DEPARTMENT OF NUTRITIONAL SCIENCES

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INVESTIGATION OF THE NUTRITIONAL PROPERTIES OF *Hydrogenomonas eutropha*

Final Report to the
National Aeronautics and Space Administration
NGR 05-003-089

1 November 1964 to 31 December 1968

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I. Introduction

Studies of the hydrogen-fixing bacterium Hydrogenomonas eutropha indicate that this organism may form the basis of a bioregenerative system for atmospheric control during prolonged space flight (1). However, maximum gain from the system would depend upon utilization of the harvested organism as food for flight personnel. The objective of this research was to determine the nutritional quality of Hydrogenomonas eutropha.

When this research was initiated the only nutritionally relevant information available was that Hydrogenomonas cells harvested from rapidly growing cultures are composed chiefly of protein containing a reasonable balance of amino acids (2), whereas cells cultured in deficient media accumulate a large amount of a lipid, poly-beta-hydroxybutyric acid (3). Based on this scanty information and on published studies of similar material, the following research questions were posed:

a. Is the protein adequately digested?
b. Is the absorbed protein of good quality?
c. Is the stored lipid digestible?
d. Is the absorbed lipid well utilized?
e. At what dietary level will nucleic acid content present a bar to use of the bacteria as food?
f. Can the bacteria serve as sole source of dietary protein for man?
g. What is the maximum amount of bacteria that can be included in the human diet?

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(1) Bongers, L. H. Aerosp. Med. 35: 139, 1964.

(2) Foster, J. F., and J. H. Litchfield. Biotech. and Bioeng. 6: 441, 1964.

(3) Schlegel, H. G., G. Gottschalk and R. von Bartha. Nature (Lond.) 191: 463, 1961.

II. Materials and Materials Analysis

All of the Hydrogenomonas cells used in these investigations were supplied by the National Aeronautics and Space Administration and its contractors. Identification codes of the various lots are:

Source	Year	Code	Dry Weight, g
University of Mississippi	Nov. 64	M0	18
Drs. Robert Tischer and	Dec. 64	M1	17
L. R. Brown	Jan. 65	M2	53

Description: M0 - Dark brown, air-dried.

M1 and M2 - Grown in Repaske's medium, without $NaHCO_3$, and with 0.1% ammonium chloride as the nitrogen source. The culture was incubated at 30° C in an atmosphere of 70% H_2, 20% O_2, and 10% CO_2. Cells were harvested by centrifugation at late log growth phase (optical density determination), suspended in 0.85% NaCl, frozen and lyophilized.

Battelle Memorial Institute			
Drs. John Foster and	Feb. 65	B1	397
J. Litchfield		B1, washed	
	Dec. 65	B2	176
	Dec. 65	B3	213

Description: B1 - The lot consisted of 24 individual samples ranging in weight from 4 to 35 grams and in color from light amber to caramel. The individual samples were blended into one composite sample (B_1). The samples submitted were obtained intermittently during nine periods of continuous culture, each of several days' duration. The culture was grown in urea-containing medium. Packed cells were suspended in distilled water (1:5, W:V), recentrifuged and freeze-dried to 1% moisture content.

B1, washed - At UCB sample B_1 was washed by repeated suspension in distilled water and centrifugation. Five washings were required to yield supernatant free of urea. The washed cells were then lyophilized and analyzed. Washing was found to reduce total nitrogen, ash, and phosphorus; lipid content was unchanged. The harvested cells thus contained about 1.2% of urea and 2.5% of salts, presumably from the medium.

B2 - B_2 consisted of 11 samples ranging in weight from 6.0 to 29.3 grams taken from seven periods of continuous culture. These samples had been washed and freeze-dried to 1% moisture content. No nitrogen was detected in the filtrate from a trichloroacetic acid precipitated composite sample of the freeze-dried bacteria.

B3 - The lot consisted of seven samples from two periods of continuous culture. These samples ranged in weight from 42 to 115 grams, approximately 600 grams total. In this case, the bacteria were sent frozen, as a wet-paste of 35.53% solids content. The samples were tested for the presence of any residual medium.

Research Institute for Advanced	Oct. 66	R1	600
Studies	Oct. 66	R3	15
Dr. Leonard Bongers	Oct. 66	R2	15

Description: R1 - Nitrogen-deficient medium. Not washed. Freeze-dried.
R3 - C^{14}-labeled. Bonger's medium. Not washed. Freeze-dried.
R2 - C^{14}-labeled. Nitrogen-deficient medium. Not washed. Freeze-dried.

Source	Year	Code
NASA Ames Research Center	Mar. 68	A1a
	June 68	A1b
	July 68	A4

Description: A1a - Grown at the Battelle Memorial Institute, the organisms were harvested from semi-continuous culture having been grown autotrophically in Repaske's medium. At Battelle the cells were washed six times in distilled water by centrifugation and resuspension. The final wet paste was frozen and shipped to the Ames Laboratories. It was stored there for several months and delivered to us still frozen in March. The cells were resuspended in distilled water and, after trichloroacetic acid precipitation, the supernatant was tested for the presence of one medium component, urea. The test was negative, indicating adequate removal of medium. A slurry of cells and distilled water was then boiled gently to kill the bacteria. This boiled material had an odd, chlorite-like flavor, not noticed in previous lots of bacteria used for animal feeding. Discussion of processing steps with Dr. Foster suggested only one, very doubtful, possibility of contamination: the use of 'Zephiran' to sanitize the centrifuge at Battelle. The flavor was not removed by repeated washing or by lyophilization. It was removed by successive treatment with cationic and anionic exchange resin (Dowex 50-4X and Dowex 21K). The boiled Hydrogenomonas eutropha was batch-processed in the cold (34° F), filtered through nylon cloth, and immediately frozen and lyophilized. This material was used for the initial phases of the human feeding study.

A1b - A portion of the bacteria produced by Battelle had been retained at the Ames Laboratories. We obtained this reserve supply and tasted each individual package of bacteria to determine if any of these were devoid of the objectionable flavor. A small number of packages without detectable flavor were identified. This material was boiled, lyophilized, and fed to human volunteers.

A4 - A4 was purchased from the Grain Processing Company, Cedar Rapids, Iowa. It was grown heterotrophically on sucrose-casein hydrolysate medium. After being washed, boiled, and lyophilized, it was used in a human feeding study.

The Ames Center also purchased from the Grain Processing Company a lot of E. coli (EC2) grown on sucrose-casein hydrolysate medium. Examination showed the cells to be dominantly Aerobacter aerogenes. The material was washed exhaustively, boiled gently, and freeze-dried.

Three other small samples, two of H. eutropha (A2, A3) and one of "E. coli" (EC1) were submitted by Ames for chemical analysis only. A2 and EC1 had been acetone-dried and were used in research at Ames. A3 was a wet paste (21.6% dry solids). Their origin was not specified.

Composition of the cells is given in Table 1. Cells harvested at log-phase growth were confirmed to be very high in protein content. Unless the cells were washed exhaustively, residual medium contributed substantial amounts of non-protein nitrogen (e.g. NPN accounted for 0.7% of dry solids in once-washed sample M1). This variable probably is a major factor in

Table 1. Composition of Bacteria, Algae, and Yeast

Constituent/ 100 g dry solids	*Hydrogenomonas eutropha*														"*E. coli*"	*Chlorella sorokiniana*	*Torula*
	M1	M2	B1	Washed B1	Washed B2	Washed B3	R1	R2	R3	Washed Ala	Ion-exchanged Ala	Washed Alb	A2	A3	EC1		
Nitrogen	11.5	11.9	14.0	13.5	11.7	11.4	8.9	8.1	13.1	11.2	13.9	14.2	13.4	14.3	12.55	11.35	8.31
Ash	5.60	5.90	5.88	2.92	----	----	2.0	----	----	----	----	----	----	----	----	----	----
Lipid, total	----	----	----	9.39	7.3	----	22.5	----	----	----	----	----	----	----	----	----	----
(ether extract)	(2.33)	(0.69)	(0.95)	(0.95)	----	----	----	----	----	----	----	----	----	----	----	----	----
Calcium	----	0.20	----	0.13	0.05	0.02	0.01	----	----	0.01	0.06	----	----	----	----	0.12	0.25
Magnesium	----	0.20	----	0.09	0.08	0.02	0.04	----	----	0.06	0.04	0.10	----	----	----	0.08	0.16
Sodium	----	1.64	----	0.23	0.13	0.01	0.11	----	----	tr.	0.01	0.02	0.56	0.02	0.70	0.01	0.01
Potassium	----	0.91	----	0.20	0.60	0.03	0.79	----	----	0.02	tr.	tr.	----	----	----	0.01	2.07
Phosphorus	1.73	1.27	1.67	0.82	----	----	----	----	----	1.73	2.02	2.23	2.99	1.09	2.83	0.74	1.69
Chloride	----	----	----	0.07	----	----	----	----	----	0.03	----	----	----	----	----	tr.	0.03

Methods: Nitrogen - microkjeldahl
Ash - muffle furnace
Lipid - ether extract, Soxhlet
total, HCl-hydrolysis, diethylether-petroleum ether-ethanol extraction
Phosphorus - Fisk and Subbarow
Chloride - Fisher titrimeter
Metals - atomic absorption spectrometry

the discrepancy between theoretical total protein (Kjeldahl N x 6.25) and the sum of measured amino acids in many studies.

We were not aware that sample R1 (nitrogen-deficient) was not washed before shipment to us and an animal test was carried out with the lyophilized cells as supplied. Later, animals were fed six times-washed R1. Growth rate and feed efficiency of animals fed the unwashed cells was poorer than that of animals given the washed preparation. This agrees with the observation of Roberts (4) relative to E. coli. It is possible that the medium adhering to the cells contains some secreted metabolites which are poorly utilized or even toxic for rodents, or it may have been only a mineral imbalance or flavor problem. The unwashed lipid-rich cells were dense and both the cells and medium were orange-colored. The washed cells were light tan and flaky after freeze-drying. Odor was much improved by washing. A sample of the washings was sent to Dr. R. G. Tischer at the University of Mississippi. There was no noticeable difference in the appearance of protein-rich sample B1 whether washed or not.

Our data confirm that cells grown in nitrogen-deficient medium do accumulate lipid. We experienced some difficulty in analysis and it is probable that our value of 23% lipid in sample R1 is low. In the protein-rich sample B1, only 9% of lipid was found. Of this, only 5% of the lipid (i.e. 0.4% of cell solids) was diethyl ether-soluble triglyceride or free fatty acid. Analysis by gas-liquid chromatography of this Soxhlet-extracted lipid revealed the presence of the following fatty acids: 12:0, 14:0, 16:0, 16:1, 17:0, 18:0, 18:1, 18:2 (18:3 or 20:0). About 35% of the total lipid was in a bound form, probably protein-bound, and could be extracted by mixed solvents (chloroform-methanol or diethyl ether-petroleum ether-ethanol) only after mild HCl-hydrolysis. Cells contain 80 mg% digitonide-precipitable sterol ($FeCl_3$-reactive) and 5.8 mg% of lipid-soluble reducing substance (tocopherol?). No carotene or vitamin A were found.

All of the cells tested (B1, R1, A1) contained about 8% of nucleic acids, irrespective of lipid content. This suggests that deposition of lipid is at the expense of non-nuclear protein. In sample B1 the nucleic acids were distributed DNA:RNA:1:25, according to analysis by Dr. David Schwartz. The only physical method for removal of nucleic acid that seemed at all feasible

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(4) Roberts, R. S. Brit. J. Nutrit. 8: 353, 1954.

in preparation of cells intended for human food was ultracentrifugation in sucrose gradient. Separation of nucleic acids from nucleoprotein was not accomplished by this technique.

The cells contained about 0.8% each of phosphorus and potassium and about 40 mg% of magnesium. Variable amounts of sodium, calcium, and chloride were present. Since the organism does not require chloride or calcium, these must be adventitious. It would be interesting to learn whether the amounts of phosphorus, sulfur, and magnesium incorporated into the cells vary if chloride and calcium are totally absent from the medium.

