trolled. A 3rd stabilization period of 1 h followed. 5 ml samples of blood were withdrawn from the left renal vein at the noted times (Figure) and measurements of plasma FFA was analyzed by the method of DOLE and MEINERTZ[10]. Sodium, hemoglobin and hematocrit estimations were determined from 50 ml blood samples taken from the left renal vein. The data were treated with the analysis of variance technique with the significance of the difference between individual means being determined by means of the Tukey's 'w' test[11].

*Results and discussion.* Table I contains the descriptive data for the experimental and control dogs. Most of the dogs in this study were somewhat dehydrated clinically although the dogs designated sodium depleted had lower plasma sodium levels. In spite of this observation it was difficult to demonstrate hemoglobin values outside of that range of values considered normal (Table II). The 12 animals which were subjected to the low sodium diet had 24 h urine sodium excretions of less than 7 mEq whereas the normal values are considered to be 20–50 mEq[7,8,12]. Urine collections were made during the procedure to ensure that urine formation was occurring, that renal function was not impaired, and that sodium depletion was evident.

Table III contains experimental renal vein plasma FFA level data. No differences were found between groups after surgical stabilization. Occlusion of the carotid artery caused increases in plasma FFA levels in the renal vein of the experimental animals. This increase persisted throughout the second stabilization period. Carotid occlusion and controlled perfusion pressure resulted in a drastic drop in FFA levels which were gradually returned to resting levels after the 3rd stabilization period. Limited variation was noted throughout the experimental cycle for the control animals. The hemodynamic changes which occurred secondary to carotid occlusion were predictable. During the phase of sympathetic response with control of the renal perfusion pressure, most dogs demonstrated decreases in renal blood flows. There is some experimental evidence[13–15] that intrarenal redistribution of blood flow can favour an increase in renal medullary flow and that changes in renal blood may not necessarily reflect a change in glomerular filtration rate. CASTENFORS[15] recently found that subjects under the stress of exercise decreased renal blood flow (RBF) more than glomerular filtration rate (GFR). It would seem that changes in RBF do not necessarily reflect changes in GFR. SAKAI et al.[16] demonstrated that noradrenaline infusion induced an immediate rise in the perfusion pressure which was sustained until the infusion was withdrawn whereby the resting level was again approached. No direct evidence was available to indicate catecholamine release as the renal response to ischemia.

The renal vein was selected as the site for the collection of blood to measure plasma FFA levels as the relationship between elevated renin levels after exercise[17] and catecholamine infusion[18] have been previously investigated under similar surgical procedures[19]. Angiotension stimulates the release of catecholamines from the adrenal medulla[20] which in turn affects the mobilization of FFA and renin release[21]. The relationship between the release of renin and FFA distribution to the kidneys is an interesting feedback reaction, however, the physiological reasons remain unanswered at this time.

The results indicate that the sympathetic nervous system (SNS) can control the release of FFA to the kidneys under the conditions of this study. The response to the SNS stimuli requires more than 10 min to increase renal vein plasma FFA levels. Such a lag phase would suggest that the stimulus is weak or that FFA are not a prime source of energy for the kidney under surgical trauma. It has been suggested[4] that a lipase, activated by circulating catecholamines, may continue to catalyze FFA mobilization after the application of the stressor with no quantitative relationship to the plasma norepinephrine level or the magnitude of the original stressor.

It may be concluded that the sympathetic response following carotid occlusion in the mongrel dog stimulates the release of FFA. Sodium depletion apparently does not affect the resting FFA levels in the renal vein[22].

*Résumé.* L'occlusion de la carotide, l'épuisement du sodium du plasma et l'effet de la pression de perfusion rénale ont été utilisés pour étudier les niveaux d'acide gras non-estérifié dans la veine rénale. Dans la présente recherche, le système nerveux sympathique controlait la libération vers les reins de l'acide gras non-estérifié. Apparemment, l'épuisement du sodium n'affecte pas les niveaux normaux de repos de cet acide dans la veine rénale.

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## The Nucleoside-Copper (II) Interaction and the Tautomeric Forms of the Nucleosides[1]

Copper(II) ions are known to interact with some nucleosides, nucleotides, polynucleotides and the corresponding derivatives of the 2'-deoxyribose[2–4]. In some papers an enolic tautomer of guanosine is postulated for the copper (II) complex, both in the solid state and in aqueous solution[5,6]. The knowledge of the tautomeric forms of the nucleosides in their copper(II) complexes is necessary for proposing a correct model of the DNA-copper(II) inter-

action. In our present study, an attempt was made to investigate the complex formation of the deoxynucleosides with copper(II), both in aqueous solution and in the solid state, using IR- and partially ESR[7] spectrometry. Attention was focused on structural differences which seemed to exist between the complex in aqueous solution and the complex in solid state.

$D_2O$ solutions were prepared as described previously[4]. The IR-spectra were recorded by a doublebam IR-spectrophometer UR 10 (Carl Zeiss, Jena) using a NaCl prism.

A superheterodyn ESR-spectrometer ERS-X (Akademie-Werkstätten für Forschungsbedarf, Berlin) working in the X-band region was used for ESR measurements. The approximated $g$ values were estimated using the DPPH and $Mn^{2+}$ signals.

