compound does not arise directly from the 3 carbon atoms units such as alanine itself or pyruvic acid. The origin of this molecule is unknown, although it could take place by decarboxylation of an hypothetical α-methyl-L-serine, which has been found in nature as a constituent of the molecule of the antibiotic amicetin[13], and which has been shown to be non enzymically decarboxylated by pyridoxal[14].

II is not an intermediate in the biogenesis of III and IV as can be deduced by the lack of incorporation of ^{3}H-ergometrine, in contrast with the high incorporation rate of ^{3}H-lysergic acid. The relative incorporation rates of ^{14}C-alanine into the α-hydroxy-α-aminoacid fragments of III and IV would be in agreement with the proposed mechanism of the formation in vivo of the corresponding non-hydroxylated aminoacids[15]. However, the efficiency of ^{14}C-alaninol to act as a source of the carbon atoms of these fragments suggests that the formation of the said α-hydroxy-α-aminoacids in vivo may follow a different route.

Riassunto. Dall'esame della incorporazione di possibili precursori negli alcaloidi prodotti da *C. paspali* e *C. purpurea* risulta che non vi è una diretta correlazione fra la biogenesi delle catene laterali della ergometrina e della α-idrossietilammide dell'acido lisergico, e che l'ergometrina non è un precursore dei derivati peptidici dell'acido lisergico.

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Temperature and Acidity for Maximal Fluorescence of Serotonin and Serotonin-O.P.T.

Numerous techniques are available for the determination of serotonin[1]; however, fluorometry has emerged as the technique of choice due to its inherent sensitivity and selectivity[2]. The most commonly employed fluorometric method is that of Bogdanski et al.[3]; however, recently Maickel and Miller[4] have reported increased detection sensitivity after reaction of serotonin standards with *o*-phthaldialdehyde (O.P.T.). The fluorescence of a compound is profoundly influenced by the temperature and pH of the solution read[5]. Control of these 2 parameters will result in greater method sensitivity and precision. Since no detailed data are available in the literature describing pH and temperature effects on the fluorescence of serotonin or serotonin-O.P.T. these were studied.

Instrumentation. Fluorescence was recorded by using a spectrophotofluorometer (S.P.F.)[6]. Activation wavelengths were 295 nm and 360 nm, respectively, for the serotonin-direct and serotonin-O.P.T. methods. Wavelengths versus fluorescence diagrams (spectra) were recorded[7] from 200–800 nm. Serotonin-direct and serotonin-O.P.T. fluorescence were read at 545 nm and 470 nm wavelengths, respectively.

Experimental, serotonin-direct. 13 analytical duplicates of serotonin standards were prepared from a 520 ng/ml standard in 0.003 N HCl. 5 ml aliquots were diluted with 4 g/100 ml (w/v) ascorbic acid, double distilled water (D.D.W.) and conc. HCl. Final concentrations obtained were 200 ng/ml serotonin, $5.6 \times 10^{-3} M$ ascorbic acid and graded normalities from 1.50–6.00 HCl. Individual blanks were also prepared containing $5.6 \times 10^{-3} M$ ascorbic acid for each normality. Each of the 13 standards and blanks were divided into 8 fractions and refrigerated at 4 °C until tested.

Experimental, serotonin-O.P.T. 12 analytical duplicates of serotonin standards were prepared from a 1560 ng/ml standard in 0.008 N HCl. 1.59 ml of this standard were diluted with 0.4 ml of 0.05 g/100 ml (w/v) O.P.T., D.D.W. and 10 N HCl. Final concentrations obtained were 200 ng/ml serotonin and graded normalities of HCl from 4.50–7.50. Individual blanks were prepared from 0.008 N HCl and were treated similarly. Specimens were complexed with O.P.T.[4]. Each of the 12 standards and blanks were then divided into 5 fractions and refrigerated at 4 °C until tested.

Fluorometry. In sequence, a single set of 13 or 12 samples and blanks representing the entire normality ranges of the direct and O.P.T. series were placed in a waterbath to bring the tubes to the desired temperature (5–50 °C range). The waterbath was connected to the flow-through compartment of the S.P.F. cuvette housing to maintain temperature. Dry, filtered nitrogen gas was used to continuously flush the cuvette housing to reduce condensate formed on the cuvettes at low temperatures and to reduce optical scattering. Samples were permitted to equilibrate in the waterbath for 5 min prior to fluorometry.

