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THE SOURCE AND NATURE OF THE NON-PROTEIN NITROGEN OF TETRAHYMENA PYRIFORMIS*

D. L. HILL+ AND JAN VAN EYS±
Department of Biochemistry, Vanderbilt University School of Medicine
Nashville, Tennessee

ABSTRACT

An investigation of the non-protein amino acids of *Tetrahymena pyriformis* revealed that no single peptide is present in quantity. Distinct peptides were found in *Tetrahymena* grown on proteose-peptone. However, these appeared to have come from the medium. The medium also influenced the concentration of free amino acids in the cell, as the concentration inside approached the concentration of the amino acids in the medium. Lysine and alanine were exceptions to this rule.

The size of the free amino acid pool was demonstrated to vary with the stage of growth of *Tetrahymena.* It was larger when the cells were not growing rapidly than when the cells were in the log phase of growth.

The presence of asparagine, glutamine, citrulline, and ornithine was confirmed, and the absence of glutathione and creatinine was demonstrated.

INTRODUCTION

In a study of the nitrogen composition of *Tetrahymena pyriformis,* Wu and Hogg (1952) reported that the amino acid nitrogen which was soluble in trichloroacetic acid accounted for one-third of the total nitrogen of this organism. Subsequently, these investigators (1956) and others (Christensson, 1959; Schleicher, 1959; Wells, 1960, and Loefer and Scherbaum, 1961) determined the free amino acids and related compounds of *Tetrahymena* by means of paper chromatography. Alanine, arginine, aspartic acid, asparagine, cyst(e)ine, glutamic acid, glutamine, glycine, histidine, leucine, isoleucine, methionine, phenylalanine, lysine, proline, serine, threonine, and valine were consistently found; on occasion, glutathione (Scherbaum *et al,* 1959), ornithine (Wu and Hogg, 1956), taurine (Wu and Hogg, 1956; Loefer and Scherbaum, 1961), and tryptophan (Wu and Hogg, 1956) were reported.

Wu and Hogg (1956) further observed that the free and the total non-protein α-amino nitrogen are 18.3 and 26.7%, respectively, of the total nitrogen of the cells. Conjugated amino acids, or peptides, accounted for the difference between these values.

Since these amounts seemed unusually large, the present investigation on *Tetrahymena* was undertaken to establish further the nature and origin of this non-protein nitrogen. Specifically, it was hoped that this sizable quantity of conjugated amino acids might contain isolable quantities of individual peptides.

MATERIAL AND METHODS

Growth of the organism. Cultures of *Tetrahymena pyriformis* E were maintained and grown on either of two media: a proteose-peptone medium as described by Warnock and van Eys (1962) and a defined medium as described by Baker *et al.* (1960). Stock cultures were maintained by weekly loop inoculation into ten ml of proteose-peptone medium. For experimental studies requiring large quantities of cells, aspirator bottles adapted for aeration (Warnock, 1962) and containing 1-2 liters of medium were inoculated with 1/100 of their volume of a full-grown stock culture. The cells were harvested after 5 days of growth by gentle centrifugation at a speed of 2000 rpm in an International Size 1 centrifuge and were washed 4-5 times with 10 volumes of Ringer phosphate solution 0.047N in NaCl, 0.001M in $MgSO_4$ and 0.012M in phosphate buffer (pH 7.3). After storing the washed organisms at —20°, the cellular protein was precipitated by adding ice-cold 10% trichloroacetic acid. The precipitate was washed 3 times with water, and the supernatant and washings were combined. The extract was adjusted to 0.1N in HCl and the trichloroacetic acid was extracted with 3 equal portions of ether.

Chromatography and amino acid analysis. For determination of the total amino acids in the trichloroacetic acid extract, a portion was dried in *vacuo,* taken up in 6N HCl, and sealed in an ampule under reduced pressure. Hydrolysis was accomplished by heating the ampule at 110° for 18 hours, after which the HCl was removed in an evacuated desiccator over NaOH. The sample was dissolved in buffer for chromatographic analysis on the Beckman/Spinco Model 120 Amino Acid Analyzer (Beckman Instruments, Inc., Palo Alto, California). A similar sample, unhydrolyzed, was used for determination of the free amino acids. Conjugated amino acids were calculated by difference.

To determine the amount of ninhydrin-positive material present at different stages of growth, each of 50 tubes containing 10 ml of sterile medium was inoculated with a small drop of a full-grown stock culture. Growth was measured turbidimetrically in an Evelyn colorimeter (Rubicon Company, Philadelphia, Pennsylvania) with a 540 mμ filter. Cells were harvested at several stages of growth, washed, and frozen until ready for use. Protein was precipitated as before; and the ninhydrin color of the supernatant (freed of ammonia by adjusting to 0.01N in NaOH) was measured on the Technicon Autoanalyzer (Technicon Instruments Corp., Chauncey,

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± Investigator, Howard Hughes Medical Institute.

New York). The procedure outlined in the manual for this instrument was used. Total ninhydrin color after hydrolysis in 6N HCl was measured in the same manner. The contribution from conjugated amino acids was calculated by difference.

