Metabolism Changes in *Tetrahymena pyriformis* W Adapted to Potassium Cyanide.* (27924)

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Tetrahymena pyriformis W, grown in Proteose Peptone-glucose medium and in the presence of increasing concentrations of KCN, gains resistance against cyanide inhibition of growth and respiration(1,2). Similar adaptation may be observed in *Chilomonas paramecium* if it is grown in medium containing Proteose Peptone and glucose, plus the usual sources of carbon (acetate), nitrogen (ammonium salt) and trace substances(3). However, Chilomonas does not adapt if the more complex organic compounds are omitted. This suggests a dependence upon different metabolism for cyanide adaptation and was the stimulus to this study. Since Tetrahymena may be grown upon completely synthetic medium(4), the means are availed for following disappearance of essential materials during growth, and to analyze cell composition derived from known precursors.

Materials and methods. All work was done upon a clone culture of *Tetrahymena pyriformis* W, grown in synthetic medium(4) in darkness at 25°C. Parent cultures were maintained in 5 ml of medium, contained in silicon-stoppered 15 × 125 mm pyrex test tubes, by 0.1 ml transfers to fresh medium every 5 days. Details of the adaptation procedure, in which cyanide concentration gradually is increased to a maintenance level of 10^{-4}M KCN, have been described(1). Cells for experimental purposes were obtained from 5-day, 20 ml cultures in silicon-stoppered 50 ml pyrex Erlenmeyer flasks.

Respiration was measured manometrically by the direct and indirect methods of Warburg(5). The indirect method employed flasks of approximately 12 ml total capacity, containing 3 or 5 ml for the "small" and "large" flasks, respectively. Smaller flasks (approximately 5 ml total capacity) were used for the direct method. Main compartments contained 1.5 ml of cell suspension, side arms 0.2 ml of 8 N/H_2SO_4 and center wells 0.1 ml of KOH or KOH-KCN solutions (6). For all tests, cells were suspended at such concentration to give a reading of 60 on a Klett-Summerson colorimeter employing a blue filter.

Cellular fat content was determined from cells concentrated and twice washed in distilled water by gentle centrifugation. Total lipids were extracted with petroleum ether, and weight determined by evaporation at room temperature in previously tared weighing bottles. Samples of the cells, in suspension, were dried overnight at 85-95°C in similar containers to determine total dry weight. Glucose in the medium was determined in uninoculated samples and in cell-free, 5-day culture medium with Glucostat Reagent (Worthington Biochemical Corp., Freehold, N. J.). Samples of the cell-free medium were also analyzed for lactic acid by the method of Baker and Summerson(7). Cellular dry weight in the culture was determined as previously described.

Amino acids were determined singly or in groups or as total ninhydrin-positive material. Cells were separated from cultures by centrifugation and washed twice in distilled water. Aliquot samples were taken for dry weight determinations. A portion was also frozen to disrupt cells and the remaining material subjected to H_2SO_4 or $Ba(OH)_2$ hydrolysis for 2 hours as described by Block *et al.*(8), salts removed with an Electric Desalter (Research Specialties Co., Berkeley, Calif.), and evaporated to dryness *in vacuo* at temperatures not exceeding 40°C. The residue was taken up in a volume of 10% isopropanol 1/200 that of the original culture. Cell-free medium was desalted, concentrated to dryness, and taken up in 10%

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isopropanol at 1/50 of the original volume.

Total ninhydrin-positive material was noted by addition of 1.0 ml of 0.2% ninhydrin solution to 9 ml of a 1:30 dilution of fresh medium or culture supernatant, heating in live steam for 12 minutes and determining the optical density of the developed color at 570 mμ, using a Beckman Model DU Spectrophotometer.

TABLE I. Potassium Cyanide Influence Upon Respiration in Normal (WS) and Cyanide-Adapted (WSA) Tetrahymena. Figures in parentheses are % of control condition.