Results. Serotonin-direct. Results corrected for individual blanks (Figure 1) indicated that (a) maximal serotonin fluorescence was achieved at an acidity of 3.25–3.50 N HCl; this was found to be independent of temperature. (b) There was an inverse relationship between temperature and serotonin fluorescence for all normalities tested. At the normality of maximal fluorecence (3.5 N HCl), this relationship was exponential between 5–20 °C (Figure 3). At temperatures greater than 20 °C, this relationship was no longer linear due to serotonin destruction. In support of this, the curve obtained from samples at 50 °C (Figure 1) was irregular; this irregularity was still present when these samples were cooled and reread at 20 °C.

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[6] American Instrument Co., Inc., Aminco-Bowman, 8030 Georgia Avenue, Silver Springs (Maryland 20910, USA).
[7] Honeywell 320 Solid State XY Recorder.

Results. Serotonin-O.P.T. Results corrected for individual blanks indicated (Figure 2) that (a) maximal serotonin-O.P.T. fluorescence was achieved at an acidity of 6.25–6.50 *N* HCl. This was found to be independent of temperature. (b) There was an inverse relationship between temperature and serotonin fluorescence for all normalities tested. At 6.5 HCl, this relationship was linear (Figure 3) between 5–35 °C, and could be described as follows:

$$F.I._2 = -1.4\,(T_1 - T_2) + F.I._1$$

where,

$F.I._1$ = Fluorescence intensity read, in F.I.U.
$F.I._2$ = Fluorescence intensity desired, in F.I.U.
T_1 = Temperature of reading, in °C.
T_2 = Temperature desired, in °C.
-1.4 = Derived correction factor.

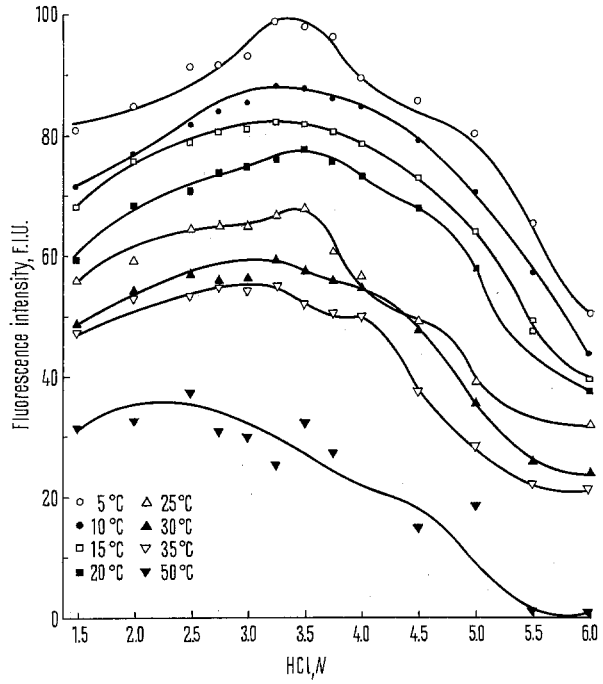

Fig. 1. Acidity and temperature for maximal serotonin fluorescence. Fluorescence intensity for 200 ng/ml serotonin standards and blanks made from spectra at 545 nm wavelength. All readings corrected for individual blanks. Instrumental conditions were activation wavelength 295 nm, attenuation 1.0% full scale and sensitivity 60 sensitivity units.

Discussion. The intensity of serotonin and serotonin-O.P.T. fluorescence is pH and temperature dependent. Many investigators routinely read serotonin fluorescence at a normality of 2.8 HCl[3]. The work reported here indicates that maximal fluorescence is achieved at a concentration of 3.25–3.50 *N* HCl. However, the curve is rather flat and fluorescence at 20 °C in 3.5 *N* HCl is only 5% greater than that in 2.8 *N* HCl. This is similar to the normality originally reported, but widely different from that of 6.0 *N* reported by SENTENAC-ROUMANOU et al.[8]. However, the curve reported by these latter authors has one point at 6.0 *N* with adjacent points at 4 and 12 *N*.

