into small segments with a scalpel. The segments were then incubated at 37°C with 5 mM phenylalanine-U-C^{14} in Krebs bicarbonate buffer. In experiments in vivo 20 ml of 5 mM Phe-U-C^{14} (Phe*) solution in physiological saline was perfused through a closed loop of intestine for a period of 1 h. Uptake is expressed as μM Phe absorbed / g tissue. The essential amino acid mixture used was that of Rose i.e. mg/ml Tryptophan 1.25, Phenylalanine 5.5, Lysine 4.0, Threonine 2.5, Methionine 5.5, Leucine 5.5, Isoleucine 3.5, Valine 4.0. The radioactivity absorbed was measured in a liquid scintillation counter.

Results. Table I demonstrates the effect on the uptake of phenylalanine of an injection of 2 ml of the essentia- amino acid mixture into the stomach. Segments of intesl tine were removed every 20 min and incubated in Krebs bicarbonate buffer (KBB) for a period of 20 min in the

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presence of 5 mM Phe*. There is a gradual increase in absorption starting 40 min after the injection of the mixture in rats fasted for 1 day as compared to the control animals. After 2 days fasting the rats showed the same increase in absorption when treated in a similar way.

Table II shows the effect of treatment with 5 or 1 ml essential amino acid mixture and 2 ml distilled water. The mixture clearly stimulates the uptake of phenylalanine whereas the distilled water has no effect. In Table III the results of an experiment in vivo are given. The segment of intestine was perfused for 60 min with 20 ml 5 mMPhe*, then a 2 ml sample of the amino acid mixture was injected into the stomach; 30 min later the same segment was perfused with a fresh solution of 20 ml 5 mM Phe*. As can be seen, there was no effect on the Phe absorption. When 2 ml of the amino acid mixture was added to the perfusion medium, there was an inhibition of Phe absorption (Table III). Similar results were obtained when 5 ml of the amino acid mixture were added to the medium.

Table I. Effect of ingestion of an essential amino acid mixture and the absorption of phenylalanine (Phe)

Time of removal (min)	Control	μMPhe/g tissue	
		1 day fasted	2 days fasted
0	12.0 ± 1.2	12.5 ± 1.1	12.4 ± 0.9
20	11.4 ± 0.9	12.8 ± 1.4	12.4 ± 1.3
40	12.1 ± 1.1	15.4 ± 1.6	15.8 ± 1.7
60	12.1 ± 1.3	16.0 ± 1.5	16.9 ± 0.9
80	—	16.0 ± 0.8	—
100	—	16.8 ± 1.0	—
120	—	16.6 ± 0.9	—

Injection of 2 ml essential amino acid mixture into stomach. Segments of intestine removed every 20 min and incubated for 20 min in 5 mM Phe-U-C^{14}. 8 rats/group.

Table II. Effect on the absorption of phenylalanine (Phe) of two levels of an essential amino acid mixture as compared to water

Time	μMPhe/g tissue absorbed		
	5 ml EAA	1 ml EAA	2 ml H_2O
0	13.0 ± 0.8	14.3 ± 1.2	11.9 ± 0.9
60	17.6 ± 1.1	18.9 ± 1.3	10.6 ± 1.0

Injection of 5 ml, 1 ml essential amino acids (EAA) and 2 ml water into rat stomach. Segments of rat intestine removed at 0 and 60 min and incubated with 5 mM Phe-U-C^{14} for 20 min. 8 rats/group.

Table III. In vivo measurement of the influence of pre-perfusion of phenylalanine (Phe) solution in the total absorption of Phe

Time	Control	2 ml EAA in stomach	2 ml EAA in medium	5 ml EAA in medium
0	34.0 ± 2.1	35.2 ± 1.8	33.0 ± 1.3	55.0 ± 2.7
60	33.2 ± 2.3	34.4 ± 2.0	19.0 ± 0.8	33.0 ± 1.6

Absorption of Phe measured in vivo over a period of 60 min. Radioactive Phe perfused through closed loop of intestine. EAA, essential amino acid mixture.

Table IV. Influence of an essential amino acid mixture on the incorporation of phenylalanine (Phe) in the proteins of the intestine

	nM Phe*/g proteins
Fasting	71 ± 3.2
Fed	73 ± 2.9
Fasting + essential amino acids	78 ± 4.0

Phe-U-C^{14} measured in perchloric acid precipitate after perfusion of intestine for 60 min with 5 mM Phe-U-C^{14}.

Table IV shows the incorporation of Phe-U-C^{14} into the proteins of the intestine. The intestine was perfused with 5 mM Phe*, after 1 h the intestine was removed, homogenized, the proteins were precipitated with 0.6 N perchloric acid. They were solubilized in 30% KOH at 100°C. The solution was then counted in a liquid scintillation counter. Treatment with the amino acid mixture did not significantly increase the amount of Phe* incorporated into proteins.

Discussion. From the above results it is clear that the first amino acids released during digestion have an effect on the absorption of the other amino acids. However, it is still difficult to say how this increase is brought about. From the experiments in vivo one can say that a direct contact with the intestine is necessary to bring about this increase. An injection of the mixture into the stomach does not bring about an increase in vivo. When the mixture is perfused with the medium there is an inhibition of absorption. This is to be expected as it is due to competition between amino acids sharing a similar carrier. An effect of exchange diffusion[7,8] would seem to be ruled out, since, when the segments are removed from the rat after treatment, they are left for 15 min in Krebs bicarbonate buffer solution before the incubation so as to remove the major part of the non-labelled Phe remaining in the tissue.

Although there seems to be no significant increase in the incorporation of Phe-U-C^{14} into the proteins in the intestine, it is possible that an increase in the number of carriers with respect to the rest of the protein would not be possible to measure as simply as this. Therefore, it is still possible that there would be a local increase in the quantity of carriers in the membrane surface thus bringing about an increase in absorption.

It is also possible that the amino acids themselves stimulate a mechanism similar to that proposed for insulin[9]. Perhaps these amino acids or one alone inhibit the production of 3′–5′ cyclic AMP making possible a greater effect of insulin on this system. A possible reason why the mixture has no effect when it is not in contact with the tissue is because these amino acids are quickly taken up by the liver and other tissues, for the synthesis of proteins, before reaching the segment involved.

Résumé. L'effet d'un mélange des acidés aminés essentiels sur l'absorption de phénylalanine par l'intestin de rat a été étudié. On a trouvé que le mélange, s'il est en contact direct avec l'intestin, peut augmenter l'absorption de phénylalanine. Ces résultats sont interprétés et plusieurs hypothèses sont émises pour expliquer ce phénomène.

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