Amino acid composition of lot B1 is given in Annex I, Table 1 (Calloway and Kumar, Applied Microbiology, 17: 176, 1969).
Our analysis revealed essentially the same pattern as reported earlier by Foster and Litchfield (2) but content was about one-third greater. Lysine was notably higher in our sample, perhaps because the material was lyophilized rather than being hot-air dried. The sum of our determined amino acids accounted for 96% of the theoretical protein.

Microscopic examination of B1 revealed the presence of a minor contaminant organism, a small coccus, that was not identified. Other contaminants were identified and are given in Annex VI (Waslien, Calloway, Margen, Nature, 221: 84, 1969).

A number of different methods for rupturing the cells were tried, with poor success. The lyophilized cells do not rupture in cold or hot distilled water and are resistant to repeated slow freezing and thawing in aqueous suspension. Hand-grinding with sand, levigated alumina and glass beads is ineffective. Sonification (20 kc Branson sonifier, 6-10 min) ruptured only about 60% of the cells. Seven hours of grinding with glass beads in a colloid mill failed to rupture the cells but damaged them sufficiently that they then fragmented on standing in aqueous suspension in the cold. Soxhlet extraction of cells did not render them susceptible to subsequent osmotic rupture. Fortunately, rat feeding studies showed that cells killed by gentle boiling were equally as digestible as those ruptured by sound waves (See Annex I). In all subsequent animal and human experiments, boiled, intact cells were fed.

In our final experiment, a test of the protein quality of alternate biomasses was conducted. Chlorella sorokiniana was prepared and supplied to us by Dr. R. L. Miller, USAF, Brooks AFB. The algae was debittered by 24-hour extraction with boiling ethanol, washed five times with water and twice

with ethanol, dried, and ground. As a representative fungi, we selected Torula food yeast which we obtained from the Lake States Yeast Division of the St. Regis Paper Company, Rhinelander, Wisconsin.

Details of diets will be found in the descriptions of individual experiments.

The human feeding studies reported herein were made possible only by multiple support for the metabolic unit. Portions of the cost were derived from NIH grant AM 10202 and NASA grant NsG 243, Supplements 7 and 11. Support for one of the graduate students involved in this research was derived from NIH Training Grant GM 1188.

III. Results

The questions posed in Part I have been answered as follows:

(a) Is the protein adequately digested? Yes. Digestibility in rat feeding studies was 93% compared to 99% of casein. See Annex I (Calloway and Kumar, Applied Microbiology, 17: 176, 1969).

(b) Is the absorbed protein of good quality? Yes. Biological value compares favorably with casein, in rat studies. Amino acid pattern is adequate for human needs if the protein could be fed at probable total nitrogen requirement levels. See Annex I (Calloway and Kumar, Applied Microbiology, 17: 176, 1969).

(c) Is the stored lipid digestible? No. Most of the lipid from lipid-rich cells fed to rats and mice was recovered in feces. Findings from the mouse test are given in Annex II (Waslien and Calloway, submitted to Applied Microbiology, April 1969).

(d) Is the absorbed lipid well utilized? Even though the lipid polymer was not absorbed, methods of depolymerizing the compound are known, so a study was conducted to determine if substantial amounts of the monomer, β-hydroxybutyric acid, could be utilized. Rats fed this material as 19% of the diet or, as a control, butyric acid ate less and grew more slowly than rats fed corn oil. No other adverse effects were noted. See Annex II (Waslien and Calloway, submitted to Applied Microbiology, April 1969).

(e) At what dietary level will nucleic acid content present a bar to the use of bacteria as food?

Human diets composed solely of Hydrogenomonas eutropha would contain about 25 times more nucleic acid than is present in the normal diet. If all the purine bases of this nucleic acid were absorbed and metabolized, blood uric acid levels would become elevated and there would be risk of urate crystallization in the urinary tract and elsewhere in the body. In addition, the high level of protein provided by these bacterial diets may accentuate even further this elevation in blood uric acid. Recent research (5) has revealed major increases in urinary uric acid when dietary protein was elevated to 600 g per day, the amount that would be ingested with 2400 kcal of protein-rich Hydrogenomonas eutropha.

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(5) Calloway, D. H., and S. Margen. Fed. Proc. 27: 725, 1968.

Human feeding trials using four levels of yeast ribonucleic acid indicated that dietary preformed nucleic acids should be less than 8 g per day in a diet providing 75 g of dietary protein (approximately the combined contributions of 100 g of protein-rich Hydrogenomonas eutropha). In regular use, not more than 3-4 g of nucleic acid should be taken daily, and this not by men with a tendency toward hyperuricemia. See Annex III (Waslien, Calloway, and Margen, American Journal of Clinical Nutrition 21: 892, 1968).

Another experiment was conducted to determine the effect of other crude cells high in nucleic acids, yeast and algae on uric acid levels, with minimally adequate amounts of protein in the diet. Elevation of uric acid followed the predictive equations developed in the model system study (Annex III), irrespective of source of nucleic acid or total dietary protein level. See Annex IV (Waslien, Calloway, and Margen, submitted to Journal of Food Science, May 1969).

It is possible partially to block endogenous uric acid formation by giving regular doses of allopurinol, an inhibitor of the enzyme xanthine oxidase. Our research has demonstrated that allopurinol also blocks the final oxidation of fed nucleic acids. See Annex V (Bowering, Margen, Calloway, and Rhyne, accepted by American Journal of Clinical Nutrition, April 1969). The urinary output of uric acid is halved by the drug, but permissable levels in the diet could not be increased by this amount. One of the altered conversion products, xanthine, is also poorly soluble, so the dietary RNA level should be increased by not more than 50%, i.e. to less than 5-6 g per day. This amount of RNA is provided by about 75 g of dry Hydrogenomonas eutropha cells.

(f) Can bacteria serve as sole source of dietary protein for man? Possibly, based on amino acid composition and rat feeding tests. However, this point has yet to be proved because all bacterial samples fed to men thus far have caused serious gastrointestinal and systemic reactions. See Annex VI (Waslien, Calloway, and Margen, Nature 221: 84, 1969).

(g) What is the maximum amount of bacteria that can be included in the human diet? Much less than 6 g, based on present evidence.

IV. Recommendations for Future Work

Humans regularly consume reasonable amounts of bacteria in food products such as yoghurt and cheese. This suggests that some bacteria are innocuous. Search for such strains might be rewarding, if the organisms could utilize by-products of chemical atmospheric regeneration systems.

It might also be possible to remove or destroy the toxin present in *Hydrogenomonas eutropha*. Chemical methods would probably involve substantial loss of material and nutritive value, but could provide for removal of purine bases at the same time.

V. Publications

In addition to the manuscripts annexed to this report, the following publications have resulted from this grant.

Paper:

Calloway, D. H. Nutritional properties of harvested Hydrogenomonas eutropha. Proc. AIBS/NASA Conference on Bioregenerative Systems (Wash., D. C., November 1966), NASA SP-165: 49-53, 1968.

Abstracts:

Calloway, D. H., and A. M. Kumar. Hydrogenomonas eutropha as a source of protein. Proc. 2nd Int. Congr. Food Sci. and Tech. (Warsaw) 1966.

Waslien, C. I., and D. H. Calloway. Hydrogenomonas eutropha as a space food source. Proc. 3rd Annu. Grad. Nutrition Symp. (Santa Barbara 1967).

Waslien, C. I., and D. H. Calloway. Hydrogenomonas eutropha as a space food source. Proc. 10th Annu. Meet. ICSU - COSPAR (London 1967) Ed. A. H. Brown and F. G. Favorite, p. 47-48. North-Holland Pub. Co., Amsterdam, 1968.

Calloway, D. H., and C. I. Waslien. Hydrogenomonas eutropha as human food. Proc. 2nd Int. Conf. on Global Impacts of Applied Microbiology (Addis Ababa, Ethiopia, 1967).

Waslien, C. I., and D. H. Calloway. The effect of dietary nucleic acid and protein on blood and urine uric acid levels in normal men. Fed. Proc. 27: 486, 1968.

VI. Personnel

Many staff members participated in this research. They are:

Nursing staff:
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- Helen Sowa
- Helen Locker
- Art Miller
- Frank Barnett
- Joe Crim
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Protein Quality of the Bacterium *Hydrogenomonas eutropha*

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Hydrogenomonas eutropha cells harvested from semicontinuous autotrophic culture and washed free of substrate contain about 13% of nitrogen on a dry-solids basis. Biological value and digestibility of the bacterial nitrogen were determined in the rat by use of an abbreviated Mitchell-Thomas nitrogen balance technique and casein as the standard protein. Casein nitrogen was 99% digestible, and that of both whole boiled and sonically ruptured bacterial cells was 93%. Biological value of casein and the bacterial preparations was uniformly 77%. Amino acid composition of the bacteria, as in the case of casein, indicates a first limitation of sulfur-containing amino acids. These compositional features suggest that *H. eutropha* may be potentially valuable as a protein supplement in animal feeds.

The demand for new food sources more independent of conventional agriculture has directed attention to the potential of single-cell proteins. A hydrogen-fixing bacterium, *Hydrogenomonas eutropha*, has been studied in this context. Foster and Litchfield (2) found that *H. eutropha* is 11.87% nitrogen on a dry-solids basis and that amino acid composition is slightly superior to other bacterial proteins. This investigation shows, by amino acid analysis and rat assay, that *H. eutropha* protein is nearly equal to the milk protein, casein.

MATERIALS AND METHODS

One lot of bacteria, weighing approximately 400 g, was obtained from the Battelle Memorial Institute. The lot consisted of 24 individual samples, ranging in weight from 4 to 35 g and in color from light amber to caramel. These were harvested intermittently during nine periods of continuous culture, each of several days' duration. The culture was grown autotrophically in urea-containing medium (2). Harvested cells had been resuspended in distilled water (1:5, w/v), centrifuged, and freeze-dried to 1% moisture content prior to shipment. The individual samples were then blended into one composite.

Because of Roberts' (6) report that the culture fluid of *Bacterium coli* is toxic to chicks and rats, the *H. eutropha* was washed free of residual medium. This was accomplished by repeated suspension in distilled water and centrifugation. Five washings were required to yield supernatant fluid free of urea. Washing reduced the ash content from 5.88 to 2.42% of dry solids and the phosphorus content from 1.67 to 0.82%. Lyophilized washed cells were analyzed for content of total nitrogen (micro-Kjeldahl; 1), nonprotein nitrogen (after trichloroacetic acid precipitation) and, after hydrolysis (HCl, 110 C, 22 hr), amino acids (Beckman amino acid analyzer).

It is not known if *H. eutropha* will grow in the intestinal tract, but conditions appear reasonable since gaseous hydrogen, oxygen, and carbon dioxide are present, nutrient medium is available, and temperature is within the tolerance range of the organism. Therefore, two methods were used to prepare a nonviable sample suitable for animal feeding studies, boiling for 15 min in aqueous suspension, and exposure to high-frequency sound. This latter process ruptured only about 60% of cells initially, but fragmentation was nearly complete on standing in aqueous suspension in the cold (4 C) for 24 hr.

