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GAS CHROMATOGRAPHIC ANALYSIS OF FREE AMINO ACIDS IN THE HYALOPLASM OF THE HYPOPHYSIS, PINEAL GLAND, THYROID GLAND, SPINAL CORD, THYMUS AND LYMPH NODES OF THE COW

JANUSZ GABRYŚ* and JANUSZ KONECKI

Department of Cytology and Histology, Biological and Morphological Institute, Silesian School of Medicine, K. Marksa 19, 41-808 Rokitnicy (Poland)

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SUMMARY

Studies of the qualitative and quantitative composition of free amino acids in the hyaloplasm of the hypophysis, pineal gland, thyroid gland, spinal cord, thymus and lymph nodes of the cow are described. The following findings are reported: the highest levels of alanine, valine, glycine, isoleucine, histidine, leucine, threonine, serine, phenylalanine, tyrosine and lysine are found in the thyroid gland, methionine and aspartic acid in the spinal cord, tryptophan and hydroxyproline in the pineal gland, and proline and glutamic acid in the thymus gland. The highest level by weight is that of glutamic acid in all tissues. The presence of α-aminobutyric acid combined with sarcosine and 4-aminoisobutyric acid with 2-AOA and citrulline with cystine was demonstrated. α-Aminoisobutyric acid and isovaline were found in the spinal cord.

INTRODUCTION

The present work extends our investigations of the quantitative and qualitative composition of free amino acids* in the hyaloplasm of various tissues. In our previous work we traced the content of amino acids in the hyaloplasm of the liver, kidneys, frontal lobes of the brain, cerebellum, eyeball without muscles, heart muscle and skeletal muscles. This present paper deals with the results obtained from the following organs of the cow: hypophysis, pineal gland, spinal cord, thyroid gland, thymus gland, and lymph nodes.

Analysis of free amino acids by gas—liquid chromatography was performed according to the methods of Gehrke et al. [1,2] and Kaiser et al. [3].

*Abbreviations of amino acids: α-ABA = α-aminobutyric acid; 4-AIBA = 4-aminoisobutyric acid; α-AIBA = α-aminoisobutyric acid; ALLOTHR = allothreonine; 2-AOA = 2-aminooctanoic acid; ARG = arginine; CITR = citrulline; CYS = cystine; CYSH = cysteine; HIS = histidine; IVAL = isovaline; ORN = ornithine; SARC = sacrosine (see also Table I).

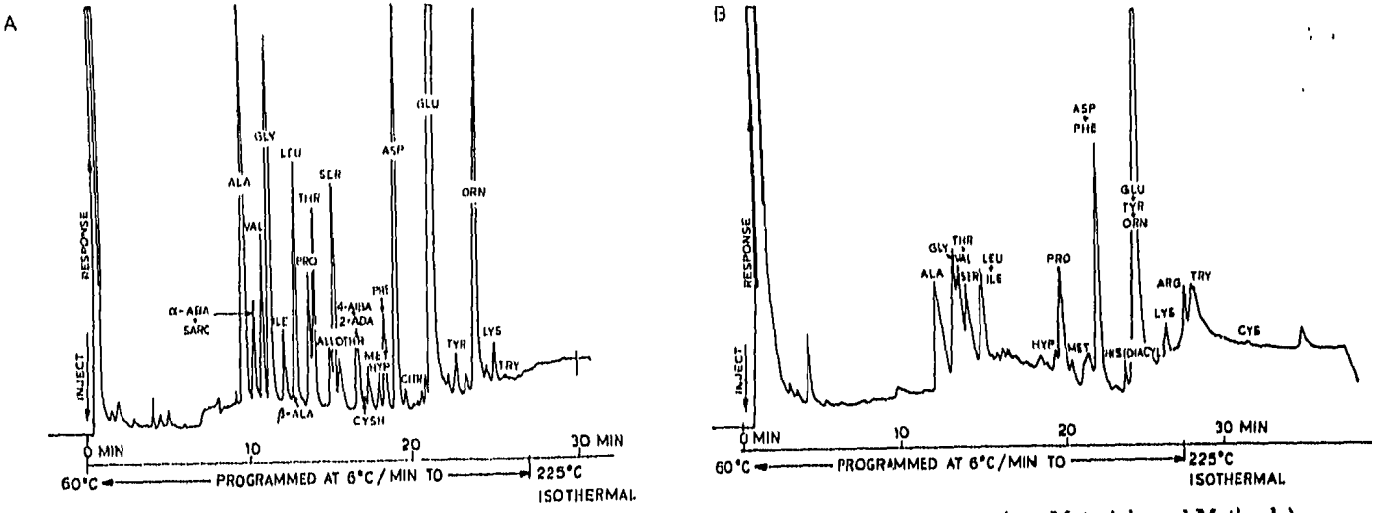

Fig. 1. GLC analysis of amino acids in the hyaloplasm of hypophysis on columns A and B (see Materials and Methods).

