DISTRIBUTION OF FREE AMINO ACIDS AND RELATED SUBSTANCES IN ORGANS OF THE RAT*

by

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Free amino acids in organs have been measured by methods that are specific for the entire group[1]. Attempts to estimate individual components of this group have met with many technical difficulties, and only in isolated cases have some chemical procedures been used successfully.

The methods developed in the fractionation and analysis of protein hydrolysates have not been used in the estimation of free amino acids of tissues. Microbiological assay which is very sensitive and in many cases quite specific has been used for the estimation of free amino acids of blood and urine[2, 3, 4].

In a recent communication one of the authors reported that in rat liver[5] only four amino acids could be detected by means of one-dimensional paper chromatography, and approximate values for their concentrations were given. Since it appeared quite obvious that other amino acids may exist in the liver and other organs, the present investigation was undertaken to establish the existence of other possible components, hitherto undetected, and to determine the distribution of amino nitrogen among those fractions measured by paper chromatography.

METHODS

Organs were obtained from rats of the SPRAGUE-DAWLEY colony, weighing 120–150 g. In all cases the animals were fasted for 24 hours, sacrificed by decapitation and the organs removed. Samples weighing 0.5 to 1.0 g were taken, rinsed to remove blood and then extracted by a procedure previously described[6]. The extracts were made up to volumes such as to represent 1 g/ml of fresh tissue.

Chromatographic analyses of the extracts were carried out on filter paper Whatman No. 4. It was necessary to resolve the components of the extracts by means of two-dimensional chromatography, using phenol and 2.4-lutidine. When chromatography is performed on strips of filter paper and one solvent is used, only some of the components will be resolved[5]. For quantitative estimations, the chromatograms were developed with 0.05% ninhydrin solution in butanol. The various components to be measured were then cut out from the paper, placed in test tubes and further analysed by a procedure previously reported[7]. The accuracy of this method was tested with mixtures of known amino acids. The chief limitation is that in some organs the distribution of free amino acids is such that one or two components exist in quantities many times as great as some other components. Under these circumstances, resolution of all the amino acids is badly impaired. In most cases resolutions were complete and only those components shown as discrete spots were measured.

In addition to R_f values, further evidence for the identity of amino acids was obtained by hydrolysing the extracts and repeating the analysis qualitatively. In this manner, the existence of hydrolysable substances was determined and some conclusions drawn as to their nature.

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TABLE I

DISTRIBUTION OF FREE AMINO ACIDS AND RELATED SUBSTANCES IN RAT ORGANS

Organ	Total Amino Nitrogen in γ per gram Fresh Tissue	Fraction of Total Amino Nitrogen in Percent*							
		Glutathione	Aspartic Acid	Glutamic Acid	Glycine	Alanine	Taurine	Glutamine	"Under" Glutamic Acid
Liver	401 ± 17.5	5.2 ± 0.17	4.0 ± 0.16	9.7 ± 0.72	8.3 ± 0.40	2.8 ± 0.16	8.2 ± 1.90	4.3 ± 0.26	1.9 ± 0.13
Kidney	662 ± 20.0	—	11.7 ± 0.67	20.9 ± 1.12	15.7 ± 0.45	10.4 ± 1.24	14.1 ± 0.41	—	—
Muscle	491 ± 22.8	6.6 ± 0.65	—	5.0 ± 0.32	16.3 ± 0.72	12.6 ± 0.51	33.1 ± 0.67	8.5 ± 0.14	4.0 ± 0.18
Heart	410 ± 13.4	5.7 ± 0.25	9.2 ± 0.59	18.2 ± 0.57	5.6 ± 0.12	6.0 ± 0.57	47.1 ± 1.80	6.4 ± 0.34	4.7 ± 0.17
Spleen	857 ± 59.0	5.4 ± 0.28	9.3 ± 0.43	13.4 ± 0.61	7.5 ± 0.35	4.8 ± 0.46	16.0 ± 0.56	4.9 ± 0.54	13.8 ± 0.59
Testis	334 ± 14.0	8.9 ± 0.14	5.0 ± 0.32	17.1 ± 0.45	6.0 ± 0.42	—	4.8 ± 0.14	—	8.5 ± 0.21
Brain	518 ± 15.6	—	9.8 ± 0.40	24.8 ± 0.68	—	—	6.4 ± 0.23	—	3.0 ± 0.28
Ileum	492 ± 40.0	—	3.4 ± 0.14	11.3 ± 0.39	4.6 ± 0.27	3.4 ± 0.16	13.5 ± 0.50	—	—