Biological value and digestibility of protein were determined by an abbreviated Mitchell-Thomas method (4). A diet containing 4% of protein from dry whole egg was fed for 1 week to 20 weanling male albino rats (Simonsen strain, cesarean-delivered from Sprague-Dawley stock). These were then assigned on a matched-weight basis to four dietary treatment groups: boiled bacteria, sonically ruptured bacteria, casein, and 4% egg protein. Bacteria and casein were incorporated into otherwise nutritionally adequate, purified diets at levels to yield 1.6% of nitrogen on a dry-solids basis. As fed, the diets contained 40% of moisture. Because of the limited amount of bacteria available, the test diets were fed for only 1 week; urine, feces, and rejected food were collected quantitatively for the last 5 days. These samples and all diets were analyzed for nitrogen content. The following factors were computed: NI = nitrogen intake; FN_m = fecal nitrogen of the 4% protein group (metabolic); FN_t = fecal nitrogen of the test group; UN_e = urinary nitrogen of the 4% protein group (endogenous); and UN_t

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TABLE 1. *Amino acid composition*[a]

Amino acid	*H. eutropha*	Published compositions		
		Casein (5)	*H. eutropha* (2)	*P. saccharophilia* (8)
Tryptophan		1.34	1.05	
Threonine	4.52	4.30	2.90	5.37
Lysine	8.61	8.06	3.57	5.73
Methionine	2.69	3.10	1.54	2.03
Cystine		0.38	0.11	0.36
Isoleucine	4.58	6.59	2.92	4.14
Leucine	8.52	10.11	5.44	8.35
Phenylalanine	3.96	5.42	2.96	3.56
Tyrosine	3.26	5.86	2.41	2.32
Valine	7.13	7.44	4.08	7.55
Histidine	2.48	3.04	1.28	1.81
Arginine	8.00	4.10	4.59	5.01
Alanine	8.80	3.38	6.02	13.57
Aspartic acid	9.57	7.44	5.82	9.72
Glutamic acid	11.17	2.32	10.35	10.52
Glycine	5.47	2.00	3.72	9.65
Proline	3.46	11.82	2.77	5.59
Serine	3.47	6.69	2.42	4.64
Total	95.69	93.39	63.93	99.92

[a] Values expressed as g/16 g of nitrogen.

TABLE 2. *Biological quality of H. eutropha nitrogen*

Protein source	Nitrogen digestibility	Biological value
	%	%
Casein	98.9 ± 0.7	77.1 ± 7.6
H. eutropha boiled	93.8 ± 3.9	77.6 ± 4.6
H. eutropha, sonically broken	93.3 ± 4.0	77.0 ± 6.6

= urinary nitrogen of the test group. Absorbed nitrogen (AN) = NI − (FN_t − FN_m); nitrogen true digestibility (ND), % = AN/NI × 100; biological value (BV), % = AN − (UN_t − UN_o)/AN × 100; net nitrogen utilization (NNU), % = ND × BV/100.

RESULTS

The bacteria as finally prepared contained 13.45% of total nitrogen and 0.86% of nonprotein nitrogen. Measured amino acid content (Table 1) accounted for 95% of a protein assumed to contain 16% of nitrogen. (Nucleic acids make up 7.8% of bacterial solids. A trace of galactosamine was also present.)

The essential amino acid pattern is quite comparable to that of casein, except that the bacterial protein contains much less of the aromatic amino acids and slightly less of the sulfur-containing ones (Table 1). Histidine content is somewhat less than in casein, and the nonessential amino acids are much higher, with the exceptions of proline and serine, which are lower.

Biological quality of *H. eutropha* nitrogen (mean and standard deviation) is indicated in Table 2. The net utilization of the bacterial protein was slightly below that of casein, 72% in contrast to 76% of casein.

DISCUSSION

Our finding that there is no difference in digestibility of whole, boiled cells and sonically ruptured ones agrees with data for *Escherichia coli* (3) and differs from results with *Bacillus megaterium* (7).

The high biological value of our bacterial preparation accords well with its amino acid pattern. Sulfur-containing amino acids appear to be limiting, as is the case with casein and with other bacteria. This is true of *E. coli*, for example, which has protein of good biological value (69%), although it is less well digested (81%) than is *H. eutropha* (3). The high lysine content of our freeze-dried *H. eutropha* is especially interesting, suggesting that the bacteria would form an excellent supplement to cereal grains, which are low in this essential amino acid.

Amino acid composition of *H. eutropha* reported in this study (Table 1) is about 50% higher than the values reported by Foster and Litchfield (2). This difference may be due simply to incomplete hydrolysis of protein in the earlier study, but more probably reflects dilution of bacterial protein with nonprotein nitrogen (urea) from residual substrate. Our values for lysine, histidine, and arginine differ even more widely than do those of the other amino acids; they were about double the Battelle values.

Our amino acid values (Table 1) compare quite favorably with those reported for a facultative hydrogen bacterium, *Pseudomonas saccharophilia* (8). The heterotrophically grown pseudomonad contains more threonine and less lysine and methionine than does the autotrophically cultivated *H. eutropha*.

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Nutritional Value of Lipids in *Hydrogenomonas eutropha* as Measured in the Rat

Running Title: Nutritional value of *H. eutropha* lipid

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ABSTRACT

Hydrogenomonas eutropha is known to accumulate lipid, comprised largely of polymerized β-hydroxybutyric acid, when maintained in nitrogen-deficient medium. This lipid was very poorly absorbed from bacterial diets by mice, even though nitrogen absorption was adequate. The monomer, free β-hydroxybutyric acid, was well absorbed from purified diet. Rats fed the monomer or butyric acid ate less food and grew more slowly than rats fed corn oil.

INTRODUCTION

Hydrogenomonas eutropha grows rapidly in phosphate-buffered medium containing minerals and urea, in an atmosphere of 20% oxygen-10% carbon dioxide (5). Cells harvested during log-phase growth contain 12-14% nitrogen and 9% lipid (3). The protein is of high quality (3), but the protein-to-energy ratio of this organism is too high for it to be considered as a primary feed source for animals. If nitrogen, oxygen, or phosphorus are made limiting for cell division, a polymer of β-hydroxybutyric acid is accumulated, constituting up to 50% of the cellular solids (9). There is no evidence that the lipid polymer can be used by animals as an energy source, though small quantities of the monomer are readily metabolized by mammalian extrahepatic tissue. When excessive amounts of β-hydroxybutyric acid are formed (as in ketoacidosis), the acid is excreted in the urine with an accompanying loss of base.

MATERIALS AND METHODS

H. eutropha was grown autotrophically both in nitrogen-rich and nitrogen-deficient media. Conditions of growth and subsequent processing of the high-protein lot (HPC) were described previously (3). Lipid-rich cells (LRC) from nitrogen-deficient Bonger's medium* were harvested from batch cultures and lyophilized at the Research Institute for Advanced Studies. Both lots were analyzed for nitrogen (2), lipid (6), and mineral constituents (1) (Table 1).

Experiment 1. Digestibility of Bacterial Lipid and Protein. HPC and LRC were boiled and then incorporated into mouse diets having approximately 25% protein and 11% lipid. These levels of protein and lipid were produced by addition of dry bacterial cells to a purified protein-free, glucose-cornstarch basal diet that

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*Bonger's medium consists of one gram of $CO(NH_2)_2$; 100 mg of $MgSO_4 \cdot 7H_2O$; and 8 mg $Fe(NH_4)_2SO_4$, in one liter of 0.025-0.040 M P-buffer (0.03 M Tris plus 0.002 M phosphate), pH 6.0.

contained 1% of corn oil to provide essential fatty acids and adequate amounts of all other essential nutrients. The LRC diet contained 43.5% of bacterial solids, providing 25 g protein and 10 g lipid per 100 g diet.

An equal amount of protein and 4 g of lipid was provided by 30% of HP cells in the diet. Lipid content of HPC diet was made comparable to LRC diet by addition of corn oil (6 g/100 g diet). An isonitrogenous casein control diet with all the lipid from corn oil was also fed, and a low-protein (3%), low-fat (1%) diet was used to determine endogenous levels of fecal lipid and nitrogen.

Twenty-four weanling male Swiss mice were fed the low-protein diet for one week and then divided into four groups of equal total group weight and individually housed in metabolic cages. Each group received one of the test diets for 10 days. Food consumption and weight records were kept for each animal, and feces were collected for nitrogen and lipid analyses.

Experiment 2. Utilization of Monomeric β-Hydroxy-Butyric Acid.

Pure sources of β-hydroxybutyric acid (βOHBA), the free acid and sodium salt, were used in isonitrogenous casein diets that contained 20% of total lipid (Table 2). The lower level tested included 10 parts of test lipid (7 as free acid, 3 as sodium salt) and the higher, 19 parts (13 and 6) with the remainder (10 or 1 part) from corn oil to provide essential fatty acids. Several control diets were included in the test designs: one (BA) contained the same amount of total short-chain fatty acid as the test diets, from butyric acid and its sodium salt. A sodium control diet (NaB) contained no free acid but enough sodium butyrate to match the sodium content of the βOHBA test diet. A conventional casein diet containing only corn oil was also fed.

Male, weanling, Charles River rats were individually housed and fed the 20% corn oil, casein control diet for 10 days and then divided into weight-matched groups. One of these groups continued the same standard casein diet. Animals fed the βOHBA, BA, and NaB diets

received the lower level of test lipids for 3 weeks to allow for possible metabolic adaptation to the ingested ketone body, βOHBA. Then, three animals from each group were killed, and the remaining four animals were fed for another 3 weeks diets with the higher level of test lipid.

Gross anatomical observations were recorded and samples of liver, kidney, small intestine, adrenal, and spleen were preserved for histologic examination. Moisture content of the liver, kidney, and small intestine was determined. Weight gain and food consumption records were kept throughout the experiment, and urine and feces were collected quantitatively the last 3 days of each experimental week. Diets, feces, and urines collected during the last 3 weeks were analyzed for nitrogen (2), energy (6), and total mineral content (4). All of the urines were analyzed for sodium, potassium, calcium, and magnesium content (1).

RESULTS

H. eutropha cells grown on nitrogen-deficient medium had less nitrogen and more lipid than cells grown in full medium. The composition of the dry cells is given in Table 1. Only 5% of the lipid in HP cells is ether-extractable (Soxhlet) and this fraction contains the following fatty acids: 12:0, 14:0, 16:0, 16:1, 17:0, 18:0, 18:1, 18:2 (18:3 or 20:0). About two-thirds of the total lipid found could be extracted by mixed solvent systems (chloroform-methanol or diethyl ether-petroleum ether-ethanol) from dry cells. The remaining one-third was extracted only after mild HCl-hydrolysis of the cells.

Experiment 1. The bacterial diets were not as acceptable to mice as the control diet. Control mice ate 3.4 g of diet per day and increased in weight 4.2 g during the 10-day feeding period. The group fed HPC ate 3.0 g of food and grew more slowly, 1.7 g. With the LRC diet, food acceptance was even lower, 2.6 g, and the group lost an average of 3.2 g weight.

True nitrogen digestibility of casein was 97% and of the HPC, 90%, comparable to that found in an earlier study (3). Digestibility of the LRC nitrogen was slightly lower, 86%, but this difference was not statistically significant. In the casein control diet, 99% of the lipid, corn oil, was digestible. Lipid digestibility of the LRC diet was quite low, only 16% being absorbed. The mixture of corn oil and bacterial lipid in the HPC diet was 48% digestible. This indicates that most of the lipid from HPC must also be poorly absorbed and that corn oil was not as well absorbed in this diet as in the casein control. Some absorbable lipid may have been mechanically removed due to the bulkier feces that resulted from bacterial diets, but increased endogenous fat loss cannot be ruled out of consideration.

Experiment 2. The addition of free fatty acid, either as βOHBA or BA, to the diet of rats had an adverse effect on food intake and weight gain (Table 3). The elevated dietary sodium content was not responsible, as growth of the NaB-fed animals was equal to the overall casein control group. The two free fatty acids and sodium

salts were as well absorbed as the control, corn oil (Table 3), but energy loss in the urine was somewhat higher. This slight reduction in the percentage of energy metabolized agrees with the observed poorer growth rate.

In the urines, only sodium was elevated, from 4 mg to 32 mg per rat per day, in animals fed the lower levels of test lipids (Table 4). With higher test lipid levels that provided still more dietary sodium, a diuresis occurred (volume increased from 11 ml to 29 ml of urine per day). Urinary pH increased from 5.4 to 7.9 with elevated output of sodium (from 5 to 67 mg) and potassium (from 9 to 33 mg per rat per day). There were no significant changes in renal excretion of the divalent cations, calcium and magnesium (Table 4).

