The ability of the 2 enzyme preparations to hydrolyse some amino acid 2-naphthylamides

2-naphthylamide of	Leucocyte preparation (Pool II)	Wound tissue preparation (Pool II)
L-methionine	100[a]	100
L-valine	7	50
L-leucine	50	25
L-alanine	44	12
L-arginine	25	0
L-lysine	43	0

[a] The numbers indicate relative hydrolytic abilities, as compared with the hydrolysis of L-methionyl-2-naphthylamide (= 100). The hydrolyses were tested in 0.08 *M tris*-HCl buffer, pH 7.15.

activity displayed by the leucocyte preparations were also pooled. The Table shows that the enzyme peaks, marked 'II' in the Figure, differed in their substrate specificity.

Dithiothreitol activated the wound tissue AAP by up to 500% at a concentration of approx. $10^{-4}M$, but the thiol compound (at concentrations from $10^{-6}M$ to $10^{-3}M$) had no effect on the leucocyte enzyme.

Wound tissue AAP's thus differ qualitatively in many respects from the leucocyte enzymes. We have previously shown[9] that the wound tissue AAP's also differ from those in serum. Thus the augmented enzymes in wounds originate, to a considerable degree, in the injured tissue itself during the earliest post-operative stage. The immigrating leucocytes, showing an intense AAP activity, participate also in the enzymatic response. The view that the intensified enzyme activity is derived exclusively from the invading leucocytes[5] may be partly due to the fact that it is extremely difficult to distinguish, for example, between macrophages and fibroblasts by routine light microscopic procedures[10]. The initial increase in enzymes seems to represent an adaptive defence mechanism by the local cells as a response to injury[1,2,11].

Zusammenfassung. Die Herkunft der Wundarylaminopeptidasen wurde untersucht, indem man die Enzyme von Leukozyten und von Wundgewebe jeweils bei ein und derselben Ratte verglich. Mit Hilfe von Fraktionierung und bei der Untersuchung ihrer Substratspezifizität und bei Aktivierung durch Dithiothreit zeigte sich, dass die Arylaminopeptidasen im Wundgewebe sich qualitativ von denen in den Leukozyten unterschieden. Weil wir früher gezeigt haben, dass sich die Wundarylaminopeptidasen auch von denen im Serum unterscheiden, können wir den Schluss ziehen, dass die Zunahme von Enzymen in Wunden erheblich in dem geschädigten Gewebe selbst während der allerfrühesten Heilungsphase herstammt.

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[9] J. RAEKALLIO and P.-L. MÄKINEN, Ann. Med. exp. Fenn. *45*, 224 (1967).
[10] R. Ross, Biol. Rev. *43*, 51 (1968).
[11] This investigation was supported by grants from the Sigrid Jusélius Foundation and from the Finnish National Research Council for Medical Sciences.

The Nature of Amino-Acid Chloranil Complexes

Several studies have been made previously of the UV- and visible spectra of mixed solutions of amino-acids and chloranil (tetrachloroquinone)[1–3]. It has been suggested that the amino-acid in a neutral form forms a 1:1 charge transfer complex with chloranil. Two objections could possibly be made to these conclusions, one is that the amino-acids are almost wholly zwitterionic at the pH's used in the experiments cited[4] and the other is that the broad featureless absorption band associated with charge transfer complexes[5] was not observed. To test the validity of the conclusions, the IR-spectra of some amino-acid chloranil complexes have been determined in the solid state. The amino-acids studied were glycine, serine, valine glutamic acid, tyrosin and tryptophan. Solid amino-acid chloranil complexes were made by allowing equimolar solutions of amino-acid and chloranil in aqueous acetone or aqueous ethanol to stand in the dark for a minimum of 3 days in order that the interaction should reach an equilibrium. The solutions were then evaporated down to dryness at 30 °C and at low pressure in a rotary evaporator. The resulting residues were brownish green in appearance and appeared to be homogenous. The IR-spectra of these residues were obtained in KBr discs using a Unicam 200 G spectrophotometer. These spectra were compared with the spectra of amino-acids and chloranil separately which had also been through the same cycle of solution and evaporation. The spectra of the complexes showed the following differences from the individual components. Whereas the amino-acids appear to be completely zwitterionic in the solid state possessing absorption bands at 1600 cm^{-1} and 1400 cm^{-1} arising from the COO^- group and bands at 3130–3030 cm^{-1}, 2150 cm^{-1}, around 16,500 cm^{-1} and between 1550 and 1485 cm^{-1} associated with the NH_3^+ group[6], the complexed amino-acids have a very strong band at 1730 cm^{-1} which arises from the unionized COOH group together with weaker COOH bands at 1410 and 1250 cm^{-1}. In addition the bands associated with $-NH_3^+$ and COO^- groups are absent in the complex, all other bands of the amino-acids are retained in the complex. A band

[1] J. B. BIRKS and M. A. SLIFKIN, Nature *197*, 42 (1963).
[2] M. A. SLIFKIN, Spectrochim. Acta *20*, 1543 (1964).
[3] M. A. SLIFKIN and J. G. HEATHCOTE, Spectrochim. Acta *23A*, 2893 (1967).
[4] R. C. WEAST, *Handbook of Chemistry and Physics*, 49th Ed. (The Chemical Rubber Co., Cleveland 1968), p. C 703.
[5] R. S. MULLIKEN, J. Am. Chem. Soc. *74*, 811 (1952).
[6] L. J. BELLAMY, *The Infra-red Spectra of Complex Molecules Ch. 13* (Methuen, London 1966).

seen at 1580 cm^{-1} in the complex might arise from a unionized NH_2 group. The identification of the COOH bands at 1730 cm^{-1} etc., has been made by examining the spectra of amino-acid hydrochlorides which have the structure COOH RCO NH_3^+ Cl^-[6].