* Arithmetic mean of values from five animals, plus or minus the standard errors, corrected for small numbers

EXPERIMENTAL

The values obtained for the concentration of individual amino acids are presented in Table I; these are expressed in percent of the total amino nitrogen which was measured by the ninhydrin method as follows: 0.1 ml aliquot of tissue extract was pipetted into a test tube; 1 ml of ninhydrin reagent, prepared according to MOORE AND STEIN[8], was added and the tube shaken for a few seconds. The mixture was heated in boiling water for exactly 20 minutes. In most cases the colour yield was too intense for direct reading and dilution to 100 ml was necessary. Readings were made in the Coleman Junior Spectrophotometer, Model 6-A. Values obtained by this method were read against a DL-leucine standard. The accuracy of the ninhydrin method has been questioned many times on the basis that there is no adequate standard. This limitation of the ninhydrin method would affect the present results if they were considered as absolute values. Actually, the total colour given by the reaction with ninhydrin is used as an arbitrary baseline. Furthermore, the variations in the color produced by the different components of the extracts are in most cases fairly small, as shown by MOORE AND STEIN[8]. The ninhydrin method was chosen in order to determine the possible number of components which do not show in the chromatogram.

Table I only shows those substances that were in sufficient amounts to be measured quantitatively. Other substances that gave a positive ninhydrin reaction were present, but in quantities too small to measure. In Table II are shown all substances identified by means of two-dimensional chromatography.

In an earlier paper[5] AWAPARA reported that in rat liver only four amino acid fractions could be detected, and the approximate concentrations were given. Those values have been found to be much higher than the values herein presented. Observations on the amino acid composition of liver extracts have revealed several unsuspected facts. Taurine was present in large quantities in some livers, whereas in other samples there was an absolute absence of this compound. Large amounts of taurine tend to interfere with glycine in one-dimensional chromatograms. The variations in all the other fractions were quite large for different animals.

Acid hydrolysis (24 hours with 6 *N* HCl at 100° C in sealed tubes) of the extracts, followed by two-dimensional chromatography, showed that peptides were probably present in the original extracts, since several new substances made their appearance. Ileum showed the largest number of these new substances, many of which could not be identified. The appearance of β-alanine and histidine upon hydrolysis of liver and skeletal muscle extracts points to the existence of carnosine in these organs.

An interesting substance, thus far unidentified, was found in a position under glutamic acid. This component, which was resistant to acid hydrolysis, was present in significant amounts in testis and spleen. It was also present in all other organs, but in lower concentration. A similar or perhaps identical substance was found in twelve human tumours studied. The concentration in some of the tumors was as high as 15% of the total amino nitrogen. A compound occupying the same position has been reported in hydrolysates of *E. coli*[9], and *C. diphtheria*[10]. It has also been reported in mouse skin, and mouse skin tumours[11]. Preliminary studies on the nature of this substance have indicated that it is highly insoluble in ethanol and very soluble in water. Further work is being carried out to determine the identity of this unknown substance.

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TABLE II

FREE AMINO ACIDS AND RELATED COMPOUNDS DETECTED IN RAT ORGAN EXTRACTS

Compound	Liver	Kidney	Muscle	Heart	Spleen	Testis	Brain	Ileum
Aspartic Acid	×	×	×	×	×	×	×	×
Glutamic Acid	×	×	×	×	×	×	×	×
Glycine	×	×	×	×	×	×	×	×
Serine	—	×	—	×	×	—	—	—
Threonine	—	—	—	—	—	—	—	—
Alanine	×	×	×	×	×	×	×	×
Methionine	×	×	×	×	×	×	×	×
Phenylalanine	×	×	×	×	×	×	×	×
Tyrosine	×	×	—	—	—	—	—	—
Valine	—	×	—	—	—	—	—	—
Arginine	—	×	—	×	×	×	—	—
Lysine	—	—	—	×	—	×	—	—
Proline	—	×	×	—	—	—	—	—
Hydroxyproline	—	—	×	—	—	—	—	—
"Under" Glutamic	×	×	×	×	×	×	×	—
Glutathione	×	×	×	×	×	×	×	×
Glutamine	×	×	×	×	×	×	×	×
Taurine	×	×	×	×	×	×	×	×