There were no grossly visible pathological changes in any group. The kidneys were absolutely smaller and made up a smaller percentage of total body weight in the animals fed high levels of βOHBA and BA (Table 4), but histopathologic studies did not reveal any abnormality in the kidney or other tissues examined.

DISCUSSION

The lipid, most of which is poly β-hydroxybutyrate, in nitrogen-starved Hydrogenomonas eutropha we found not to be absorbed by mice. This confirms unpublished observations of Schlegel (personal communication, Professor H. G. Schlegel, University of Göttingen, 1967). Cell rupture is evidently not the factor limiting absorption because the bacterial nitrogen is well absorbed from diets containing these cells. The monomer, β-hydroxybutyric acid, is also well absorbed, so we conclude the polymer is not adequately degraded by digestive enzymes.

Rodents fed the monomer or, as a free fatty acid control, the unhydroxylated 4-carbon acid (butyric acid) were equally slightly smaller than animals fed corn oil. This may have been due in part to the volatility and noxious odor of these acids and an associated reduction in food consumption. The test lipids did not appear to have other adverse short-term effects. Although urinary Na and K were elevated in the groups fed the BA and βOHBA diets, loss of

urinary electrolytes was not greater than that seen with the sodium-control diet containing only sodium butyrate (NaB diet). This indicates that dietary sodium, and not an independent metabolic effect of the free acids, was responsible for the high concentrations of these electrolytes in the urine. Thus, if food consumption could be maintained, βOH butyric acid may be a useful nutrient.

Methods are known for depolymerizing the bacterial lipid into D(-)-β-hydroxybutyric acid, either enzymatically (10) or chemically (8). If these methods could be adapted for low-cost production, lipid-rich H. eutropha could be a useful feed supplement, as a source of both protein and energy.

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Table 1. Composition of *Hydrogenomonas eutropha* Cells

Code	Constituents, per 100 g dry solids					
	Nitrogen	Lipid	Ca	Mg	Na	K
	g	g	mg	mg	mg	mg
HPC	13.0	9.4	66	98	280	590
LRC	8.9	22.5	11	41	110	790

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Diet	Casein	βOHBA		BA		NaB	
		Low Level	High Level	Low Level	High Level	Low Level	High Level
				g/100 g diet			
Casein	40.0	40.0	40.0	40.0	40.0	40.0	40.0
Corn oil	20.0	10.0	1.0	10.0	1.0	17.4	14.8
β-hydroxybutyric acid (free acid)	----	7.0	13.0	----	----	----	----
3-hydroxybutyric acid (Na salt)	----	3.0	6.0	----	----	----	----
Butyric acid (free acid)	----	----	----	7.4	13.8	----	----
Butyric acid (Na salt)	----	----	----	2.6	5.2	2.6	5.2
Basal diet[a]	40.0	40.0	40.0	40.0	40.0	40.0	40.0

(Table 2 continued)

[a] Basal diet provided: 5.0 g salt mix (4 g USP XIV and 1 g trace mineral mix which provides the following additional minerals, in mg/100 g diet: $ZnCO_3$, 2.0; $MnSO_4 \cdot H_2O$, 32.0; $CuSO_4$, 4.0); 1.0 g vitamin mix (in mg/g, thiamine HCl, .25; riboflavin, .5; pyridoxine HCl, .25; niacin, 2.0; Ca pantothenate, .2; inositol, 10; para-amino benzoic acid, 5; biotin, .01; folic acid, .1; vitamin B_{12}, .005; choline chloride, 100); and 1.0 g glycerol; .5 g ascorbic acid, 8.0 g sucrose; and 24.5 g starch. Fat-soluble vitamins were given orally in two 25-mg drops per week, providing: vitamin A, 600 I.U.; vitamin D, 10 I.U.; vitamin E, 3.0 mg; vitamin K, 0.3 mg.

Table 3. Growth and Nutrient Utilization of Rats Fed βOH Butyric Acid and Control Diets

Diet Code[a]	βOHBA	BA	NaB	Casein Control
Weight gain, g/rat/day				
Weeks 1-3, n = 7	3.4	3.5	4.3	4.8
Weeks 4-6, n = 4	3.4	2.0	5.0	4.2
Food intake, g/rat/day				
Weeks 1-3	10.6	10.7	11.0	11.2
Weeks 4-6	10.9	9.0	11.0	11.1
Gross absorption, % of intake				
Nitrogen	97.4	96.9	97.8	95.4
Energy	96.3	97.5	97.5	97.1
Metabolizable energy, % of absorbed	92.1	94.1	93.7	95.8

[a]See Table 2 for diet key.

Table 4. Kidney Weight and Urinary Characteristics of Rats Fed Test Lipids

Diet	Kidney	Urine Volume	pH	Ca	Mg	Na	K
	g/100 g body weight	ml/rat/day			mg/rat/day		
10% Test Lipids, Weeks 1-3							
Casein	1.08 ± .07[a]	15.7 ± 3.7	6.2 ± .7	1.5 ± .9	3.0 ± .7	4.0 ± 1.3	16.1 ± 5.0
βOHBA	1.39 ± .51	19.3 ± 2.7	7.3 ± .8	1.3 ± .5	2.3 ± .9	26.2 ± 11.4[b]	15.2 ± 4.1
BA	1.10 ± .06	21.0 ± 4.3	7.3 ± .9	1.3 ± .5	2.6 ± 1.1	27.0 ± 8.9[b]	17.8 ± 7.3
NaB	1.19 ± .08	21.0 ± 3.3	7.0 ± .4	1.4 ± .9	2.6 ± .9	31.6 ± 8.1[b]	18.9 ± 9.0
19% Test Lipids, Weeks 4-6							
Casein	1.05 ± .10	11.0 ± 2.7	5.4 ± .5	2.4 ± .8	2.1 ± .5	5.0 ± 2.4	9.2 ± 4.6
βOHBA	.85 ± .03	23.3 ± 4.3[b]	6.9 ± .7[b]	4.4 ± 2.2	2.7 ± .6	66.7 ± 28.4[b]	32.9 ± 8.2[b]
BA	.85 ± .06	23.3 ± 4.3[b]	7.0 ± 1.1[b]	1.6 ± .9	2.1 ± .8	64.8 ± 22.7[b]	26.2 ± 2.9[b]
NaB	.99 ± .04	28.7 ± 3.3[b]	7.9 ± .3[b]	1.7 ± .6	1.5 ± .9	60.1 ± 16.1[b]	33.3 ± 13.1[b]

[b]Mean ± standard deviation. For kidney weight after 3 weeks, n = 3; after 6 weeks, n = 4. Other data for weeks 1-3, n = 7 and for weeks 4-6, n = 4.

[b]These values are significantly different from those of the casein group.

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Uric Acid Production of Men Fed Graded Amounts of Egg Protein and Yeast Nucleic Acid[1,2,3]

CAROL I. WASLIEN, DORIS HOWES CALLOWAY AND SHELDON MARGEN

ALGAE AND BACTERIA form the basis of candidate bioregenerative systems for atmosphere control and food supply in space missions. These and other microorganisms could also serve as economical sources of protein to meet existing regional needs and predicted world deficits. However, consumption of such foods may be restricted by their high nucleic acid content. In man the purine portion of these compounds is degraded to uric acid, which has low solubility at the pH of body fluids and is relatively poorly excreted by the kidney. If the blood uric acid content is elevated, crystals may form in the joints, as in gout, and with excessive renal clearance loads, stones may be deposited in the urinary tract.

Studies have shown that plasma and urinary uric acid levels are influenced both by the amounts of nucleic acids (1–6) and protein (7–14) in the diet. In most of these reports, few subjects were studied; often, dietary protein and nucleic acid varied simultaneously or the diets were incompletely described. Nugent and Tyler (5, 6) used yeast nucleic acid as a supplement to the normal diet thus eliminating the increase in protein that occurs when less refined sources of nucleic acid are fed, but the basal diet of their subjects was not stipulated beyond the specification of "low-purine" foods. We have now evaluated these two factors separately, using carefully controlled diets, and the data have been used to derive predictive equations describing the response to foods high in nucleic acid.

METHOD OF STUDY

The subjects were healthy male volunteers ranging in age from 21 to 38 years, in height from 168 to 199 cm, and in weight from 56 to 106 kg. They were housed in a closed metabolic unit and given a basic formula diet adequate and constant in all known essential nutrients, except when protein was deliberately reduced (Table 1). Caloric needs to maintain constant body weight were met by additions of pure fats and carbohydrates. When protein was reduced, an isocaloric equivalent of carbohydrate was substituted. Egg albumin was the only source of protein and thus the diet was free of nucleic acid unless it was added in the form of pure yeast ribonucleic acid.[4] Minimum fluid intake was stipulated but subjects were allowed free access to deionized water beyond the minimum. Total

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[3] Presented at the 52nd Annual Meeting of the Federation of American Societies for Experimental Biology, Atlantic City, April 1968.

[4] Purchased from Calbiochem, Los Angeles, Calif.

fluid intake was recorded and there was no food rejection.

The effect of variation in protein intake, at several levels from 0 to 75 g/day, was evaluated in a total of 20 different subjects, some of whom were studied on more than one occasion. Each dietary level of protein was administered for a minimum of 9 days (usually 12–15), and the first 6 days were allowed for adjustment to the changed intake. Data on urinary uric acid are the averages of individually pooled 24-hr outputs for the last 3–6 days of study, and plasma uric acid concentrations are in fasting bloods drawn the final morning of each period. Two of the men were fed the control (75-g protein) diet for 66 consecutive days, as an additional methodologic check. Their blood was sampled intermittently and 72-hr urine collections were made during the entire period.

In a separate study, five men were fed the control diet supplemented with 0, 2, 4, and 8 g of RNA. Each dosage of RNA was given for 5 consecutive days, distributed equally among four equal meals per day. The sequence in which the dosages were administered was varied among the subjects.

Urine was quantitatively collected and stored in the cold without preservative. Its weight was recorded daily and the total diluted to volume with distilled water. Urinary and plasma uric acid was determined by the enzymatic method of Kalckar (15).[5]

RESULTS

Average daily urinary uric acid excretions of the two long-term control subjects were 327 ± 28 and 382 ± 50 mg, based on fourteen 72-hr specimens. There was no significant difference between excretion levels at the beginning of the period of measurement and at the end, indicating that no unintentional feature of the experimental diet or regimen systematically affected synthesis or excretion of uric acid. The daily output of these men fell within the range of the total population of 20 men studied. Average output of the larger group was 392 ± 66 mg/day (Table II). Mean control plasma uric acid concentration was 4.7 ± 0.6 mg/100 ml.

Urinary uric acid output fell and plasma levels rose when dietary protein was reduced (Table II). In the 10 subjects who received both protein-free and control diets, urinary uric acid excretion at 0-protein intake differed significantly ($P < 0.01$) from paired values at the 75-g daily intake level of protein. Plasma uric acid concentration was significantly ($P <$

[5] The enzyme, uricase, was purchased from Worthington Biochemical Corp., Freeland, N. J., or from Sigma Chemical Co., St. Louis, Mo.