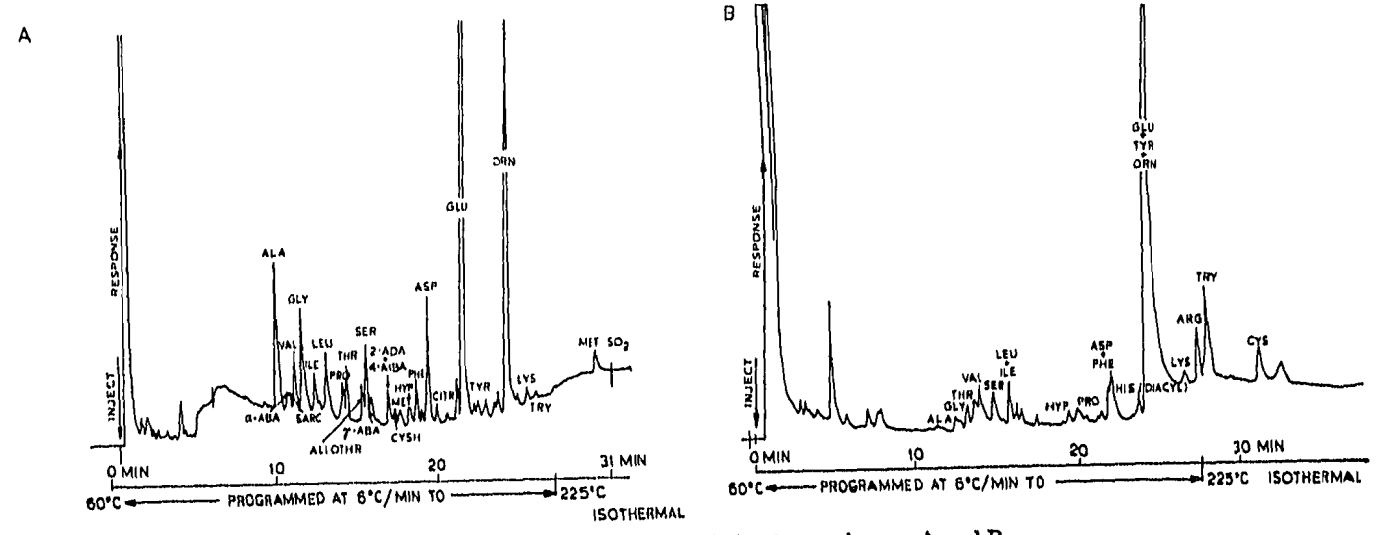

Fig. 2. GLC analysis of amino acids in the hyaloplasm of pineal gland on columns A and B.

Preparation of the hyaloplasm was performed according to the description of Chauveau et al. [4].

MATERIAL AND METHODS

Animals

The material, obtained from the slaughter house, was fixed in saturated picric acid. The tissues obtained were homogenized in picric acid in the ratio 1:5.

Apparatus

The following equipment was used: glass homogenizer (25-ml capacity) with a Teflon piston; K-24 and VAC 601 centrifuges produced by Janetzki Heins, Ilmenau, G.D.R.; ion-exchange columns 12 mm × 150 mm and 15 mm × 300 mm; lyophilizing apparatus produced by VEB MLW Labortechnik, Engelsdorf, G.D.R.; reaction vessels of our own design equipped with Teflon-lined screw-caps; automatic ultrasonic disintegrator UD-11 produced by Techpan, Warsaw, Poland; oil-baths with a thermoregulator; evaporator produced by Büchi, Flawil, Switzerland; gas chromatograph (Varian 3700) with data analyzer CDS 111 C and a recorder A 25 (Varian, Palo Alto, CA, U.S.A.); 10-μl syringes from Hamilton, Reno, NV, U.S.A.; and 1000-μl and 100-μl automatic pipettes produced by Eppendorf, Hamburg, G.F.R.