Cell	MKCN	No. tests	Q_{O_2}	Q_{CO_2}	R.Q.
WS	0	18	−34.1	+36.5	1.07
	10^{-3}	18	−20.2 (59)	+25.4 (70)	1.26
WSA	0	18	−32.7	+33.7	1.03
	10^{-3}	18	−31.2 (95)	+31.7 (94)	1.02

Individual amino acids in disrupted (free amino acids) or digested cells (total amino acids), or concentrated fresh or conditioned medium were separated by paper chromatography. Three methods were employed: (1) one-dimensional development employing a n-propanol:water (4:1) system, (2) two-dimensional development, both dimensions with n-propanol:water in order to increase resolution on a diagonal, and (3) two-dimensional development with n-propanol:water employed in the first dimension and liquified phenol (Fisher) in the second. Chromatograms were treated with ninhydrin to locate amino acids. These were compared with similar tests upon known amino acids for establishing Rf values.

TABLE II. Total Lipid Content in Normal (WS) and Cyanide-Adapted (WSA) Tetrahymena. Figures are averages of 12 tests each condition.

Cell	MKCN	Fat as % of dry wt
WS	0	13.5
	10^{-3}	14.1
WSA	0	12.3
	10^{-3}	13.7

Quantitative estimation was accomplished by measurement of spot area(9) of all chromatograms and by use of a Photovolt Densitometer (Photovolt Corp., New York) on those chromatograms with linear amino acid displacement (methods 1 and 2).

TABLE III. Glucose Utilization and Lactate Production by Normal (WS) and Cyanide-Adapted (WSA) Tetrahymena. All cultures at 7 days except 2 WS in 10^{-4}M KCN, kept 14 days to obtain better growth. WS in 10^{-4}M at 7 days showed .116 mg (dry)/ml.

Cell	No. tests	mg (dry)/ml	μM/mg Glucose	μM/mg Lactate
WS	21	.358 ± .034	−15.0 ± 2.6	+15.0 ± 1.4
WS +10^{-4}M KCN	12	.230 ± .029	−21.3 ± 2.4	+ 3.6 ± .5
WSA	21	.259 ± .032	−18.9 ± 3.7	+20.0 ± 2.5

Results. Cyanide inhibits respiration in normal cells and causes an elevated respiratory quotient (R.Q.); in adapted cells such effects are not seen (Table I). This suggests that normal cells in cyanide might be in oxygen debt or depositing fat.

Normal and adapted cells, grown in presence or absence of cyanide, showed similar amounts of Sudan III-positive material in the anterior region. Fat extraction from cells of mass cultures showed similar lipid content in both cell types (Table II).

Glucose is utilized more rapidly by adapted than by normal cells (Table III), although both produce proportionally similar amounts of lactic acid (approximately 50% of that anticipated). Cyanide causes normal cells to use more glucose, but considerably reduces lactate yield (8% of anticipated). It does not appear that the increased R.Q. of normal cells in cyanide is due to lipid formation or oxygen debt.

As amino acid is used from the medium (0.03M total molarity) during growth, the color reaction by ninhydrin is reduced. This allows calculation of total amino acid utilization (Table IV). Adapted cells use more

TABLE IV. Utilization of Amino Acids by Normal (WS) and Cyanide-Adapted (WSA) Tetrahymena. WS in 10^{-4}M KCN at 10 days for better growth. All others at 7 days.

Cell	No. tests	mg (dry)/ml	μM amino acid used/mg
WS	6	.525 ± .077	18.1 ± 2.9
WS +10^{-4}M KCN	6	.456 ± .082	12.8 ± 2.4
WSA	6	.368 ± .052	44.7 ± 6.6

TABLE V. Amino Acid Utilization and Cell Content (μM/mg dry wt) in Normal (WS) and Cyanide-Adapted (WSA) Tetrahymena. Each figure represents average of 14 chromatographic analyses. Avg growth: WS, .470 and WSA .370 mg (dry)/ml. Figures in parentheses represent WSA as % of WS.

	Removed from medium		To cells-bound		To cells-free		Total in cells		Total change in culture	
	WS	WSA	WS	WSA	WS	WSA	WS	WSA	WS	WSA
Alanine	.84	2.28	.79	.63	.13	.38	.92	1.01	+ .08	−1.27
Aspartic acid	.11	.44	.81	.43	.05	.10	.86	.53	+ .75	+ .09
Glutamic "	+.23	.04	1.17	.43	.37	.65	1.54	1.08	+1.77	+ .94
Glycine-serine	3.59	6.32	1.18	1.66	.05	.13	1.23	1.79	−2.36	−4.53
Leuc.-isol.-phenylal.	5.05	8.85	1.16	1.35	.75	1.64	1.91	2.99	−3.14	−5.86
Lysine-threonine	2.64	5.63	.99	.82	.44	.94	1.43	1.76	−1.30	−3.87
Methionine-valine	2.98	5.06	.52	.34	.23	.48	.75	.82	−2.23	−4.24
Arginine	.49	1.21	++	+	+	++	+	+		
Tryptophan	.03	.24	++	+	+	++	+	+		
Proline	.53	1.43	++	+	+	++	+	+		
Total	16.03	31.50 (196)	6.62	5.66 (86)	2.02	4.32 (208)	8.64	9.98 (116)		