TABLE I

Typical composition of a diet providing 75 g of protein and 2,800 kcal[a]

Component	g/day
Egg albumin[b]	103
Sucrose	99
Dextri-Maltose (Mead Johnson)	177
Cornstarch	150
Corn oil	44
Crisco (Procter & Gamble)	49
Citric acid	5
NaCl	5
$K_2HPO_4 \cdot 3H_2O$	4.378
$CaHPO_4 \cdot 2H_2O$	3.000
MgO	0.670
Synthetic flavoring[c]	0.400

[a] Subjects also received daily: 10 g of decaffeinated coffee powder (Sanka, courtesy of the General Foods Corp.); a vitamin preparation (courtesy of Hoffmann-LaRoche, Inc.) containing 2 mg thiamine mononitrate, 3 mg riboflavin, 20 mg niacinamide, 5 mg vitamin B_6, 10 mg calcium pantothenate, 50 μg *d*-biotin, 2 μg vitamin B_{12}, 4,000 IU vitamin A palmitate, 400 IU vitamin D, 35 mg *dl*-α-tocopheryl acetate, 1 mg menadione, 50 mg ascorbic acid, and 0.5 mg folic acid; and a mineral supplement containing, in milligrams, 16.7 $FeSO_4 \cdot 7H_2O$, 1.79 $CuCl_2 \cdot 2H_2O$, 14.6 $ZnSO_4 \cdot 7H_2O$, 5.12 $MnSo_4 \cdot H_2O$, 0.21 $Na_2MoO_4 \cdot 2H_2O$, 1.07 $Cr(SO_4)_3 \cdot 15H_2O$, 0.008 Na_2ScO_3, 28.3 $AlK(SO_4)_2 \cdot 12H_2$, 2.0 NaF, and 0.2 KI. The total diet provided 600 mg of nitrogen in addition to the nitrogen from egg albumin.

[b] Additional biotin, 200 μg/day, was added to formulas containing this amount of dried egg white.

[c] This amount of flavoring (courtesy of Firmenich) was nearly devoid of nitrogen so individual selection was permitted.

TABLE II

Urinary and plasma uric acid of healthy men fed graded levels of egg albumin

Avg Protein Intake, g/Man per Day	Urinary Uric Acid		Plasma Uric Acid	
	Number of subjects	mg/24 hr	Number of subjects	mg/100 ml
0	14	354 ± 67	8	6.0 ± .7
22	6	337 ± 48	10	5.2 ± .7
28	6	352 ± 72	6	5.6 ± .7
37	5	331 ± 28	5	5.8 ± .5
75	20	392 ± 66	13	4.7 ± .6

0.05) higher with the protein-free diet than matched control values for five subjects. A few of the men were fed intermediate levels of dietary protein, ranging from about one-half (22 g) to the full minimum need for dietary protein (37 g). Average urinary excretion and plasma concentrations did not differ significantly from the protein-free diet condition.

Typical response to added dietary RNA is portrayed in Fig. 1. Urinary uric acid excretion rose promptly and reached a steady level of output at a higher level than with the control diet, by the 2nd or 3rd day of 2-g dosage. Excretion rose sharply on the 1st test day and more slowly for the remainder of the time when the 4- and 8-g doses were given; the rate of rise was greater with 8 than with 4 g.

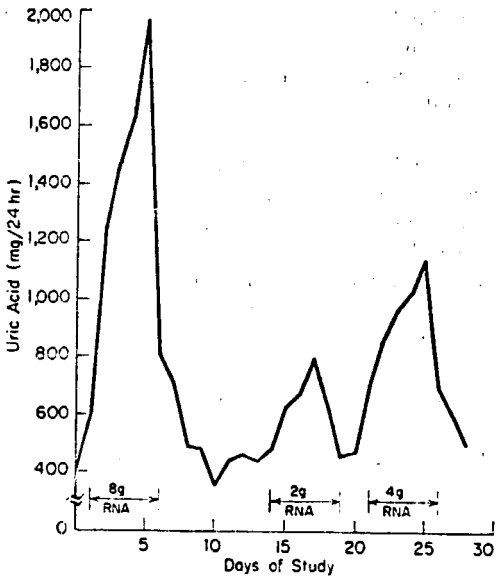

FIG. 1. Daily urinary uric acid excretion of *subject 1005.*

After RNA administration ceased, urinary output fell, sharply on the 1st day and more slowly thereafter, until control levels were again attained, by the 3rd day. One man did not behave in this typical fashion in that his urinary uric acid output was essentially the same at 4- and 8-g dosages of RNA. Excretion values, shown in Table III and used to compute the regression equation diagrammed in Fig. 2, are averages of the last 3 days of each treatment

TABLE III

Plasma and urinary uric acid of healthy men fed various amounts of yeast nucleic acid with a constant, 75-g egg-protein diet

Nucleic Acid, g/Man per Day	Plasmic Uric Acid, mg/100 ml				Urinary Uric Acid, mg/Man per 24 hr			
	0	2	4	8	0	2	4	8
Subjects								
1001	5.0	6.0	8.8	10.2	405	663	1,123	1,522
1002	4.7	6.0	7.7	9.5	430	765	867	1,317
1003	5.2	6.1	6.8	7.2	332	668	963	1,676
1004	5.5	6.6	8.0	10.2	316	542	713	755
1005	3.9	5.3	7.1	9.7	378	698	1,028	1,697
Average	4.9	6.0	7.7	9.4	373	667	939	1,393

period. Plotted in this way, the relationship between dietary RNA and urinary uric acid is linear, which suggests that excretion must have been at least near the maximum at the end of the 5-day treatment periods. In four of the men, urinary uric acid increased linearly (r = 1.000), by 147 mg/g of yeast RNA (Fig. 2); in the aberrant subject, this value was markedly decreased at the two higher RNA levels.

Plasma uric acid concentrations increased with each increase of dietary RNA (r = 0.996). Again, four men responded similarly and a fifth was different, but not the same subject as differed in urinary output. The regression of plasma uric acid with RNA was 0.65 mg/100 ml

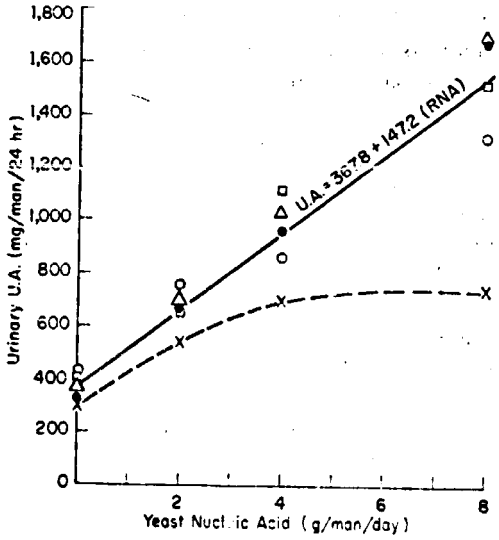

FIG. 2. Change in urinary uric acid with supplemental yeast nucleic acid. □ = *Subject 1001;* ○ = *Subject 1002;* ● = *Subject 1003;* × = *Subject 1004;* △ = *Subject 1005.*

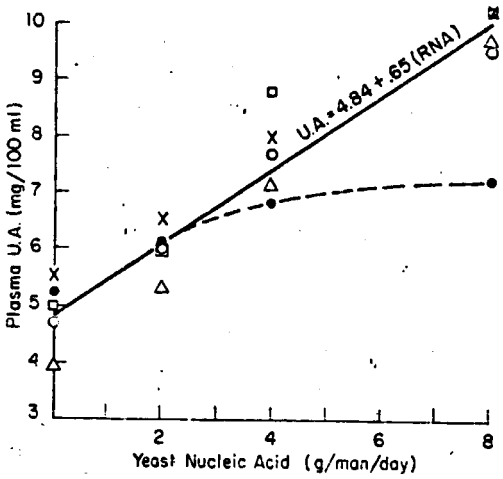

FIG. 3. Change in plasma uric acid with supplemental yeast nucleic acid. □ = *Subject 1001;* ○ = *Subject 1002;* ● = *Subject 1003;* × = *Subject 1004;* △ = *Subject 1005.*

per gram in the uniform set (Table III and Fig. 3). In the fifth man the slope was much lower.

DISCUSSION

If one groups data from other studies involving low purine (but not absolutely purine-free) diets (Table IV) containing 0–75 g of protein, a trend toward increased urinary uric acid excretion is clear in spite of the broad range and overlapping of values.

The lower plasma uric acid and higher urinary uric acid with a normal protein allowance (75 g), compared with a protein-free diet, have been ascribed by earlier workers to increased renal clearance (14). The elevation of urinary uric acid might also reflect increased endogenous syn-

TABLE IV

Published uric acid excretion of men fed low purine diets

Protein ingested, g	0–24	25–43	44–62	63–75
Average urinary uric acid, mg	218	364	428	436
Range	120–430	282–475	291–680	277–750
Number of observations	7	11	5	8
References	9, 10, 14	8, 10, 12, 13	8, 11, 12	9–11, 14

thesis, which others have shown to occur at higher levels of dietary protein (7).

The plasma uric acid concentration of all subjects fed the control diet alone or with 2 g of yeast RNA fell within the accepted range of normal values. However, after the 4-g dosage of RNA, three, and possibly four, of the men attained abnormally high levels. The rise in plasma uric acid reported here (2.8 mg/100 ml) is almost identical to the elevation due to 4 g of yeast RNA reported by Nugent and Tyler (5). Four of our men had greatly elevated plasma values after the 8-g dosage. The plasma level of the fifth man was only approaching the abnormal range, even at this highest dosage of RNA: this subject did not differ in any obvious way from the other subjects.

Urinary uric acid excretion of our subjects fed the control diet is within the range of values for men receiving low purine diets (16). Excretion with 2 g of RNA in the diet is similar to that reported for subjects given diets with moderate amounts of meat and vegetables. Most studies indicate production of 0.5–0.75 mg of urinary uric acid per milligram of purine added to the diet in the form of foods (1, 3). Based on published compositional data of yeast nucleic acid (17), our four uniform subjects appear to have excreted 0.62, 0.61, and 0.59 mg uric acid per milligram yeast purine with the 2-, 4-, and 8-g dosages of RNA, respectively. The subjects of Nugent and Tyler (5) excreted 0.45 and 0.14 mg uric acid per milligram yeast purine at the 4- and 7-g dosages, respectively. Their value for the 7-g dosage is based only on one urine collection from one subject and is similar to the 0.22 mg uric acid/mg ingested purine shown by the one deviant subject in our study. We did note one difference between this subject and our other men and that was the regular presence of a substantial amount of methane in his breath. This could indicate different bacterial activity in his intestinal tract (18), offering an alternate means of uric acid removal.

For practical purposes of supplementation to diets containing inadequate amounts of protein, the nucleic acid contribution of microorganisms should not constitute a serious bar to their use. Yeast and bacteria vary in composition depending upon conditions of growth, but both contain about 1 g of nucleic acid per 10 g of protein. It is not likely that the remainder of a low protein diet would be rich in purines, since most foods high in one are high in the other. Therefore, with low protein diets, a daily supplement of 10–20 g of microbial protein could be used to advantage and without undue hazard. However, addition of crude microorganisms to typical American diets, containing larger amounts of muscle and organ meats, should be approached with caution.

SUMMARY

Healthy male subjects were fed purine-free basal diets containing 0–75 g of protein and, at the highest protein level, 0–8 g of added yeast ribonucleic acid in order to differentiate effects of these dietary components on plasma and urinary uric acid production. Urinary uric acid levels were significantly higher and plasma levels lower with 75 g of protein than with a protein-free diet. When nucleic acid was fed, plasma and urinary uric acid increased linearly in four of five subjects. Predictive equations were derived describing this response to dietary nucleic acid.

We wish to thank Mrs. Melinda Buchanan for performing urinary uric acid determinations and Dr. Amy Odell for her cooperation in the conduct of the experiment.

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URIC ACID LEVELS IN MEN FED ALGAE AND YEAST AS PROTEIN SOURCES.

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Microorganisms grown on human or industrial waste may be economical, nutritious food sources. Algae (Chlorella sorokiniana) and yeast (Torulopsis utilis) were compared with casein at two nitrogen levels as sole sources of protein for men. Biological value of algal protein was superior to casein, but yeast protein was not quite as good as algae, in diets containing 25 g protein. When 50 g of algae protein was consumed, true nitrogen digestibility was reduced, but nitrogen balances indicated that 50 g of any of these proteins met or exceeded dietary requirements. Urinary uric acid increased considerably with algae and yeast. Renal clearances were not sufficient to prevent abnormally high plasma uric acid levels, comparable to those found in gout.