For chromatography of the ninhydrin-positive compounds, a large column (3 x 150 cm) was packed with Dowex 50-X2, a peptide resin (Hirs *et al.*, 1956), which had been washed previously first with 4N HCl, followed by 2N NaOH, and then equilibrated with 0.2N sodium citrate buffer (pH 3.1). An extract, prepared as described above and adjusted to pH 2 with 1N NaOH, was placed on the column. The effluent was analyzed by the use of the Autoanalyzer, which took a continuous sample from a T-joint at the base of the column. The remainder of the effluent (94% of the total) was collected in 13-ml fractions.

Elution was begun by passing 800 ml of the pH 3.1 buffer over the column; then a gradient was started with this buffer in a 2-liter mixing flask and a 1N sodium acetate-citrate buffer of pH 5.1 in an upper chamber. After 2400 ml of effluent had been collected, a 2N buffer (pH 5.1) was substituted in the upper chamber; and after 4400 ml of effluent had been collected, a 2N sodium acetate buffer (pH 6.6) was introduced directly to the column. The elution was completed with this buffer.

Desalting of the ninhydrin-positive fractions was accomplished by use of columns containing Dowex 50-X8 (H^+), 20-30 mesh. The sample was placed on the resin, and the column was washed with 3 column volumes of water. The amino acids and peptides were eluted with 30% pyridine-4% acetic acid. The first few ml of the eluate contained the ninhydrin-positive material. Some of the more basic compounds were desalted by using Amberlite IRC-50(H^+), 20-50 mesh. In this case, the sample was placed on the resin, and the column was washed with 0.1% acetic acid until the effluent was free of the sodium ion. The ninhydrin-positive material was then eluted with the pyridine-acetic acid buffer.

The desalted, ninhydrin-positive compounds were identified by paper chromatography. After desalting, each peak was concentrated to a small volume by lyophilization, and aliquots were chromatographed in several solvents. Those used were butanol: acetic acid: water (73:10:17); lutidine: water: ethanol: diethylamine (55:25:20:2); phenol: water (4:1), containing 0.004% 8-hydroxyquinoline; propanol: ammonia: water (6:1:3); and tert-amyl alcohol: formic acid: water (3:1:3). The Rf values of the ninhydrin-positive spots were compared with those of standard amino acids.

Preparations containing unidentified material as determined by paper chromatography were streaked on paper and chromatographed in the solvent offering the best resolution. The unidentified material was cut out and eluted; the eluate was lyophilized and hydrolyzed. The preparation was then chromatographed in two dimensions using the latter two solvents. After spraying with 0.5% ninhydrin in water-saturated *n*-butanol, the spots were then compared to the known amino acids of a chromatogram of hydrolyzed bovine serum albumin which had been treated in the same manner. The appearance of several amino acids in the hydrolyzate of the unidentified material was taken as an indication that the original compound was a peptide.

Other analytical procedures. Nitrogen was measured by the Folin-Farmer microkjeldahl technique (Folin and Farmer, 1912). In addition to analysis by the Technicon Autoanalyzer, ninhydrin-positive materials were determined manually by the method of Moore and Stein (1954). The method of Archibald (1944) was used to measure citrulline. Citrulline was detected on chromatograms by use of either *p*-dimethylaminobenzaldehyde (Dalgliesh), 1952 or ninhydrin.

Creatinine was measured by the Jaffé test after treatment with Lloyd's reagent (Hare, 1950) and detected on chromatograms by the Jaffé reaction (Acher *et al.*, 1955).

Glyoxalase activity was measured by the method of Woodward (1955). Glutathione was determined by the same method and by the use of alloxan (Patterson *et al.*, 1949). Protein was measured by the Lowry method (Lowry *et al.*, 1951).

Results

Amino acid analyses. Table I compares the quantitative amino acid and nitrogen analyses of the trichloroacetic acid-soluble fractions of *Tetrahymena* grown for 132 hours on two different media. Other compounds which possibly were present in trace amounts are: cysteic acid, glycero-phosphoethanolamine, methionine sulfoxide, phosphoethanolamine, and phosphoserine. Compounds which were not present in detectable amounts were: β -alanine, α -aminoadipic acid, α -aminobutyric acid, γ -aminobutyric acid, β -aminoisobutyric acid, anserine, carnosine, creatinine, galactosamine, glucosamine, glutathione, hydroxylysine, hydroxyproline, 1- and 3-methylhistidine, sarcosine, tryptophan, and urea.

Free arginine was absent in the preparation of *Tetrahymena* grown on proteose-peptone which is reported in Table I. However, it has been demonstrated by column and paper chromatography to be present in other preparations of *Tetrahymena* grown on the same medium. The citrulline found in the sample reported in Table I was identified not only by position of elution, but also by paper chromatography (Table 2), using either *p*-dimethylaminobenzaldehyde or ninhydrin to visualize the spots. Further support for the identification of citrulline was given by quantitative analysis of this preparation. Using the Amino Acid Analyzer, 7.2 micromoles of citrulline per gram dry weight was found; using diacetylmonoxime, the value was 7.5 micromoles per gram dry weight. In other samples, however, citrulline could not be determined by paper chromatography. Ornithine was not determined for the extract of cells grown on proteose-peptone but was present in the extract of cells grown on the defined medium. It was identified only by position of elution. The variability of arginine, citrulline, and ornithine was found to be due to an active arginine dihydrolase system in *Tetrahymena* (Hill and van Eys, 1964.).

