fluorescence at 5 °C was 30% greater than at 20 °C; however, the peak of maximal fluorescence remained constant for the range studied.

The authors routinely read fluorometrically in the serotonin-direct and serotonin-O.P.T. methods at 20 °C since it is easier to maintain samples at this temperature than at 5 °C; furthermore, temperatures above 20 °C result in accelerated amine destruction in the direct method and inability to correct in a simple way for temperature variations (Figure 3). It was found that if samples are frozen in liquid nitrogen and read in the frozen state fluorescence could be increased approximately 300-fold. However, no detailed studies have been undertaken to optimize serotonin fluorescence by sample freezing[9].

Résumé. Dans la détermination fluorimétrique de la sérotonine elle-même et du complexe sérotonine-O.P.T. (*o*-phthaldialdéhyde) un maximum de fluorescence a été atteint pour des normalités de HCl de 3.25–3.50 et 6.25–6.50, respectivement. Pour les deux procédés, on a noté des rapports en sens inverse entre la fluorescence produite et la température de l'échantillon, pour des températures comprises entre 5° à 35 °C. Entre la température ambiante (de 20 °C) et 5 °C, la fluorescence s'est accrue de 30% environ.

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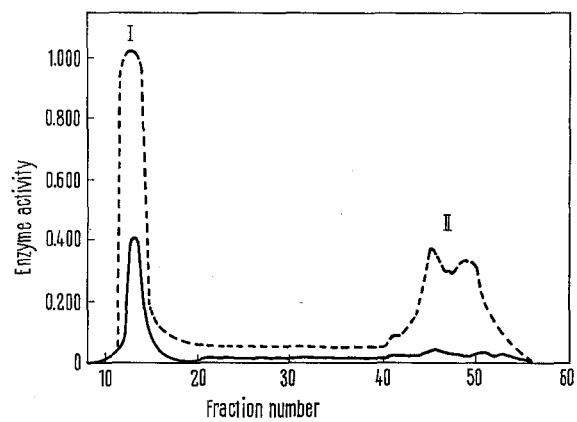
On the Origin of Wound Arylaminopeptidases

An increase in arylaminopeptidase (AAP) activity during the very first post-operative hours has been demonstrated both histochemically[1] and biochemically[2]. The intensified AAP activity involves an actual increase in the amount of enzymes, i.e. in the number of enzyme molecules appearing in the wound periphery[3,4]. It seems possible that enzyme activity is partly responsible for the mediation of the increased vascular permeability and tissue leucocytosis in the early stage of inflammation. The origin of these enzymes – whether from the local connective tissue cells, blood plasma, or leucocytes – is therefore fundamental. It has recently[5] been claimed that the intensified enzyme activity is derived solely from the invading leucocytes.

If the increase in AAP's is really due to the leucocytes, their enzyme pattern ought to be qualitatively similar to that in the wound tissue of the same individual. To elucidate this we made an experimental study on rats. Blood and tissue samples from each animal were processed separately and the results were compared reciprocally. The preparation of the wound tissue sample, the column chromatographic procedures, and the method for the estimation of the AAP activity have been described earlier[3,4,6]. The blood leucocytes were obtained from the rats, given 10 U of heparin/g body weight i.v. The animals were decapitated 5 min after the injection. About 10 ml of whole blood was obtained in this way from rats weighing 250–300 g. The final collecting of the leucocytes was accomplished by the Ficoll flotation method[7]. The remaining erythrocytes were then lysed by hypotonic shock in 0.21% NaCl for 30 sec[8]. The lysis was terminated by addition of KCl to a final concentration of 0.15 *M*. The leucocytes obtained were suspended to a small volume of 0.01 *M* *tris*-HCl buffer, pH 7.15, and disintegrated by 3 times rapid freezing (– 20 °C) and thawing. After centrifugation at 23,000 *g*, the clear supernatant fluid was used for further studies.

The Figure shows an example of the fractionation of leucocyte and wound tissue AAP's. The results consistently showed that the leucocyte preparations were almost always devoid of the last AAP peak. When the active fractions, forming the last peak and shown in the Figure, were pooled and the enzyme preparations resulting tested for their ability to hydrolyze various amino acid 2-naphthylamides, the results given in the Table were obtained. The corresponding fractions with the very low enzyme



Fractionation of arylaminopeptidases of rat blood leucocytes (—) and wound tissue (– – –), acting on L-methionyl-2-naphthylamide. Column: 10 × 850 mm, CM-Sephadex C-50. Salt gradient: NaCl gradient from 0 to 1 *M* (mixing volume 150 + 150 ml). Fraction volume: 3.2 ml.

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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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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

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