URIC ACID LEVELS IN MEN FED ALGAE AND YEAST AS PROTEIN SOURCES

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Short Title: Algae and Yeast as Human Food

SUMMARY--Microorganisms grown on human or industrial waste products may be economical and nutritious food sources. Algae (Chlorella sorokiniana) harvested from continuous culture and extracted with ethanol) and yeast (food grade Torulopsis utilis) were compared with casein at two levels of nitrogen as the sole source of protein for men. Biological value of algal protein was superior to casein and yeast protein was not quite as good as algae, in diets containing 25 g of protein. When 50 g of algae protein was consumed, true nitrogen digestibility was reduced from 89 to 82%. Nitrogen balances were not significantly different in groups of men fed the higher protein level, indicating that 50 g of any of these proteins met or exceeded dietary requirements. Urinary uric acid excretion was doubled when the higher level of algae was ingested; with yeast, excretion was nearly four times as high as the amount voided with a purine-free diet. During ingestion of these materials, renal clearance rates were not sufficient to prevent plasma uric acid concentrations from reaching abnormally high levels, comparable to those found in gout.

INTRODUCTION

Microorganisms are technologically attractive in that they offer promise of producing food without dependence on traditional agricultural methods. Some may serve a second role, as biological processors of industrial and human wastes. Algae can provide edible protein-rich cells as a by-product of sewage treatment and some yeasts of the Torulopsis genera flourish in residues from sugar and paper production.

Several studies have proved that the quality of these algal and yeast proteins is reasonably good (Dirr, et al., 1942, Goyco, et al., 1959, Kondratiev, et al., 1966, Dam, et al., 1965, Lee, et al., 1967). However, some adverse responses do occur when large amounts of crude products are incorporated into the human diet so some food processing may be required. For example, gastrointestinal disturbances have been attributed to poor digestibility of the algal cell wall (McDowell, et al., 1963) and there is evidence that utilization is improved if the dried cells are first extracted with alcohol (Kondratiev, et al., 1966, Dam, et al., 1965).

A major limitation to the use of microorganisms as food sources is their high nucleic acid content. Uric acid, the end-product of the purine portion of nucleic acid catabolism, is only slightly soluble at the pH of body fluids and there is some risk that salts may be deposited in the renal tract and possibly other tissues (e.g. joints) if the diet contains excessive purines. Ingestion of large quantities of yeast has been shown to produce elevated blood uric acid concentrations (Dirr, et al., 1942) but no similar information is available concerning the effect of algae.

An experiment was conducted in which yeast and algae were compared with casein, a purified protein of established biological value, as the

sole protein sources in a human diet. Their effect on blood uric acid and other indices of tolerance was measured.

METHODS AND MATERIALS

Seven healthy men were fed seven test diets during sequential 9-day periods, according to the plan shown in Table 1. Three of the men received

(table)

all of the diets and completed 56 days of study. The other men participated for fewer periods, totaling 27 to 45 continuous days. During the experiment, the men were confined to a metabolic unit except for brief periods of supervised activity at bi-weekly intervals. Exercise was provided by means of a motor-driven treadmill, the men being required each day to walk 30 min at 3.5 mph and to run 15 min at 5 mph, both at a 10% grade. The men were freely ambulatory but largely sedentary.

All of the men received during one period a protein-free (1 g N per day) but otherwise nutritionally adequate diet. Protein sources--casein, algae, and yeast--were fed at two dietary levels, to provide 25 and 50 g of protein (N x 6.25) per day. Pure yeast ribonucleic acid (RNA) was added to the casein at the approximate level found in several microorganisms, 8 g RNA per 100 g protein. Both algae and yeast contained nucleic acid naturally.

Algae was provided by Dr. R. L. Miller, Brooks Air Force Base. The cultivated strain, Chlorella sorokiniana, was contaminated at a level of about one bacterium per five algal cells. Harvested cells were processed as follows: Extracted with boiling ethanol (1/4 W/V) for 24 hours, washed with ethanol, dried. Batches combined, washed five times with distilled

water, allowed to settle, decanted, washed twice with ethanol, re-dried, and ground. The final product was dark green and gritty in texture but did not taste bitter, as unprocessed cells do. The desired protein levels were obtained by adding 31 and 64 g of dry algae to the protein-free basal diet.

The yeast, Torulopsis utilis, was purchased from a commercial supplier (Lake States Yeast Co., Rhinelander, Wisconsin). The two levels of dietary protein required consumption of 43 and 90 g of yeast per man per day, plus the basal diet.

Composition of the single-cell protein sources is given in Table 2.

(table)

Nutritional adequacy of the diets was assured, despite the varying contributions from the proteins, by addition of macrominerals to a formula given as one meal a day. Trace minerals and vitamins were given by capsule separate from the diet (Waslien, et al., 1968).

The diet was administered as four major meals daily with snacks mid-morning and mid-afternoon. At breakfast, protein was added to a special low-protein hot cereal; at lunch and dinner, the protein was served as flavored sauces with low-protein pastas; and in the evening, it was added to a spread served with a protein-free muffin. All diets included constant amounts of applesauce, orange-flavored beverage, carbonated beverages, decaffeinated coffee and tea. Additional calories were available, as required to maintain body weight, from protein-free cookies, candies, and salt-free, milk-free margarine.

Because of the risk of urate crystallization in the urinary tract with high levels of RNA in the diet, fluid intake of at least 3 liters per day was maintained. As necessary, sodium bicarbonate tablets were prescribed

to maintain urine pH above 6.5 and thereby to promote solubility of salts of uric acid.

Urine was collected quantitatively every day of the experiment and feces were pooled by 3-day periods. Blood samples were taken at the completion of each metabolic period and were analyzed for uric acid, total protein and albumin, urea nitrogen, glucose, cholesterol, and triglyceride. Urinary volume, pH, and osmolality were measured every day, and qualitative tests were made for the presence of protein, sugar, and acetone. Urinary nitrogen (Block, et al., 1956), uric acid, and creatinine (Technicon AutoAnalyzer) were determined daily on the last 3 days of each period. The urines from the last 3 days of each period were pooled by subject before analysis for content of phosphorus (Technicon AutoAnalyzer), sodium, potassium, calcium, and magnesium (atomic absorption spectroscopy). The feces from the last 3 days of the period also were analyzed for these minerals and nitrogen, as were composite samples of the total diets.

Feces and composite diets were lyophilized prior to combustion in a ballistic bomb calorimeter to compute digestible energy value of the diets (Miller, et al., 1959). Concentrations of hydrogen and methane in expired air and total volume of rectally passed flatus were recorded at intervals, as an indication of bacterial activity in the intestine (Calloway, et al., 1968).

RESULTS

There were no marked physiologic disturbances due to diet in any of the subjects. Fluctuations of body weight were recorded but these were not associated with any particular diet and were usually corrected by minor changes in caloric allowances (Table 1).

Fluid intake and urine volumes and concentration were uniform for all diets (Table 3). The pH was lower with yeast diets, presumably because

(table)

their potassium content was lower than in the casein and algae diets (Table 4). Some men had to be given additional sodium (as $NaHCO_3$) to

(table)

maintain urinary pH above 6.5 while consuming the yeast diets. These urines also showed a marked tendency to form precipitates on standing.

Fecal wet and dry weights were increased when the men ate algae and yeast (Table 3), but there were great differences between subjects in this respect. Only one man, 1304, complained of subjective discomfort such as "stuffiness" and "bloating" with the 50-g algae protein diet, and he passed a large amount of soft stools. Dietary energy was well absorbed from all diets. About 7% of caloric intake from casein diets was recovered in the feces and 10% from algae and yeast diets.

Volumes of rectally egested flatus were somewhat increased with the algae diets but, again, there was much variation within dietary groups (Table 3). Breath hydrogen and methane patterns failed to reflect any change in microfloral activity, except in one case. Subject 1303 gradually lost his initially high level of breath methane and reached a steady low level after the third metabolic period.

When the men ate the protein-free diet, they were in negative balance of all minerals except phosphorus (Table 4). With the casein diets, only calcium and magnesium balances were negative, taking the subjects as a whole, and some men were in slightly positive balance. Calcium balance was negative with the higher level of algae, but not at the lower intake. With

the yeast diets, calcium, magnesium, and phosphorus excretions all exceeded intake, at both protein levels.

The protein digestibility values given in Table 5 have been corrected

(table)

in the conventional way for fecal endogenous nitrogen output, that is, by subtracting from the test series the fecal nitrogen found with the protein-free diet, to give "true" digestibility. On this basis, casein is seen to be 95 to 99% digestible; but, as fecal nitrogen with casein in the diet was not significantly different ($p >> .05$) from the protein-free condition, the digestibility could be regarded as 100%. Fecal nitrogen was significantly higher with algal diets than with casein and varied directly with intake. Digestibility was 89% at the lower dietary level and 82% at the higher. Yeast-containing diets resulted in slightly more fecal nitrogen than did casein, but the difference was not statistically significant with the small number of cases and large variability within groups. At the 25-g level, true digestibility was computed to be 83% and, with 50 g of yeast protein in the diet, 87%.

Comparing the lower levels of dietary nitrogen, Chlorella protein was significantly ($p < .05$) better in promoting nitrogen balance than was either of the other sources (Table 5). This is reflected in the higher biological value computed for the algae, 79%, than for casein, 66%, and yeast, 70%. Utilization of absorbed algal nitrogen was superior to casein at the higher dietary level also, but balance was less positive (though not significantly so) because of the poorer nitrogen absorption with the larger dose of algae. Yeast protein produced about the same nitrogen balance as did casein.

Figure 1 presents the regression equations derived from nitrogen

(figure)

balance data at varying intake levels. The predicted total range of minimum requirements for balance in men of this population was 5.0 to 8.8 g of casein nitrogen per day. The mean value is 6.6 g. Fewer subjects are available for the other proteins, but the mean need for algal protein appears to be 6.2 g N per day, within a range of 5.1 to 6.4 g per day. Yeast values are 8.3 g N per day, mean requirement for assured balance, and range, 7.0 to 8.8 g.

Urinary uric acid output was 394 mg per day with the protein-free, purine-free diet, and plasma levels were in the normal range (Table 6).

(table)

Addition of 1.8 g RNA with casein caused urinary excretion to increase to 562 mg; plasma values, though elevated, were still in the normal range. At double this intake, renal excretion rose to 886 mg per day and plasma levels reached the lower limits of the abnormal range. Responses to the algae diet were not significantly different from the reaction to the RNA-supplemented casein. Algae contributed the same amount of nucleic acid as was added to the control casein diets. Yeast used in this experiment contained 11.4% nucleic acid which was much higher than was anticipated from published values for other yeasts. Urinary excretion rose to a mean of 1536 mg and plasma levels to 12.6 g% with 50 g of protein and 10.3 g nucleic acid present from yeast. Fortunately, none of the men experienced symptoms of gout, either during or after the feeding period.

Blood urea nitrogen varied directly with protein intake but was unaffected by protein source (Table 7). All of the men had normal blood

(table)

protein, lipid, and glucose levels initially and these did not vary due to either amount or source of protein in the diet.

DISCUSSION

The high biological values shown for yeast and algae in this study are good indications of their usefulness as a protein supplement for man. However, if yeast were to be used as the major source of protein at levels sufficient to maintain nitrogen equilibrium, plasma and urinary uric acid concentrations would be dangerously elevated. With algae as the major source of protein, the risk is substantially diminished, both because nucleic acid concentration is lower on an equivalent protein basis and slightly less protein is required to assure balance. Even so, extreme caution is indicated because any normal diet to which the supplement might be added would almost certainly contain some other purine bases.

The effect of long-term consumption of such diets has yet to be demonstrated. However, in leukemia, plasma uric acid is elevated to these same levels and secondary gout is a common finding in such patients. The experience of Kondratiev, et al. (1966) is illustrative of the problem. Some of their male subjects developed edema and irritation of the great toe (a prime target for gouty attacks) on being fed 150 g of dried algae per day for 14 to 16 days.