Table 1

Free and Conjugated Amino Acids and Related Compounds in *Tetrahymena Pyriformis**

	Grown on Proteose-Peptone		Grown on Defined Medium	
	Free	Conjugated	Free	Conjugated
Alanine	25.0	11.6	25.8	9.8
Arginine	0.0	1.9	9.4	4.5
Aspartic acid	5.7	9.6	6.9	36.8
Asparagine	Present	—	(14.8)	—
Glutamine	Present	—		—
Citrulline	7.2	—	Present	—
Cystine (1 2)	0.0	0.8	4.0	2.9
Glutamic acid	16.8	11.7	17.9	44.4
Glycine	6.0	16.0	26.4	22.5
Histidine	1.8	0.0	24.0	5.0
Leucine	4.0	1.4	16.6	8.1
Isoleucine	2.8	1.8	9.0	6.3
Lysine	16.3	8.2	20.9	20.1
Methionine	1.0	3.7	10.5	2.1
Ornithine	—	—	0.9	0.0
Phenylalanine	2.2	1.3	10.2	3.1
Proline	Present	Present	14.0	10.7
Serine	3.1	0.0	17.0	7.2
Taurine	Present	Present	1.2	1.4
Threonine	1.8	1.3	12.4	9.7
Tyrosine	1.0	0.0	8.7	0.0
Valine	7.8	0.4	7.1	14.3
Identified amino acids	102.5	69.7	257.7	208.9
Identified amino acid N	136.6	83.6	370.5	252.5
Ammonia	20.8	32.2	30.4	52.6
TCA-soluble N (by analysis)	333		945	
Total N	4433		4095	

* All values are expressed on micromoles/gram dry weight.

Quantitatively, alanine, glutamic acid, and lysine are the most prevalent free amino acids in *Tetrahymena* grown on the proteose-peptone medium. Glycine, glutamic acid, and alanine are the most prevalent conjugated amino acids. For *Tetrahymena* grown on the defined medium, glycine, alanine, and histidine are the free amino acids in highest concentration. Glycine, aspartic acid, and glutamic acid are the predominant conjugated amino acids.

A greater percentage of the total nitrogen is trichloroacetic acid-soluble for the *Tetrahymena* grown on the defined medium than for *Tetrahymena* grown on proteose-peptone. This is probably due to the growth stage at which the organisms were harvested, as will be seen in Fig. 3.

The amount of trichloroacetic acid-soluble nitrogen in these preparations is 7.5 and 23.0 per cent of the total nitrogen for the extracts of cells grown on proteose-peptone and on the defined medium, respectively. These values are lower than the 32-35 per cent reported by Wu and Hogg (1952). Accordingly, the free amino acid pools are smaller; but the value for *Tetrahymena grown* on the defined medium (257.7 micromoles per gram) approaches that observed by Scherbaum *et al.*, (1959), who harvested *Tetrahymena* grown on proteose-peptone at their maximum growth. Their value was 345

Table 2

Chromatographic Identification of Citrulline

Material	Color developing agent	Solvent* 1	2	3
		Rf	Rf	Rf
Standard Citrulline	Dimethylamino-benzaldehyde	0.61	0.12	0.08
Unknown	Dimethylamino-benzaldehyde	0.61	0.12	0.07
Unknown	Ninhydrin	0.61	0.12	0.08

* Solvent 1 = Phenol: water (4:1), with 0.004% 8-hydroxyquinoline
Solvent 2 = Propanol: ammonia: water (6:1:3)
Solvent 3 = Butanol: acetic acid; water (73:10-17)