Reagents

7 *N* pure ammonia solution and Dowex 50W-X8 100—200 mesh (H^+) were products by Fluka, Buchs, Switzerland; pure picric acid was from P.O.CH, Gliwice, Poland. HCl gas was produced by Fluka; *n*-butanol Seq. grade and trifluoroacetic anhydride (TFAA) were from Pierce, Rockford, IL, U.S.A. Standard amino acids were obtained from BDH (Poole, Great Britain), Mann Labs. (New York, NY, U.S.A.), Merck and Pierce. A calibration mixture was prepared by adding the successively estimated amino acids. Column supports were 2% OV-17 + 1% OV-210 on 100—120 Supelcoport (column B) and 0.65% EGA-PS on 80—100 mesh Chromosorb W AW (Supelco, Bellefonte, PA, U.S.A.) (column A).

Preparation of amino acids

Homogenization. The tissues were homogenized in the ratio of 1:5.

Centrifugation and ultracentrifugation. The investigations were performed at +4°C according to the method of Chauveau et al. [4]. The homogenate was centrifuged at 600 *g* for 15 min; the supernatant was then decanted and centrifuged at 105,000 *g* for 60 min. Cell hyaloplasm was obtained in the supernatant, and 1% of picric acid was added in the ratio 1:5. The solution was centrifuged at 3500 *g* for 15 min, the proteins being separated in the sediment. The deproteinized supernatant containing an excess of picric acid was introduced into the ion-exchange column.

Ion exchange. In the following ion-exchange procedure a 60-fold or greater excess of resin capacity to exchangeable ions placed in the column was maintained, i.e. 6 g of Dowex 50W-X8 (100—200 mesh) for 25 ml of the examined

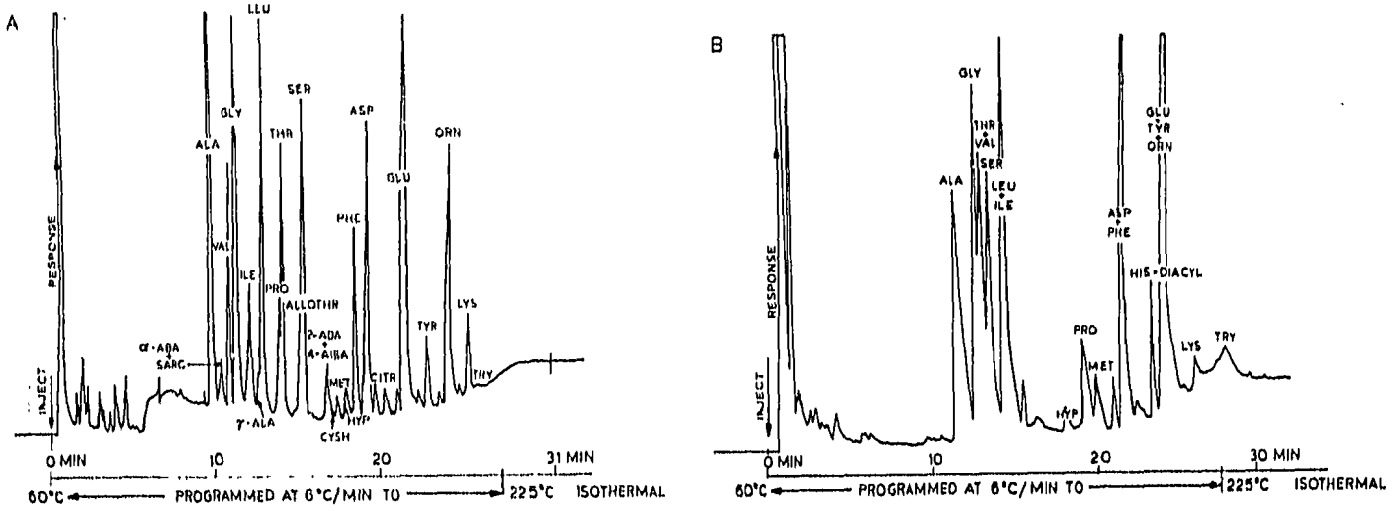

Fig. 3. GLC analysis of amino acids in the hyaloplasm of thyroid gland on columns A and B.