With algae, the adverse gastrointestinal response to large doses must also be considered. In our case, symptoms were not so severe as to impair nutrient balances significantly but indications for caution were present. Pokrovskaia, et al. (1968) have reported that calcium and magnesium assimilation were depressed by continued consumption of 120 g of dry cells per day. Our balance data indicate a similar trend when the amount of algae fed was 64 g per day.

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Table 1. Characteristics of subjects and their diets, caloric intake, and weight change during 9-day metabolic periods.

		1301	1302	1303	1304	1305	1306	1307
Age, years		27	22	23	30	23	26	30
Height, cm		185	183	178	183	188	178	180
Weight, kg		96	80	73	75	68	70	67
Creatinine, mg/kg[a]		20.5	22.0	23.7	23.2	25.2	23.0	23.9
Period 1	Diet Code[b]	50C	25C	50C	25C	---	50C	---
	Intake, kcal	2867	2829	2925	2829	----	2874	----
	Weight Change[c]	-.07	-.02	+.03	-.16	----	-.06	----
2		25C	0	25C	50C	50C	0	---
		2989	2847	2917	2999	3005	3111	----
		-.07	-.11	-.04	-.04	-.01	-.03	----
3		0	50C	0	25A	25C	---[d]	---
		3176	2940	2935	3034	2949	----	----
		-.07	0	-.04	+.02	-.04	----	----
4		25Y	50Y	25A	50A	25A	25C	---
		3319	2968	2999	2941	3023	3296	----
		0	-.03	-.02	+.03	+.02	+.02	----
5		50Y	25Y	50A	0	0	---	25A
		3521	3093	3068	3111	2985	----	2773
		0	+.06	+.06	-.02	+.02	----	0
6		---	25A	25Y	50Y	50A	---	0
		----	3049	3110	2901	3000	----	2847
		----	-.02	-.04	-.06	-.01	----	-.06
7		---	50A	50Y	25Y	---	---	50A
		----	3171	3068	3096	----	----	2800
		----	+.10	+.02	+.02	----	----	+.01

(Footnotes, Table 1)

[a]Creatinine in urine, average last day of each period studied, mg/kg body weight on those days.

[b]Codes 0, 25, and 50 = g protein in daily diet; sources C = casein, A - algae (*Chlorella sorokiniana*), Y = yeast (*Torulopsis utilis*). The casein contained 8 g RNA per 100 g protein.

[c]kg/day, mean for 9 days.

[d]Subject had 25 g protein diet in period 3, but from an unrelated protein source.

Table 2. Composition of yeast and algae.

	Chlorella sorokiniana	Torulopsis utilis	Casein
g/100 g			
Nitrogen	11.35	8.01	15.50
Nucleic Acid	5.6	11.4	[a]
mg/g			
Calcium	1.19	2.45	0.78
Magnesium	0.78	1.49	0.03
Sodium	0.11	0.13	0.88
Potassium	0.12	20.0	2.12
Phosphorus	7.4	16.3	6.15
Chloride	0.031	0.26	2.64

[a]None present; for diets pure yeast RNA was added, 8 g/100 g protein.

Table 3. Fluid intake and output, urinary pH and concentration, fecal weight, and intestinal gas production of men fed algae, yeast, and casein.

		Casein + RNA		Chlorella sorokiniana		Torulopsis utilis	
	Prot.-free	25 Prot.	50 Prot.	25 Prot.	50 Prot.	25 Prot.	50 Prot.
Fluid Intake, ml/day	3332±125[a]	3366±91	3405±80	3515±304	3529±172	3641±403	3728±515
Urine volume, g/day	2097±504	1824±578	1933±283	2017±576	1938±564	2155±443	2188±404
Urine pH	7.0±0.2	7.0±0.2	6.8±0.2	7.0±0.2	6.9±0.4	6.6±0.2	6.6±0.3
Urine Osmolality, mOsm/l	226±49	282±75	297±50	241±76	257±65	207±50	248±47
Feces Weight, g/day							
Wet	142±65	125±86	114±61	151±48	202±86	206±95	195±104
Dry	26±6.1	28±7.3	25±2.4	31±5.4	44±7.2	40±9.6	39±3.9
Flatus Volume, ml/12 hr[b]	393±69	374±46	252±36	479±11	446±95	401±54	310±39
Breath Hydrogen, ppm							
at 0730	2-17[c]	3-15	4-9	3-4	2-11	5-13	2-10
Maximum[b]	4-38	6-39	5-20	5-30	4-13	5-22	3-19
Breath Methane, ppm[d]							
at 0730	3-4	3-5	3-5	2-3	2-3	3	3

(Footnotes, Table 3)

[a]Mean and standard deviation.

[b]Gas egested from the rectum 1030-2230 hrs. Maximum breath H_2 is average of 3 highest values recorded during this period.

[c]Range of individual values.

[d]Subject 1303 omitted. His data show marked period effects, from initial 32 ppm to 17 ppm by period 3 and 3 ppm by period 5 and after.

Table 4. Mineral balances of men fed algae, yeast, and casein.

		Casein + RNA		*Chlorella sorokiniana*		*Torulopsis utilis*	
	Prot.-free	25 Prot.	50 Prot.	25 Prot.	50 Prot.	25 Prot.	50 Prot.
g/day							
Sodium							
Intake	2.90	2.57	2.52	3.22	3.13	3.41±.27	3.47±.24
Balance	-.05±.28[a]	.07±.29	.07±.28	.31±.37	.52±.24	.24±.45	.31±.40
Potassium							
Intake	3.43	3.28	3.28	3.52	3.34	1.86	2.46
Balance	-.32±.27	.25±.81	.30±.41	.19±.34	.38±.23	.42±.23	.47±.20
Calcium							
Intake	0.80	0.75	0.77	0.79	0.87	0.78	0.88
Balance	-.11±.16	-.14±.25	-.04±.12	.00±.13	-.07±.14	-.34±.19	-.24±.16
Magnesium							
Intake	0.52	0.44	0.48	0.60	0.64	0.59	0.61
Balance	-.04±.10	-.09±.15	-.03±.06	.08±.10	.07±.07	-.02±.10	-.06±.07
Phosphorus							
Intake	1.57	1.60	1.67	1.47	1.40	1.41	2.00
Balance	.12±.29	.27±.18	.25±.10	.16±.16	.30±.09	-.10±.27	-.07±.24

(Footnote, Table 4)

[a]Mean and standard deviation. A single entry for dietary intakes indicates that there was no known deviation.

Table 5. Utilization of algae, yeast, and casein nitrogen at two levels of dietary intake.

Dietary Protein	Nitrogen, g/day				Nitrogen True Digestibility[b]	Biological Value[c]
	Intake	Fecal	Urinary	Balance[a]		
					%	%
Prot.-free	1.08	1.02±.16	2.52±.26	-2.61±.36	------	------
Casein + RNA	4.25	1.13±.31	3.97±.40	-1.00±.49	95±8	66±4
	7.84	1.08±.11	6.27±.85	+0.33±.88	99±1	52±6
Chlorella sorokiniana	4.83	1.58±.15	3.32±.30	-0.28±.41	89±4	79±12
	7.81	2.61±.52	5.00±.45	+0.05±.83	82±6	60±6
Torulopsis utilis	4.51	1.79±.42	3.75±.13	-1.23±.36	83±7	70±5
	8.20	2.04±.28	5.62±.16	.39±.28	87±3	58±6

[a]Includes a correction for blood samples taken but no correction for sweat and integumental losses. Balance = Intake - (Fecal + Urinary + Blood). True balance is probably underestimated by 0.2-0.5 g/day if all routes of loss were accounted.

[b] $$\frac{\text{N Intake} - (\text{Fecal N} - \text{Fecal N at 0 protein intake})}{\text{N Intake}} \times 100$$

[c] $$\frac{\text{Absorbed N} - (\text{Urinary N} - \text{Urinary N at 0 protein intake})}{\text{Absorbed N}} \times 100$$

Table 6. Urinary and plasma uric acid levels of men fed nucleic acid with casein and as algae and yeast.

Diet	Nucleic Acid Intake, mg/day	Uric Acid, mg Urine per day	Uric Acid, mg Plasma per 100 ml
Prot.-free	0	394±50[a]	5.4±1.0
Casein + RNA			
25 g prot.	1.8	562±47	6.9±1.0
50 g prot.	3.7	886±77	8.7±0.9
Chlorella sorokiniana			
25 g prot.	1.7	605±122	7.4±1.1
50 g prot.	3.6	872±209	9.7±1.1
Torulopsis utilis			
25 g prot.	5.0	942±65	10.2±1.7
50 g prot.	10.3	1536±65	12.6±2.0

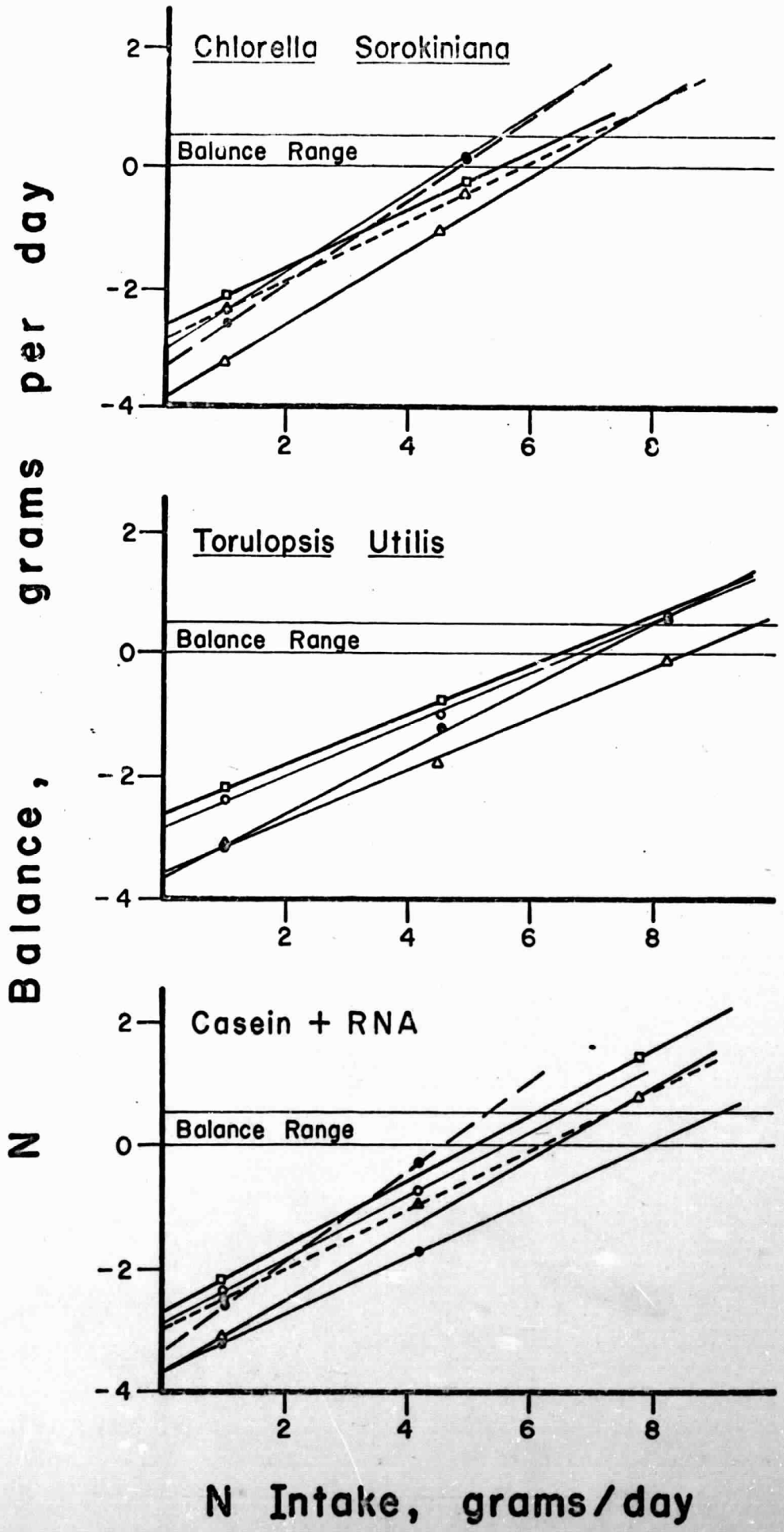
[a]Mean and standard deviation.