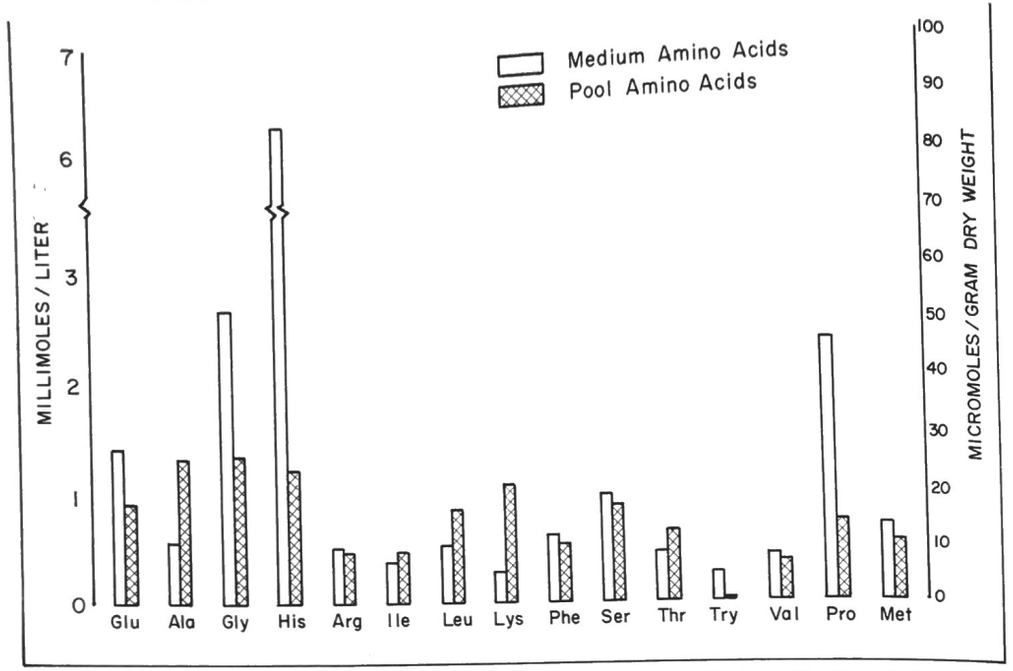

Fig. 1. Relative Concentration of Amino Acids in the Medium and in the Amino Acid Pool. Concentrations of the medium amino acids were calculated from the amounts added in making the defined medium. These values are expressed as millimoles per liter. Values for the pool amino acids, as determined by the Beckman/Spinco Amino Acid Analyzer, are expressed as micromoles per gram dry weight.

micromoles of free amino acids per gram dry weight.

Influence of the medium on amino acid pools. The free amino acid pool is related to the composition of the medium used. The composition of the defined medium compared to the free amino acids inside cells grown on this medium is seen in Fig. 1. The relative amounts of free intracellular and free medium amino acids are similar in most cases and differ from those of proteose-peptone grown cells (Table I.). Lysine and alanine are exceptions for cells grown on the defined medium. These amino acids are more concentrated relative to the medium; glycine, histidine, and proline are relatively less concentrated, but they still reflect the overabundance of these amino acids in the medium. A semi-quantitative analysis of the proteose-peptone medium revealed that alanine, leucine, valine, aspartic acid, glutamic acid, and glycine are the main free amino acids present. These same amino acids compose over half of the free amino acids in *Tetrahymena* grown on this medium (Table I). In this case, lysine is again an exception since it is a major component of the pool but not of the medium.

The effect of growth stage on amino acid pools. Growth curves for *Tetrahymena* in the proteose-peptone medium and in the defined medium are shown in Fig. 2. Although the growth on the defined medium is slower, these cells accumulate about the same total amount of free amino acids as the more numerous cells grown on proteose-peptone (Fig. 3A, B). These figures also show the increase in ninhydrin color on hydrolysis of the trichloroacetic acid-soluble extract of *Tetrahymena.*

The free amino acid pool, expressed per milligram of protein, varies considerably during growth (Fig. 4A). It ranges from 0.3 to 0.6 micromoles of leucine-equivalents for cells in the proteose-peptone medium and from 0.8 to 3.35 micromoles of leucine-equivalents for cells in the defined medium. The size of the free amino acid pool, expressed in this manner, reaches a minimum when maximum growth is achieved. This accounts for the small amino acid pool in the extract of proteose-peptone grown cells which was analyzed on the Amino Acid Analyzer. After the same period of growth, the free amino acid pool is much larger for cells grown on the defined medium. The rise in the ninhydrin-positive material after 150 hours for the cells grown on proteose-peptone may be due to the fact that the cells have surpassed their maximum growth (Fig. 2) and that autolysis has begun.

Fig. 4B shows the amount of conjugated amino acids per milligram of protein at different stages of growth on the two media. It is of special interest to note that during the log phase of growth, the amount of conjugated amino acids remains at about 0.7 micromoles of leucine-equivalents for organisms grown on either medium.

Search for peptides. In order to see if the conjugated amino acids represented a few specific peptides or a large number of molecular species, the trichloroacetic acid-soluble fraction of cells grown for 132 hours on proteose-peptone was chromatographed on a column of Dowex 50-X2. This gave the results shown in Fig. 5A. Those peaks labeled with "P" contained peptides. The complete composition of each of the peaks was ascertained. No qualitative differences were noted for cells harvested during the log phase, as the same 33 peaks in Fig. 5A were obtained for a preparation of cells which had been growing for only 72 hours. However, the peptides present in cells grown on proteose-peptone could not be demonstrated in the extract of *Tetrahymena* grown on the defined medium (Fig. 5B). In this case, all of the peaks were found to be amino acids or unidentified compounds stable to acid hydrolysis. The identifiable peptides in the preparation of *Tetrahymena* grown on proteose-peptone had apparently been taken up by the cells from the medium. None of the peptides had an unusual composition, as determined qualitatively.

It was found that during the log phase of growth there is about 0.7 micromoles of conjugated amino acids per milligram protein in cells grown either on proteose-peptone or on the defined medium (Fig. 4B). This value could not be due to ingested peptides, nor could it reflect one or more specific small peptides since none was found when the cells were grown on the defined medium. This material must consist of a great number of different peptides which cannot be detected individually by this method, or of a small amount of a larger, trichloroacetic acid-soluble protein. If, in this conjugated amino acid fraction, there were present a very large number of different peptides or a small amount of protein, the contribution of peptides ingested from the medium would be obscured, even though they dominate a chromatogram by being fewer in number.