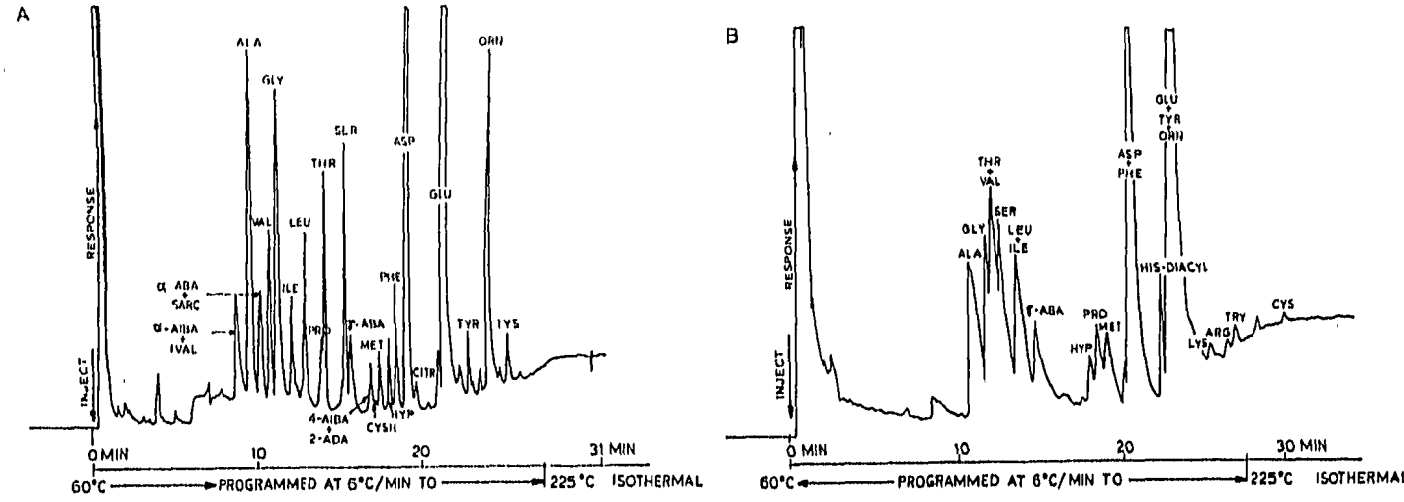

Fig. 4. GLC analysis of amino acids in the hyaloplasm of the spinal cord on columns A and B.

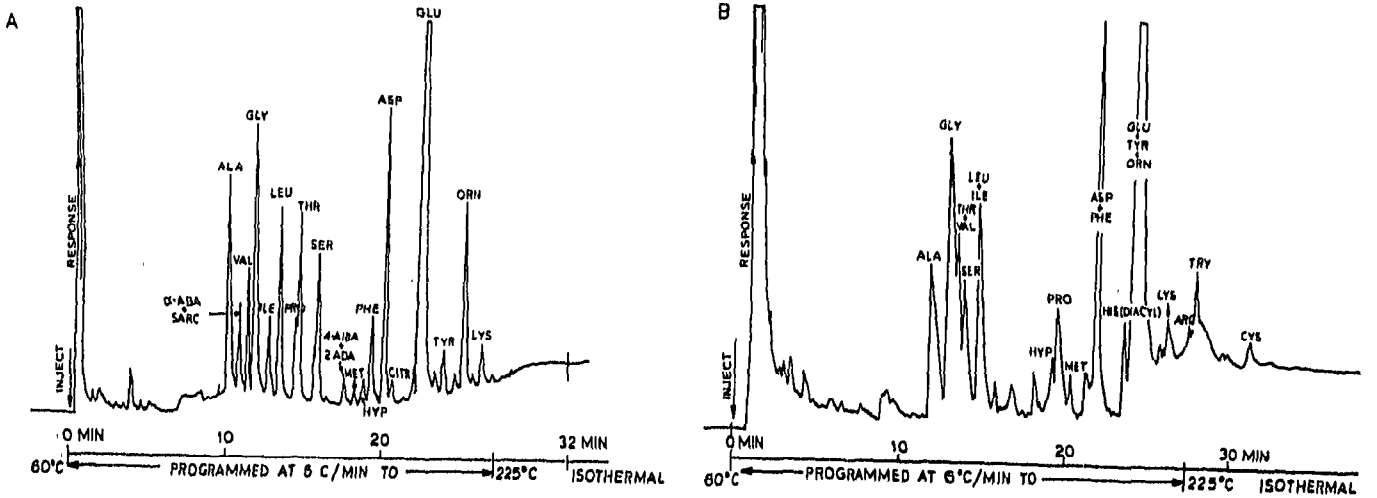

Fig. 5. GLC analysis of amino acids in the hyaloplasm of thymus gland on columns A and B.