amino acid (247%) while cyanide reduces amino acid utilization by normal cells.

Both methods for quantitative determination of individual amino acids in culture medium or cells were in close agreement. The average of 14 tests (Table V), indicates that adapted cells use more amino acid during growth (196%) than do normal cells. For both cell types, amino acids "essential" to Tetrahymena (serine, leucine, isoleucine, phenylalanine, lysine, threonine, methionine, valine, arginine, and tryptophan) (10) are used to the greatest degree. Glutamic acid production is greater in normal cells. Except for aspartic and glutamic acids, adapted cells have higher amino acid content.

Respiratory stimulation by substrates of the culture medium is indicated in Table VI. Endogenous respiration is represented as 100%, and the effect of 0.03M substrates is

TABLE VI. Influence of Various Substrates and Cyanide (10^{-3}M KCN) upon Respiration in Normal (WS) and Cyanide-Adapted (WSA) Tetrahymena as Determined by the Direct Method of Warburg. All figures are results of at least 8 tests, except endogenous condition with 144 tests. Endogenous Q_{O_2} (WS-15.3 and WSA-14.9) taken as 100% values and others expressed as percentage of these for comparison. (X) = substrate plus cyanide as a percentage of that particular substrate.

	Condition					
	WS			WSA		
	O	KCN	(X)	O	KCN	(X)
Exogenous, cond. med.	258	172	(67)	262	207	(79)
Endogenous	100%	76		100%	70	
D-glucose	132	100	(76)	104	67	(64)
Acetate	143	77	(54)	175	125	(71)
L-phenylalanine	128	105	(82)	170	162	(95)
DL-isoleucine	111	96	(86)	156	132	(85)
L-glutamic acid	127	68	(54)	114	97	(85)
L-aspartic acid	99	67	(68)	98	101	(103)
L-histidine	108	60	(56)	92	91	(99)
DL-alanine	116	67	(58)	94	70	(75)
DL-threonine	113	89	(79)	124	101	(81)
L-leucine	122	92	(75)	110	90	(82)
L-arginine	78	59	(76)	87	64	(74)
L-lysine	88	55	(63)	78	66	(85)
L-tryptophan	89	87	(98)	82	63	(77)
Glycine	109	59	(54)	98	57	(58)
DL-serine	132	80	(61)	110	74	(67)
DL-valine	126	120	(95)	97	81	(89)
L-proline	161	130	(81)	125	84	(67)
DL-methionine	184	136	(74)	122	58	(48)

presented in relation to this. Cells, removed from conditioned medium by gentle centrifugation, were washed and resuspended in the culture medium-inorganic salts solution at the pH of the culture. The range was pH 6.6-6.9.

Glucose stimulation to normal cells is cyanide sensitive. Acetate stimulation is greater in adapted cells, and less sensitive to cyanide.

Adapted cells are most stimulated by phenylalanine and isoleucine; phenylalanine stimulation is of low cyanide sensitivity. Normal cells oxidized methionine and proline best. Other amino acids were less stimulatory or even inhibitory. Adapted cells were protected from cyanide by aspartate, while tryptophan acted similarly for normal cells.

Discussion. Amino acids, as well as carbohydrates, are oxidized by Tetrahymena(11). Cyanide inhibits both metabolisms and causes an increased R.Q. Elrod(12) observed a similar R.Q. rise in *Chilomonas paramecium* exposed to cyanide, and this was associated with increased lipid storage. Lipid content of Tetrahymena, in range with reports by others(13,14,15), is not altered by cyanide exposure. Glucose oxidation is cyanide sensitive, but lactate does not accumulate. Therefore the elevated R.Q. cannot be explained on the basis of lipid synthesis or the usual type of oxygen debt.