Analysis of the proteose-peptone medium revealed only peaks for the common amino acids. Further analysis of these fractions by paper chromatography, however, showed that numerous peptides were present. Since no individual peptide in the medium was present to the extent that it gave a distinct peak on column chromatography and since such peaks were noted in the extract of *Tetrahymena* grown on proteose-peptone, it is possible that ingested peptides are concentrated inside the cells. Another possibility is that all ingested peptides are broken down to shorter fragments which cannot be further degraded and which accumulate inside the cell.

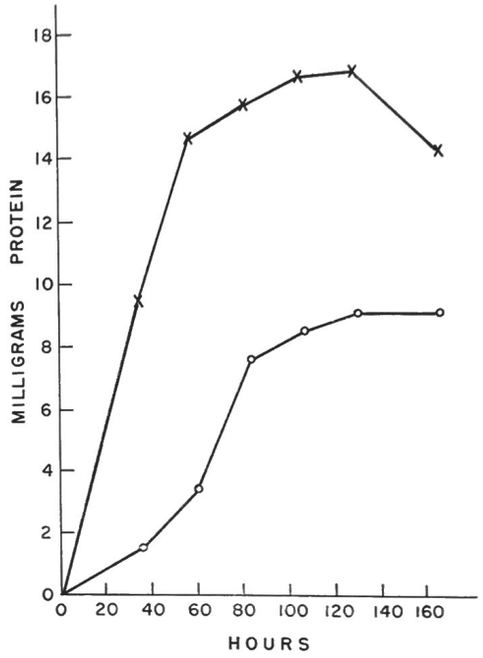

Fig. 2. Growth Curves for **Tetrahymena** in the Proteose-Peptone Medium and in the Defined Medium. Cells were grown in 10 ml of medium in large test tubes with cotton plugs. Cultures were harvested at different intervals, and the protein content of the washed cells was determined. (x—x) represents growth on the proteose-peptone medium; (o—o) represents growth on the defined medium.

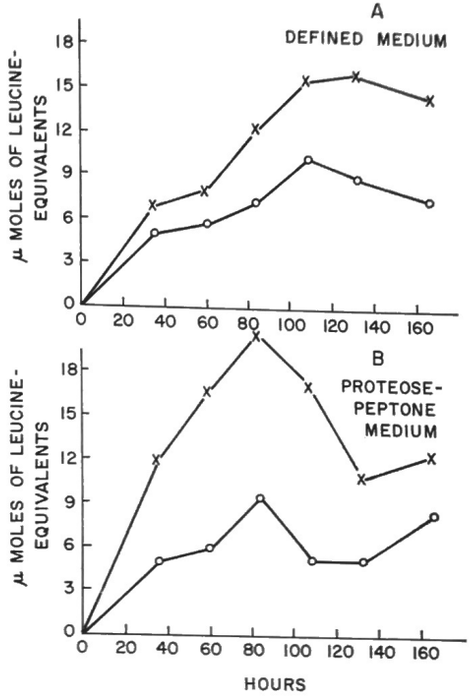

Fig. 3 A, B. Ninhydrin-Positive Material in **Tetrahymena** Grown on Two Different Media and the Increase in this Material after Hydrolysis. Cells were grown in 10 ml of medium in large test tubes with cotton plugs. Cultures were harvested at different intervals, and the total ninhydrin-positive material which was soluble in trichloroacetic acid was determined before and after hydrolysis. Results are expressed as micromoles of leucine-equivalents. (x—x) represents the amount after hydrolysis. and (o—o) represents the amount before hydrolysis.

Asparagine, glutamine, and methionine sulfoxide. In the search for peptides, asparagine and glutamine were first noted as unidentified, ninhydrin-positive spots on chromatograms, which gave on acid hydrolysis only aspartic acid and glutamic acid, respectively. They were then compared chromatographically in several solvents with standard asparagine and glutamine and found to be identical.

Methionine sulfoxide was identified by comparing its Rf values with the standard compound and by its partial oxidation on acid hydrolysis to methionine sulfone, which was also identified chromatographically. Methionine sulfoxide may be an artifact, produced during the isolation procedure by the oxidation of methionine.

Glutathione. Glutathione has been reported as present in *Tetrahymena.* Lawrie (1935) first reported that the nitroprusside test for sulfhydryl groups was positive for preparations of this organism and that no cysteine could be demonstrated. Seaman (1949), by use of the glyoxalase assay method, reported that there were 3.5 micromoles of glutathione per gram dry weight. Other workers (Scherbaum *et al.,* 1959) designate an acid-labile spot on a two-dimensional paper chromatogram as glutathione.

No glutathione, however, was found by analysis using the Amino Acid Analyzer or by column chromatography followed by paper chromatography. Subtraction of the amount of free cystine from the total non-protein cystine gave values of 2.9 and 0.8 micromoles per gram dry weight for *Tetrahymena* grown on the defined medium and on the proteose-peptone medium, respectively. These values represent the conjugated cystine present in *Tetrahymena* and are therefore maximum values for glutathione. Other analyses using more specific methods such as cofactor activity for glyoxalase or the alloxan conjugate gave maximum values of 1.0 and 0.5 micromoles per gram dry weight. These assays were limited by the sensitivity of the methods. It is concluded that there is very little, if any, glutathione present in *Tetrahymena.*

Creatinine. Since creatinine is only weakly ninhydrin-positive, its presence was checked by the Jaffé reaction. Creatinine was identified by paper chromatography of extracts of cells grown on the proteose-peptone medium. However, creatinine could not be demonstrated for cells grown on the defined medium. Subsequently, it was found to be present in the proteose-peptone medium. Apparently, the creatinine in the cells had been ingested. The uptake of creatinine by the cells is further evidence for the variation of their internal composition with the medium used.