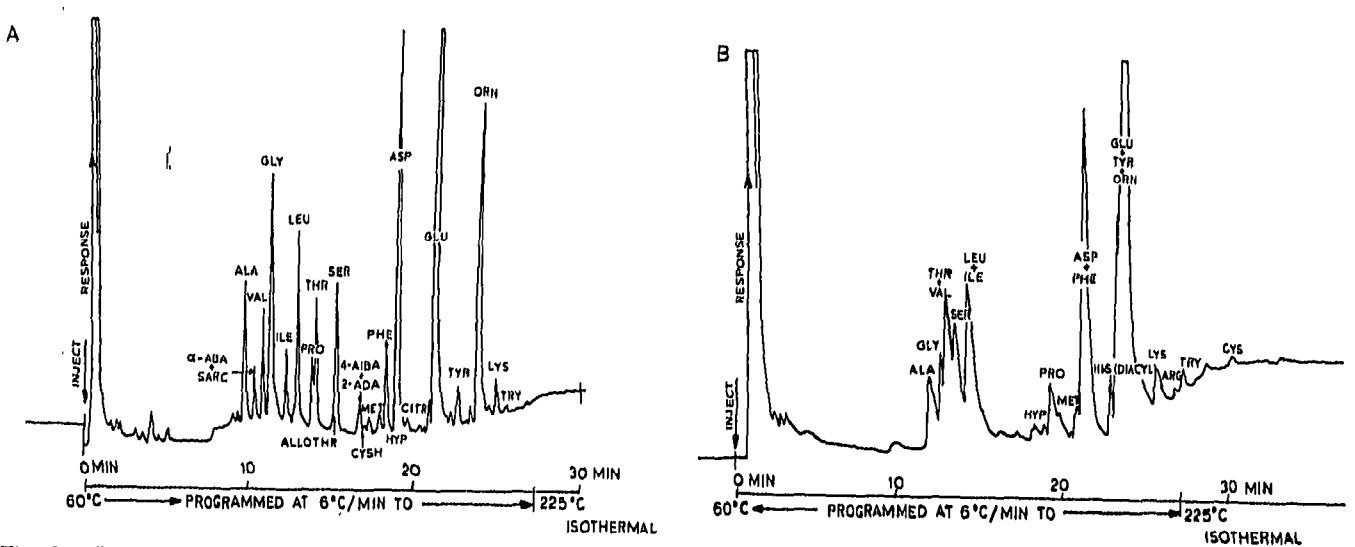

Fig. 6. GLC analysis of amino acids in the hyaloplasm of lymph nodes on columns A and B.

TABLE I

COMPARISON OF GAS–LIQUID CHROMATOGRAPHIC ANALYSIS OF HYALOPLASM TISSUES

Values are expressed in μg of amino acid per 110 mg of tissues, and represent the mean ± S.D. of five independent samples.