The IR-spectra of the $D_2O$ solutions of the 4 deoxynucleosides, Ino, 6-aza-Urd, 6-aza-Cyd, poly (C), and poly (I)[8] were studied in the region between 1450 and 1800 $cm^{-1}$. Changes of the spectra after addition of $CuSO_4$ at $r = 20$ (molar ratio copper(II) : nucleoside) are demonstrated in Table 1. The complex formation induces marked changes in the IR-spectra of dGuo, dCyd, Ino poly (C) and poly (I), but no changes can be observed in the IR- spectra of dAdo, dThd, 6-aza-Urd and 6-aza-Cyd. Figure 1 shows the IR-spectra of dGuo without and with addition of copper(II), Figure 2 those of Ino. IR- spectra of dCyd, dAdo, dThd and poly (C) can be found in figures 1 and 16, given in our previous papers[4,9]. The appearance of the 1562 $cm^{-1}$ band clearly indicates the formation of a $Cu^{2+}$-complex with Ino and poly (I) (Table II) in $D_2O$ solution in agreement with spectrophotometric results of EICHHORN and TARIEN[10]. The ESR-spectra confirm these results. Increasing amounts of nucleosides were added to a $2 \times 10^{-2}$ $M$ solution of $CuSO_4$ up to a molar ratio $r$ of 1/20. The signal of the aquocomplex $[Cu(OH_2)_6]^{2+}$ decreases when dCyd, dGuo or Ino are added to the solution. A new ESR signal of smaller line width increases at higher values of the magnetic field with increasing amounts of the nucleosides. The estimated $g$ values of the copper(II)-nucleoside complexes are presented in Table II.

The introduction of a nitrogen atom in the 6-position of the pyrimidine ring of cytosine seems to prevent the copper(II) interaction; the IR-spectra of 6-aza-Cyd are not influenced by a 20fold molar excess of copper(II) ions.

The derivatives of uracil, dThd and dUrd do not indicate any complex formation with copper(II) ions, which can be measured by IR- and ESR-spectroscopy. This is also valid for 6-aza-Urd.

The ESR-spectra yield informations about the relative stability of the nucleoside-copper(II) complexes in aqueous solution. The $g$ values in a series of equal coordination sphere should increase with increasing complex formation tendency[11]. Therefore, the results of Table II suggest a decreasing complex formation tendency in the order Ino > dGuo > dCyd > dUrd. The $g$ value of the hydrated copper(II) ion is higher than the $g$ values of the nucleoside complexes, but this is no indication of a greater stability against the drastic change of the coordination sphere (oxygens of the water instead of nitrogens and carbonylic oxygens).

Table I. Changes in the IR-spectra (1450–1800 $cm^{-1}$) of the deoxyribonucleosides and some related compounds (dissolved in $D_2O$) induced by the formation of copper (III) complexes

| | decrease in absorbancy ($cm^{-1}$) | increase in absorbancy ($cm^{-1}$) |
|---|---|---|
| dGuo (7.5 m$M$) | 1580 | 1573, 1590 |
| dCyd (15 m$M$) | 1503, 1616 | 1517, 1547 |
| dAdo (15 m$M$) | no changes observable | |
| dThd (15 m$M$) | no changes observable | |
| 6-Aza-Urd (15 m$M$) | no changes observable | |
| 6-Aza-Cyd (15,m$M$) | no changes observable | |
| Ino | 1506, 1549, 1578 | 1515, 1562 |
| Poly (C) | 1510, 1523 | 1517, 1549 |
| Poly (I) | 1505, 1550, 1581 | 1512, 1556 |

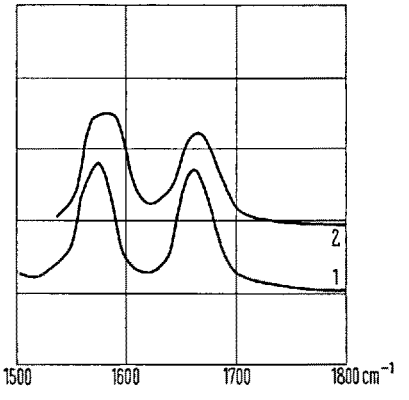


Fig. 1. IR-spectra of deoxyguanosine (7.5 m$M$) in $D_2O$. Curve 1: Without addition of Cu(II). Curve 2: Copper sulphate (150 m$M$) added to the solution.

Table II. Approximate $g$ values of the copper(II) ion in some nucleoside complexes

| Complex | $g$ value |
|---|---|
| Cu(II) × Ino | $2.12_2$ |
| Cu(II) × dGuo | $2.11_3$ |
| Cu(II) × dCyd | $2.10_5$ |
| Cu(II) × dAdo | not determined because of precipitation |
| Cu(II) × dUrd | no complex formation observed |
| Cu(II) × aqu | $2.16_8$ |

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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].

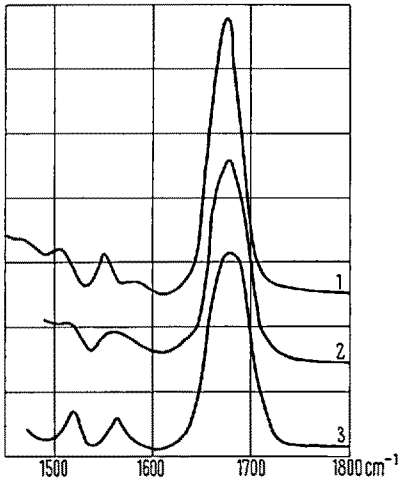


Fig. 2. IR-spectra of inosine (15 m$M$) in $D_2O$. Curve 1: Without addition of Cu(II). Curve 2: 75 m$M$ copper sulphate added. Curve 3: 300 m$M$ copper sulphate added.

*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 m$M$ phenylalanine-U-$C^{14}$ in Krebs bicarbonate buffer. In experiments in vivo 20 ml of 5 m$M$ 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 $\mu M$ 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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