Serotonin fluorescence increases with decreasing temperature and maximal fluorescence at 5 °C is 26% greater than at 20 °C; however, irrespective of temperature, the peak of maximal serotonin fluorescence remains constant at 3.25–3.50 *N* HCl. Thus, there is approximately a 2% increase in fluorescence per degree fall. The slight irregularity of readings seen in Figures 1 and 2 are presumably due to imperfect temperature control in the S.P.F. cuvette housing at the time of reading.

MAICKEL and MILLER[4] have suggested that maximal fluorescence of the serotonin-O.P.T. complex is achieved at a concentration (present authors' calculation) of 6.00 *N* HCl. The data, however, reported here show that maximal serotonin-O.P.T. fluorescence is achieved at a concentration of 6.25–6.50 *N* HCl. Serotonin-O.P.T. fluorescence increases with decreasing temperature and maximal

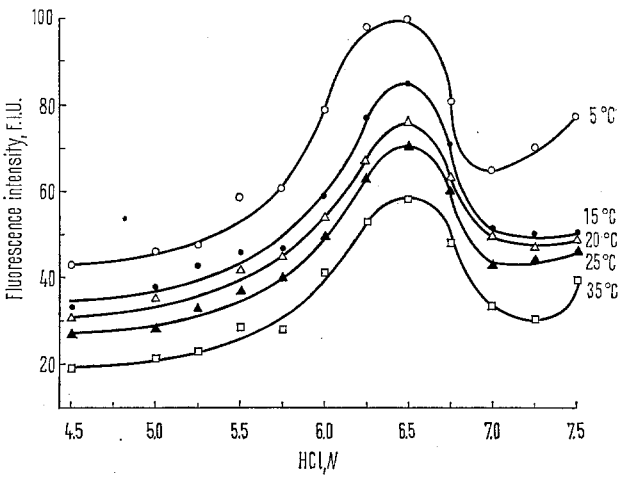

Fig. 2. Acidity and temperature for maximal serotonin-O.P.T. fluorescence. Fluorescence intensity for 200 ng/ml serotonin-O.P.T. standards and blanks made from spectra at 470 nm wavelength. All readings corrected for individual blanks and adjusted to a common attenuation and sensitivity of 10.0% full scale and 60 sensitivity units, respectively. Activation wavelength was 360 nm.

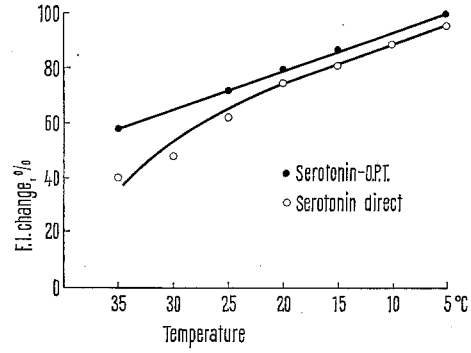

Fig. 3. Change in fluorescence of serotonin and serotonin-O.P.T. with temperature. Data obtained from readings of fluorescence intensity at the normalities of maximal fluorescence presented in Figures 1 and 2.

[8] H. SENTENAC-ROUMANOU, J.-Y. LEGALL, G. MARBLE and J.-C. MARC, Annls Biol. clin. *25*, 825 (1967).

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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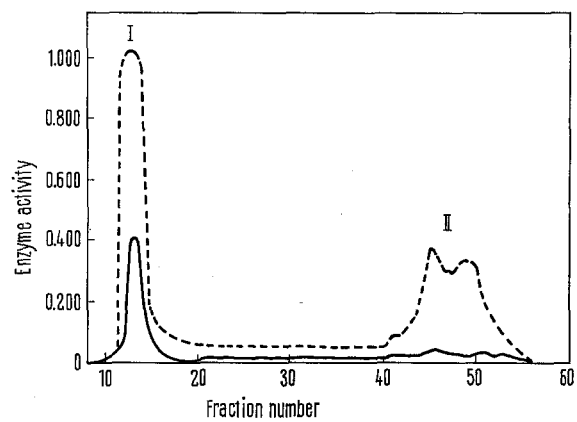
On the Origin of Wound Arylaminopeptidases

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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