Amino acid	Abbreviation	Reaction time (min)	Pineal gland	Lymph nodes	Thymus gland	Thyroid gland	Hypophysis	Spinal cord
Alanine	ALA	9.45±0.08	0.69±0.04	0.39±0.05	0.50±0.01	0.89±0.03	0.73±0.02	0.50±0.01
Valine	VAL	10.69±0.05	0.27±0.02	0.28±0.02	0.25±0.00	0.39±0.04	0.25±0.01	0.21±0.00
Glycine	GLY	11.07±0.04	0.59±0.01	0.81±0.07	0.73±0.02	0.85±0.01	0.71±0.02	0.55±0.01
Isoleucine	ILE	11.94±0.04	0.14±0.01	0.23±0.03	0.18±0.01	0.25±0.04	0.11±0.01	0.14±0.01
Leucine	LEU	12.68±0.03	0.31±0.01	0.55±0.05	0.42±0.02	0.85±0.09	0.41±0.01	0.25±0.01
Proline	PRO	13.63±0.05	0.22±0.00	0.23±0.04	0.24±0.00	0.24±0.02	0.24±0.03	0.10±0.03
Threonine	THR	13.89±0.06	0.36±0.02	0.30±0.01	0.35±0.04	0.42±0.04	0.32±0.02	0.33±0.03
Serine	SER	15.10±0.06	0.38±0.07	0.37±0.01	0.33±0.03	0.65±0.04	0.39±0.01	0.43±0.02
Methionine	MET	17.13±0.05	0.07±0.01	0.10±0.01	0.07±0.01	0.19±0.08	0.18±0.00	0.23±0.03
Hydroxyproline	HYP	17.74±0.04	0.13±0.01	0.06±0.00	0.06±0.00	0.07±0.01	0.06±0.00	0.10±0.00
Phenylalanine	PHE	18.17±0.06	0.24±0.01	0.25±0.01	0.22±0.00	0.41±0.01	0.18±0.00	0.21±0.01
Aspartic acid	ASP	18.86±0.09	0.64±0.02	1.20±0.04	0.69±0.00	0.64±0.04	0.78±0.01	1.48±0.02
Glutamic acid	GLU	21.05±0.06	5.22±0.66	4.17±0.13	5.25±0.01	3.16±0.04	4.67±0.09	4.68±0.09
Tyrosine	TYR	22.65±0.04	0.31±0.01	0.50±0.03	0.54±0.01	0.63±0.01	0.38±0.01	0.46±0.02
Lysine	LYS	25.10±0.02	–	0.34±0.04	–	0.45±0.07	0.24±0.02	0.20±0.03
Tryptophan	TRY	25.98±0.21	–	–	0.08±0.01	–	–	0.12±0.05
Total			9.57	9.78	9.91	10.09	9.65	9.99

supernatant. The 6 g of ion-exchanger were placed in a 500-ml vial, covered with 7 *N* ammonia solution and mixed for 60 min. After sedimentation the fluid was decanted. The procedure was repeated two times. Afterwards the column was washed with bidistilled water until a pH of 7.5 was attained. The ion exchanger was regenerated with 3 *N* HCl (three times), and washed with water until a pH value of 6.2 was attained. Columns 15 mm × 150 mm were filled half-full with the wet resin. The supernatant with the picric acid was passed through the column at a rate of 2 ml/min. The surface was washed with water until the eluate was decolourised. Then 25 ml of 7 *N* ammonia solution and 30 ml of bidistilled and deionized water were passed through the column. The eluate and the washings were collected and mixed, and 55 ml of the mixture were lyophilized. The procedure was carried out according to the description of Zumwalt et al. [5].

Lyophilization. A 55-ml volume of the eluate and washings was collected into a vessel and quickly frozen in liquid nitrogen. The sample was placed in a 1000-ml condenser. After lyophilization the dry sediment was retained and transferred to an esterification vessel.

Derivatization. The reaction was carried out according to the method of Gehrke et al. [1,2].

RESULTS AND DISCUSSION

The quantitative and qualitative content of amino acids and their trace amounts in the cell hyaloplasm (cytosol) of glandular tissues and the spinal cord of cows were studied (see Table I). During the investigation three independent series of analysis of amino acid composition were performed. The following conclusions were drawn: the highest levels of ALA, VAL, GLY, THR, ILE, LEU, HIS, SER, PHE, TYR and LYS were found in the thyroid gland, MET and ASP in the spinal cord and PRO + GLU in the thymus. In the tissues analyzed GLU was found to have the highest concentration of all amino acids. As a result of the separation of amino acid mixture on EGA the presence of α-ABA with SARC and 4-AIBA with 2-AOA was discovered. The works of Raulin et al. [6], Amico et al. [7], and Casagrande et al. [8] were of great help. The peak of α-AIBA with IVAL identified in the spinal cord was the only one found in the material investigated. Quantitative computerized analysis shows a lower weight level of the total bulk of the separated mixture as compared to its amount taken for derivatization purposes. This problem has been also emphasized by Adams et al. [9]. They found ions, lipids and peptides in the investigated material. Attention has been paid to tissue material stored for periods of longer than 14 days. Decreased peak heights for PRO + ALLOTHR, α-ABA, MET, CITR, TYR, ARG, TRY, CYSH and β-ALA were found.

CONCLUSIONS

The highest levels of amino acids were found in thyroid gland, lymph nodes and thymus. TRY, ARG and CYS were the amino acids with the lowest levels. The presence of small peaks indicates a trace content of unidentified compounds.

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