Discussion

Qualitatively, the free amino acids found in *Tetrahymena* in this investigation are generally those which have been reported previously (Wu and Hogg, 1956; Christensson, 1959; Schleicher, 1959; Wells, 1960; and Loefer and Scherbaum, 1961); quantitatively, the results vary considerably from previous reports. However, the variability of the non-protein amino acid pool with the medium and with the stage of growth apparently

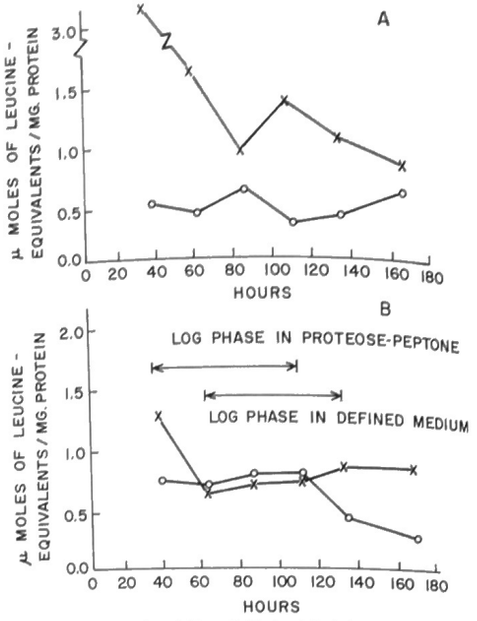

Fig. 4A, B. Free Amino Acids and Conjugated Amino Acids in **Tetrahymena** at Different Stages of Growth on Two Different Media. The results are expressed as micromoles of leucine-equivalents per milligram of protein. In Fig. 4A the free amino acids of **Tetrahymena** grown on the defined medium are represented by (x—x), and on proteose-peptone by (o—o). Fig. 4B shows the conjugated amino acids of **Tetrahymena.** The same symbols apply to this figure. Also in Fig. 4B, the periods corresponding to the log phase of growth in the different media are shown.

accounts for this discrepancy. The relationship between intracellular amino acids and the external medium has not been stressed in previous characterizations of the amino acid pool of *Tetrahymena.* Such characterizations would thus appear to be of little value.

The variation in the size of the free amino acid pool correlates well with the variation in size of *Tetrahymena* as reported by Summers (1963). The cell volume decreases from the lag phase to the log phase and reaches a minimum at the end of the log phase, before increasing again at the stationary phase. Thus, cells in the lag and stationary phases are large and have a high ratio of free amino acids to protein; cells in the log phase are small and have a low ratio. This relationship could be a reflection of the increased utilization of amino acids during active growth and of the shorter time available to increase in size during such growth.

There is one report regarding the uptake of amino acids by *Tetrahymena* (Stephens and Kerr, 1962). The results show that there is an adsorptive, rate-limiting step at low concentrations of amino acids. However, at high concentrations, similar to those of the defined medium used in this investigation, the concentration required to reach Vmax is exceeded and the adsorptive step is no longer noted. This implies, as do the results of the present investigation, that the composition of the free amino acid pool is to some extent dependent on the concentration of amino acids in the medium.