The following changes are observed for the complexed chloranil. The carbonyl band appearing in the free form at 1695 cm^{-1} is shifted to 1630 cm^{-1}, the C=C stretch band at 1575 cm^{-1} disappears. Identical behaviour has been observed in the IR-spectrum of the charge-transfer complex formed between hydroquinone and chloranil[7].

The shift of the carbonyl band to 1630 cm^{-1} and the disappearance or shift of the C=C band is attributed to charge donation into an anti-bonding orbital of the chloranil which causes a lowering of bond order and hence of frequency of vibration of these bands. It has therefore been confirmed that the amino-acid chloranil complexes show all the features of the classic charge transfer complex including a colour change which is apparent in the solid complex but not in solution[3] where the concentration is very much lower and that the amino-acid is present in the complex in the non-zwitterionic form. These observations also explain the very slow formation of these complexes in solution as over the pH range used, 4 to 9, the amount of unionized amino group present in equilibrium is very small indeed. The fact that interaction rate increases as the pH increases is also explained by the presence of more unionized amino group at the higher pH[4].

The bonding in the amino-acid complexes must arise from the donation of a lone-pair (n) electron on the nitrogen of the unionized amino group to the chloranil. Particularly interesting is the fact that the 2 aromatic amino-acids, tryptophan and tyrosin behave as n-electron donors and not as π-electron donors. This might have interesting biological consequences as lone-pair (n) electrons are fairly widely distributed in biological systems and in considering electron transport or charge transfer systems, lone pair electrons should not be excluded as possible partcipants, even in the near vicinity of π-electrons.

Zusammenfassung. Die IR-Spektren der Komplexe von Aminosäure mit Chloranil zeigen, dass zwischen Aminosäure in einer nicht zwitterionischen Form und Chloranil klassische Elektronen-Donator-Akzeptor-Komplexe gebildet werden. Die aromatischen Aminosäuren wirken als n-Elektronendonatoren.

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[7] M. A. SLIFKIN and R. H. WALMSLEY, to be published (1969).
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Renal Medullary Hypertonicity and the Pathogenesis of Acute Renal Failure

The present study was designed to evaluate a possible role of renal medullary hyperosmolarity in the development of acute renal failure as produced in rats by the injection of human methemoglobin and sodium ferrocyanide[1].

Diuresis was induced in 160–200 g male CFE rats by replacing their drinking water with 5% glucose solution. Pigment nephropathy was induced by the method of MASON et al.[1]. 48 h after methemoglobin and ferrocyanide injection the left kidney of each rat was weighed. The weights of diuresed and of control animals were compared using the student t-test to evaluate statistical significance.

In the first experiment rats were diuresed for 5 days before induction of the renal lesion. The resulting data are in Table I. In a second series, groups of rats were severally diuresed for 12, 48, 72 and 120 h before injection with methemoglobin-ferrocyanide solution. Representative data are in Table III. Histological sections of kidneys were examined.

Rats treated with sustained diuresis had significantly lighter kidneys than did control animals (Tables I and II). All rats given 5% glucose to drink showed high urine output (Table III) but kidney enlargement characterized all groups not treated with sustained diuresis. Increase in kidney weight caused by methemoglobin-ferrocyanide injection correlates closely with impairment of renal function and with the degree of histologically demonstrable renal damage[2]. Also, the heavy kidneys showed extensive cast formation, interstitial round cell infiltration and tubular hydropic changes. These data therefore show that prolonged diuresis, rather than diuresis per se, is necessary to protect rats from developing pigment nephropathy, and indicate that rate of urine was not the protecting factor.

LEVITIN et al.[3] described a continuing fall in renal medullary osmolarity during a 5-day diuresis in dogs drinking 5% glucose and water. Parallel changes in rats diuresed for 5 days have been described by MORARD[4] in the acid mucopolysaccharides of the renal medulla which show increased polymerization with a decreased negative charge. This is believed to decrease the accumulation in the medullary interstitium of cations necessary for the formation of the intrarenal osmotic gradient. PARRY et al.[5] were able to prevent experimental pigment nephrosis in rats with mannitol. Mannitol diuresis decreases medullary osmolarity[6]. TESCHAN and LAWSON[7]

[1] A. D. MASON JR., P. TESCHAN and E. E. MUIRHEAD, J. surg. Res. *3*, 430 (1963).
[2] L. MAILLOUX, S. ROSEN, N. LAWSON and P. TESCHAN, Proc. Am. Soc. Nephrol., Abstracts (Los Angeles 1967), p. 42.
[3] H. LEVITIN, A. GOODMAN, G. PIGEON and F. H. EPSTEIN, J. clin. Invest. *41*, 1145 (1962).
[4] J. C. MORARD, C. r. Acad. Sci. Paris, Series D *264*, 2166 (1967).
[5] W. L. PARRY, J. A. SCHAEFFER and D. B. MUELLER, J. Urol. *89*, 1 (1963).
[6] R. L. MALVIN and W. S. WILDE, Am. J. Physiol. *197*, 177 (1959).
[7] P. E. TESCHAN and N. L. LAWSON, Proc. Am. Soc. Nephrol. Abstracts (Los Angeles 1967), p. 66.