DISCUSSION

The distribution pattern of free amino acids and some related substances (taurine, glutamine, glutathione) varies for each organ examined. The data (Table I) suggests that taurine is a major component of the amino fraction of most organs. The heart shows the highest values, and liver shows tremendous variations in the concentration of this compound. The presence of large amounts of taurine in most organs may be interpreted as being the result of active metabolism of some or perhaps all sulfur amino acids. Unpublished data in this laboratory showed that when cysteine was given intravenously to the rat, a significant increase in the taurine and alanine concentration could be detected in the liver. The ability of the organs to remove taurine from circulation was tested by injecting taurine into four groups of rats and measuring the taurine increase by means of paper chromatography at various intervals. The results showed that taurine was readily removed from the circulation by the kidney, heart and liver. Muscle and spleen showed only insignificant increase. The heart returned to normal values 30 minutes after the injection, whereas the taurine concentration of the kidney returned slowly to normal values.

Aspartic acid, which was found in all organs examined, appeared in low concentrations in skeletal muscle. In many instances it could not be detected at all. The explanation for the absence of aspartic acid in muscle is not clear. This situation seems quite consistent, and similar results were obtained in two samples of human skeletal muscle obtained from surgical specimens. Skeletal muscle was also unique in that it was the only organ containing free proline and hydroxyproline. Kidney showed proline only on acid hydrolysis of the extract.

The kidney, as one may have expected from its excretory function, contained the largest number of components, several of which were not identified. Glutamine and glutathione were found in the kidney in trace amounts, pointing to the high enzymatic activity of kidney towards these two substances. The values for glutamine in the present investigation are not the true values, since much of this substance is converted into pyrrolidone-carboxylic acid during the evaporation of the extract. However the relative amounts detected are in agreement with known facts concerning the existence of glutamine in various organs[12].

The present work does not attempt to give the absolute amounts of each component in the organs of the rat. A close approximation of the manner in which the amino nitrogen is partitioned is quite justifiable. Many of the present data agree with known facts concerning enzymatic activity of the various organs investigated. Similarly, it seems possible to predict enzymatic activity from the distribution pattern of amino acids in the various organs investigated.

SUMMARY

1. The distribution pattern of free amino acids and related substances (taurine, glutathione and glutamine), has been determined by means of two-dimensional paper chromatography in eight organs of the rat.

2. A major portion of the amino nitrogen was found distributed among few substances, in a manner characteristic for each organ. Several unidentified substances have been detected in some of the tissues examined.

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RÉSUMÉ

1. Le mode de distribution des acides aminés libres et des substances alliées (taurine, glutathione, et glutamine), a été déterminé dans huit organes du rat au moyen de la chromatographie de partage sur papier en deux dimensions.

2. Nous avons trouvé que la plus grande partie de l'azote aminé est distribuée parmi quelques substances d'une manière charactéristique pour chaque organe en question. Nous avons recontré dans quelques-uns des tissus examinés des substances non identifieés.

ZUSAMMENFASSUNG

1. Die Verbreitung der freien Aminosäuren und verwandter Stoffe, (Taurin, Glutathion und Glutamin), wurde durch zwei-dimensionale Chromatographie auf Filterpapier in acht Organen der Ratte festgestellt.

2. Ein Hauptteil des Amino-Stickstoffes wurde zwischen einigen Substanzen, in einer für jedes Organ charakteristischen Weise verteilt gefunden. Verschiedene, bis jetzt noch nicht identifizierte Stoffe, sind in einigen der analysierten Geweben gefunden worden.

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