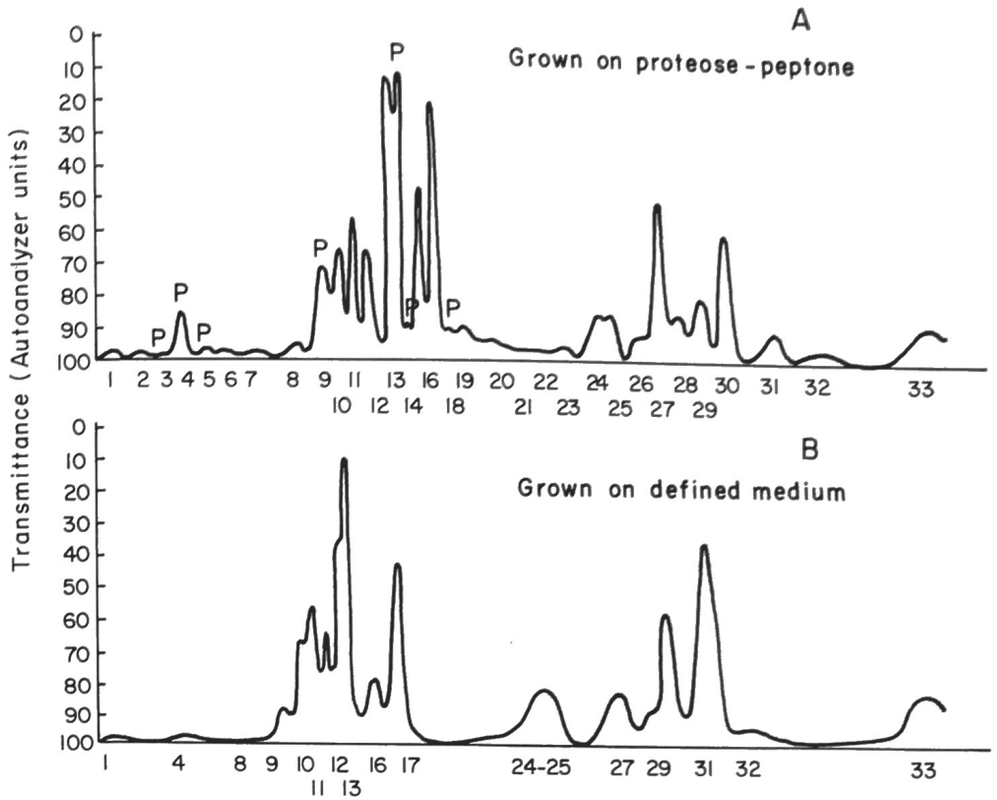

Fig. 5A, B. Elution Patterns of Ninhydrin-Positive Trichloroacetic Acid-Soluble Material from **Tetrahymena** Grown on Proteose-Peptone and on the Defined Medium. For Fig. 5A, the peaks are numbered in the order of their appearance. For Fig. 5B, a peak which on analysis was found to be analogous to a peak in Fig. A was given the same number. Those peaks containing peptides are labeled "P".

Tetrahymena are equipped with a buccal apparatus by means of which it may ingest fluid from the medium. This ingestion requires an inducer, present in proteose-peptone (Seaman, 1961). It is assumed that by this mechanism peptides from the medium are trapped inside the cells. They apparently constitute a relatively small portion of the conjugated amino acids, since in either medium cells during the log phase of growth have about the same amount of conjugated amino acids per milligram of protein.

In view of the current concepts of ribosomal protein synthesis whereby the amino acid chain is formed by sequential addition of amino acids starting at the N-terminal amino acid, it seems likely that at any particular time there would be present a very large number of peptides. Not only would there be a peptide for each protein being synthesized in the cell, but there would be amino acid sequences at different stages of completion for each protein. This being the case, it is not likely that any one peptide would be present at such a concentration as to be isolable by the techniques at hand.

A ribosomal system for protein synthesis has been demonstrated in *Tetrahymena* (Mager and Lipmann, 1958). Two other protein-synthesizing systems, one involving the mitochondria and one involving the kinetosomes have been reported also (Mager, 1960; Seaman, 1962). The mechanisms by which the latter two systems form proteins from amino acids are entirely unknown.

The large amount of conjugated amino acids in *Tetrahymena* is apparently a characteristic of the protein-manufacturing systems of this organism and is not due to the presence of a small number of discrete peptides.

LITERATURE CITED

Acher, R., Diemar, W., Pfeil, D., and Schiffner, G. 1955. Die papier-chromatographische Trennung von Kreatin und die quantitative Bestimmung von gesamtkreatinin in Lebensmitteln. Z. *anal. Chem.,* 148:10-14.

Archibald, R. M. 1944. Determination of Citrulline and Allantoin and Determination of Citrulline in Blood Plasma. *J. Biol. Chem.*, 156:121-142.

Baker, H., Frank, O., Pasher, I., Dinnerstein, A., and Sobotka, H. 1960. An Assay for Pantothenic Acid in Biologic Fluids. *Clin. Chem.*, 6:36-42.

Christensson, E. 1959. Changes in Free Amino Acids and Proteins during Cell Growth and Synthronous Division in Mass Cultures of *Tetrahymena pyriformis*. *Acta Physiol. Scand.*, 45:339-349.

Dalgliesch, C. E. 1952. The Relation between Pyridoxine and Tryptophan Metabolism, Studied in the Rat. *Biochm. J.*, 52:3-14

Folin, O., and Farmer, C. J. 1912. A New Method for the Determination of Total Nitrogen in Urine. *J. Biol. Chem.*, 11:493-501.

Hare, R. S. 1950. Endogenous Creatinine in Serum and Urine. *Proc. Soc. Exptl. Biol. Med.*, 74:148-151.

Hill, D. L., and van Eys, J. 1964. The Relationship Between Arginine, Citrulline, and Ornithine in *Tetrahymena pyriformis*. *J. Protozool.*, Submitted for publication.

Hirs, C. H. W., Moore, S., and Stein, W. H. 1956. Peptides Obtained by Tryptic Hydrolysis of Performic Acid-oxidized Ribonuclease. *J. Biol. Chem.*, 219:623-642.

Lawrie, N. R. 1935. Studies on the Metabolism of Protozoa. II. Some Biochemical Reactions Occurring in the Presence of the Washed Cells of *Glaucoma pyriformis*. *Biochem. J.*, 29:2297-2302.

Loefer, J. B., and Scherbaum, O. H. 1961. Amino Acid Composition of Protozoa. *J. Protozool.*, 8:184-191.

Lowry, O. H., Rosebrough N. J. and Farr A. L. 1951. Protein Measurement with the Folin Phenol Reagent. *J. Biol. Chem.*, 193:265-275.

Mager, J. 1960. Chloramphenicol and Chlortetracycline Inhibition of Amino Acid Incorporation into Proteins inn a Cell-free System from *Tetrahymena pyriformis*. *Biochim. Biophys. Acta*, 38:150-152.