Upon becoming adapted to cyanide, Tetrahymena shows less sensitivity to cyanide alteration of R.Q. and glucose and lactate metabolisms. Acetate becomes much more stimulatory. Whatever the basis for adaptation, there has been a pronounced change in carbohydrate metabolism.

Cellular amino acid contents, determined in this study, are similar to those reported by Wu and Hogg(16), although total free amino acid is less than their report upon cells grown in peptone medium. Others(17,18) have identified qualitative differences in Tetrahymena strains. We have observed only quantitative differences.

Roth *et al.*(19) studied 29 amino acids and found only 9 stimulatory to respiration: L-phenylalanine, L-tyrosine, D- and L-cysteine, L-proline, L-isoleucine, 1,1-DL-aminopropane-sulfonic acid, and D- and L-leucine. We find phenylalanine, proline, leucine and isoleucine stimulatory. In addition, methionine, serine, glutamate, valine, alanine, threonine, glycine and histidine were effective, contrary to Roth *et al.* It may be that growth in medium containing amino acids, rather than protein or peptide source, conditions cells to better oxidative activity.

Evaluation of our data indicates greater amino acid metabolism in adapted cells, confirming an earlier report regarding the importance of such substrates in cyanide resistant metabolism(20).

Adapted cells oxidize phenylalanine, isoleucine and threonine to a greater degree, and phenylalanine oxidation becomes more cyanide resistant. On the other hand, methionine, proline and serine are oxidized to a lesser degree in cyanide-adapted cells and, except for serine, cyanide sensitivity increases. Thus the change in metabolism is quite varied for these 6 amino acids.

Varying degrees of oxidative activity toward glutamate, leucine, valine, alanine, histidine, and glycine are lost upon adaptation. Aspartate is not utilized by normal and adapted cells and arginine, lysine and tryptophan inhibited respiration in both types.

Unusual but interesting effects are seen in the sensitization of normal cells to cyanide by histidine and the protection against cyanide inhibition of normal and adapted cells by tryptophan and aspartate, respectively.

The increased utilization of amino acids from the medium during growth of adapted cells is probably accounted for by accumulation of serine and conversion of proline, methionine, alanine, valine, arginine, lysine and tryptophan to other materials.

Summary. Cyanide-adapted *Tetrahymena pyriformis* show considerable alteration in glucose, acetate and amino acid metabolism. Total lipid metabolism is not changed. Although adapted cells remove more amino acid from the culture medium during growth, this appears more due to accumulation and/or conversion to other materials than to increased oxidation. Methionine, proline, serine and valine oxidative activities are especially lowered upon adaptation, as is glucose

oxidation. Adapted cells show marked increase in oxidation of phenylalanine, isoleucine and threonine, as well as acetate.

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Studies on Dopa Decarboxylase Inhibitors *in vivo* by Use of C^{14}-Carboxyl-Labeled Dopa.* (27925)

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Decarboxylation of amino acids is a minor but nevertheless important pathway in amino acid catabolism, since it is responsible for the formation in microorganisms, plants and animals, of biologically active amines such as histamine, 3,4-dihydroxyphenethylamine (dopamine, the precursor of norepinephrine and epinephrine), tyramine, tryptamine, and 5-hydroxytryptamine (serotonin, 5HT).

There have been many reports concerning compounds which inhibit the enzymatic decarboxylation, particularly of 3,4-dihydroxyphenylalanine (dopa). Most studies have been made *in vitro* and the variety of compounds tested has recently been reviewed (1,2). Inhibition *in vivo* has been studied by different methods, for example: pressor response of dopa on blood pressure(1-4), measurement of the dopamine formed after injection of dopa and decarboxylase inhibitors(3,5), broncho-dilation by 5HT(6), heart contractile force(7), and formation of 5HT in kidney after injection of 5-hydroxytryptophan(8).

We found that a rapid method of studying directly the effect of various compounds on decarboxylation of dopa *in vivo*, was to measure radioactivity in the respiratory carbon dioxide after injection of dopa labeled in the carboxyl group with C^{14}.

α-Methyldopa has been found to possess activity as an inhibitor of dopa decarboxylation(4,5,8,9). This report concerns mainly our investigation of different α-amino acids for dopa decarboxylase inhibition activity but also includes some other compounds which are known to possess inhibition *in vi-*

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