_____, and Lipmann, F. 1958. Amino Acid Incorporation and the Reversion of its Initial Phase with Cell-free *Tetrahymena* Preparations. *Proc. Natl. Acad. Sci.*, 44:305-308.

Moore, S., and Stein, W. H. 1954. A Modified Ninhydrin Reagent for the Photometric Determination of Amino Acids and Related Compounds. *J. Biol. Chem.*, 211:907-913.

Patterson, J. W., Lazarow, A., and Levey, S. 1949. Reactions of Alloxan and Dialuric Acid with the Sulfhydryl Group. *J. Biol. Chem.*, 177:197-204.

Scherbaum, O. H., James, T. W., and Jahn, T. L. 1959. The Amino Acid Composition in Relation to Cell Growth and Cell Division in Synchronized Cultures of *Tetrahymena pyriformis* GL. *J. Cell. Comp. Physiol.*, 53:119-137.

Schleicher, J. D. 1959. Paper Chromatography Analyses of Amino Acids in Protozoa: Some Aspects of the Metabolism of Aspartic Acid. Catholic University of America Press, Washington, p. 74.

Seaman, G. R. 1949. Cytochrome c, Diphosphopyridine Nucleotide, Glutathione, and Adenosine Triphosphatase Content of the Ciliate *Colpidium campylum*. *J. Comp. Cell. Physiol.*, 33:441-443.

_____. 1961. Some Aspects of Phagotrophy. *J. Protozool.*, 8:204-212.

_____. 1962. Protein Synthesis by Kinetosomes Isolated from the Protozoan *Tetrahymena*. *Biochim. Biophys. Acta*, 55:889-899.

Stephens, G. C., and Kerr, N. S 1962. The Uptake of Amino Acids by *Tetrahymena*. *Amer. Zool.*, 2:450.

Summers, L. G. 1963. Variation of Cell and Nuclear Volume *Tetrahymena pyriformis* with the Parameters of Growth, Age of Cell, and Generation Time. *J. Protozool.*, 10:288-293.

Warnock, L. G. 1962. Energy Expenditure and Metabolic Regulation in *Tetrahymena pyriformis*. Thesis, Vanderbilt Univ., University Microfilms, Ann Arbor.

_____ and van Eys, J. 1962. Normal Carbohydrate Metabolism in *Tetrahymena pyriformis*. *J. Cell. Comp. Physiol.*, 60:53-60.

Wells, C. 1960. Identification of Free and Bound Amino Acids in Three Strains of *Tetrahymena pyriformis* using Paper Chromatography. *J. Protozool.*, 7:7-10.

Woodward, G. E. 1935. Glyoxalase. III. Glyoxalase as a Reagent for the Quantitative Micro-estimation of Glutathione. *J Biol. Chem.*, 109:1-10.

Wu, C., and Hogg, J. F. 1952. The Amino Acid Composition and Nitrogen Metabolism of *Tetrahymena geleii*. *J. Biol. Chem.*, 198:753-764.

_____. 1956. Free and Nonprotein Amino Acids in *Tetrahymena pyriformis*. *Arch. Biochem. Biophys.*, 62:70-73.

NEWS OF TENNESSEE SCIENCE

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The appointment of Dr. Lawrence K. Akers as assistant chairman of the Special Training Division of the Oak Ridge Institute of Nuclear Studies has been announced by Dr. William G. Pollard, Executive Director of the Institute. In his new position, Dr. Akers will be responsible for coordinating the research programs of the Special Training Division, and will assist the chairman of the Division, Dr. Ralph T. Overman, in the administration of the Division.

An extensive program of courses designed to train personnel in the safe and efficient use of radioactive materials and to stimulate further interest in their applications will be offered by the Oak Ridge Institute of Nuclear Studies during 1965. The courses are presented by the ORINS Special Training Division for the U. S. Atomic Energy Commission. The calendar of courses for 1965 includes: four-week basic courses on the use of radioisotopes in research, designed to train participants in the use of radioisotopes as research tools—Jan. 11-Feb. 5, Mar. 22-Apr. 16, May 3-28, Aug. 9-Sept. 27-Oct. 22; three-week medical qualification courses (for physicians) which fulfill the minimal requirements for licensure by the U. S. Atomic Energy Commission to use radioisotopes in diagnostic procedures—Jan. 4-22, Mar. 1-19, May 3-21, Sept. 13-Oct. 1; an annual ten-week health physics course concerned with the study, evaluation, and control of radiation hazards—Sept. 13-Nov. 19; three-week advanced health physics courses for more specialized training in recent developments and new techniques in the field—Mar. 29-Apr. 16, Nov. 29-Dec. 17; and advanced medical qualification courses for physicians who have previous experience in the use of radioisotopes and desire more specialized training—Apr. 5-9 (tentative). The schedule for summer institutes and other special courses which are also regularly offered by the Division will be announced later. Additional information concerning courses, schedules, fees, and application procedures may be obtained from Dr. Ralph T. Overman, Special Training Division, ORINS, P. O. Box 117, Oak Ridge, Tennessee 37831.

Robert W. Peplies, Associate Professor of Geography, has been appointed research director at East Tennessee State University.

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