Table 7. Blood components of men fed algae, yeast, and casein.

		Casein + RNA		*Chlorella sorokiniana*		*Torulopsis utilis*	
	Prot.-free	25 Prot.	50 Prot.	25 Prot.	50 Prot.	25 Prot.	50 Prot.
Fluid Intake, ml/day	3332±125[a]	3366±91	3405±80	3515±304	3529±172	3641±403	3728±515
Urine volume, g/day	2097±504	1824±578	1933±283	2017±576	1938±564	2155±443	2188±404
Urine pH	7.0±0.2	7.0±0.2	6.8±0.2	7.0±0.2	6.9±0.4	6.6±0.2	6.6±0.3
Urine Osmolality, mOsm/l	226±49	282±75	297±50	241±76	257±65	207±50	248±47
Blood, mg/100ml							
Glucose	87±4[a]	90±6	93±4	89±4	89±4	88±7	89±5
Urea Nitrogen	5.1±.5	7.7±1.1	10.1±1.6	7.0±.3	9.3±1.1	6.4±.4	8.6±1.3
Total Nitrogen	31±1	31±1	32±2	31±2	31±1	31±2	30±1
Plasma, mg/100 ml							
Protein, total	6.4±1.0	6.4±.4	6.6±.4	6.9±.6	6.9±.6	6.2±.2	6.4±.4
Albumin	4.0±.5	4.0±.2	4.1±.2	4.2±.1	4.4±.3	4.0±.4	4.2±.4
Creatinine	1.4±.2	1.2±.1	1.3±.1	1.6±.5	1.4±.2	1.4±.2	1.3±.1
Cholesterol	160±24	166±33	165±18	154±28	155±33	154±14	163±20
Triglyceride	63±20	68±8	69±27	85±21	71±17	72±33	79±45

[a]Mean and standard deviation.

Fig. 1. Nitrogen balance as a function of nitrogen intake from casein + RNA, algae, and yeast. Regression equations are, where B = balance, g/day, and I = intake, g/day: casein + RNA $B = -3.25 \pm .55I$; algae $B = -3.2 \pm .60I$; and yeast, $B = -3.17 \pm .44I$. If $B = 0.5$, then casein $I = 6.8$, algae $I = 6.2$, and yeast $I = 8.34$.


Chlorella Sorokiniana
Balance Range
Torulopsis Utilis
Balance Range
Casein + RNA
Balance Range
N Balance, grams per day
N Intake, grams/day
2
0
-2
-4
2
4
6
8

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Brief Communication

Suppression of Uric Acid Formation from Dietary Nucleic Acid with Allopurinol

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Short Title: Dietary nucleic acid and allopurinol treatment

The high levels of serum and urinary uric acid found in gout and some other diseases can be reduced by allopurinol, a hypoxanthine analog, which inhibits xanthine oxidase (1). The block in uric acid formation causes increased excretion of hypoxanthine and xanthine, but, since allopurinol also has a feedback inhibitory action on the initial step of purine synthesis, the reduction in uric acid output exceeds the increased excretion of its precursors (2). Thus, allopurinol is used effectively to control uric acid levels in blood and urine in diseases where metabolic defects enhance endogenous purine synthesis.

Urinary and serum uric acid levels are elevated also by exogenous purines. These occur in small amounts in normal diets but are abundant in the "single-cell protein sources" (algae, bacteria, yeasts) being considered for expansion of world food resources and might constitute a bar to their use. We were led therefore to inquire if allopurinol would limit the formation of uric acid from dietary precursor ribonucleic acid.

EXPERIMENTAL

Six healthy men were confined to a metabolic unit and had participated in another study with controlled diet and activity for 2 months immediately prior to the present study. All men received in four equal meals a formula containing 8 g yeast ribonucleic acid (RNA)[3] and 50 g casein per day and nutritional supplements as reported previously (3). Four subjects received 100 mg allopurinol[4] daily on the days indicated in Table I and Fig. 1. Two men were controls and received RNA but no drug.

Daily 24-hour urine collections and fasting blood serum taken every third day were assayed for uric acid by the phosphotungstate reduction method using a Technicon Autoanalyzer. Urinary hypoxanthine + xanthine was assayed according to a revision of the enzymatic-spectrophotometric method for uric acid (4). After removal of uric acid with a cation exchange resin[5], the remaining oxypurines were converted to uric acid with xanthine oxidase.[6] Finally, the change in optical density at 292 mμ[7] following removal of uric acid with uricase[6] was a measure of the hypoxanthine + xanthine originally present expressed as 'uric acid equivalents.'

RESULTS AND DISCUSSION

In previous experiments in this laboratory[8], the 50-g casein diet without yeast RNA produced a urinary uric acid excretion of about 400 mg/day with serum uric acid in the 4 to 6 mg/100 ml range. As shown in Table I and Fig. 1, addition of 8 g yeast RNA daily to the purine-free diet gave 3- to 5-fold increases in urinary uric acid and 2- to 3-fold increases in serum uric acid over values obtained with a 50-g protein, purine-free diet. When 100 mg/day of allopurinol was given to men receiving 8 g yeast RNA, serum uric acid approached the high normal range, 6 to 8 mg/100 ml, and urinary excretion was reduced to 700 to 900 mg uric acid/24 hrs (Table I). During allopurinol treatment, urinary hypoxanthine + xanthine excretion was increased from 10 mg/24 hrs to about 500 mg/24 hrs. Fig. 1, in which daily urinary data are given for two subjects, shows an immediate rise in hypoxanthine + xanthine excretion and a slightly slower decline in uric acid excretion during continued administration of allopurinol. Fasting blood serum taken 2 days after initiating allopurinol treatment also indicated the suppression of uric acid formation. After discontinuing allopurinol, approximately 2 days were required for the drug effect to wear off completely, according to the urinary oxypurine data. Fasting serum uric acid levels also returned to pre-treatment levels in 2 days.

Total oxypurine excretion (the sum of actual uric acid and hypoxanthine + xanthine expressed as uric acid equivalents) by subjects 2 and 3 during the period of allopurinol treatment was 300 to 500 mg less than when the same subjects were untreated. This suggests that even in the presence of a large exogenous purine load allopurinol suppresses uric acid formation

from purines synthesized endogenously. However, the possibility of increased excretion of undetermined purine degradation products cannot be excluded.

SUMMARY

In a controlled human metabolic study, addition of 8 g/day of yeast RNA to a low-protein, purine-free formula diet produced elevated serum and urinary uric acid levels which could be reduced by allopurinol, an inhibitor of uric acid formation. Administration of 100 mg/day of allopurinol also led to a rise in urinary hypoxanthine + xanthine which was less than the fall in urinary uric acid. Since total oxypurine excretion was reduced, it appears that allopurinol effectively suppresses both endogenous purine synthesis and synthesis of uric acid from exogenous purines.

(Footnotes)

1. From the Department of Nutritional Sciences, University of California, Berkeley, California 94720.
2. Research supported by NASA grant NsG 243, Suppl. 11, and NIH grant AM 10202.
3. Purchased from Calbiochem, Los Angeles, California.
4. 'Zyloprim' brand allopurinol (4-hydroxypyrazolo (3,4-d) pyrimidine), Burroughs Wellcome and Co. (U.S.A.), Inc., Tuckahoe, New York.
5. AG 50 W X8 20-50 mesh, Bio-Rad, Richmond, California.
6. Purchased from Worthington Biochemicals, Freehold, New Jersey.
7. Spectrophotometer PMQII, Carl Zeiss, Germany.
8. Unpublished results.

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(Legend)

Fig. 1. Daily urinary oxypurine excretion for two subjects. Numbers above the bars refer to serum uric acid in mg/100 ml.

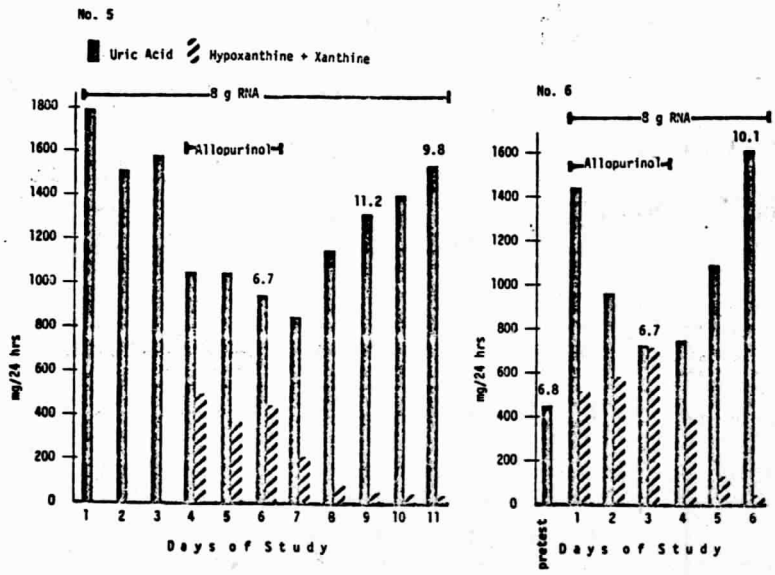

Table I

Effect of yeast RNA and allopurinol[a] on serum uric acid and urinary oxypurines in humans

Subject	Age	Weight	Day of Study[b]	RNA[c]	Allo-purinol	Serum Uric Acid	Urinary Oxypurines		
							Uric Acid	Hypoxanthine + Xanthine[d]	Total Uric Acid Equivalents[e]
		kg				mg/100 ml		mg/24 hrs	
2	22	79	pre-test	-	-	4.3	414	--	--
			1-3	+	-	12.0	1703	18	1721
			4-6	+	+	6.8	905	503	1408
3	23	68	pre-test	-	-	5.8	365	--	--
			1-3	+	-	13.8	1702	11	1713
			4-6	+	+	7.7	810	376	1188
1	28	90	--	--	--	--	--	--	--
			1-3	+	-	10.8	1244	10	1254
			4-6	+	-	9.0	1606	12	1618
4	30	69	pre-test	-	-	5.6	277	--	--
			1-3	+	-	11.8	1541	9	1550
			4-6	+	-	12.8	1399	10	1409

(Table I continued)

[a]'Zyloprim' brand allopurinol, 100 mg/day.

[b]Urine samples from days 1-3 and 4-6 were analyzed daily and reported as 3-day averages.

[c]Yeast ribonucleic acid, 8 g/day.

[d]Expressed as uric acid equivalents. Equivalents equal mg uric acid formed from total hypoxanthine and xanthine.

[e]Sum of uric acid and uric acid equivalents derived from hypoxanthine and xanthine.

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Human Intolerance to Bacteria as Food

DEMANDS for new means to solve man's nutritional needs when the pressure of population intensifies or when he attempts to travel in space have suggested the use of single cell organisms as food. *Hydrogenomonas eutropha*, a hydrogen-fixing bacterium, could effectively control the atmosphere of a space cabin as well as providing nourishment[1]. The protein is of high biological value[2] and is well tolerated even in high concentrations in rat diets[3]. Human feeding trials seemed warranted and were attempted.

At the Battelle Memorial Institute, several lots of bacterial cells were collected from a semi-continuous culture, washed free of medium[4] by repeated centrifugation and resuspension, and frozen for shipment to this laboratory. Removal of medium was verified by examination of the supernatant for non-protein nitrogen before a slurry of the cells and distilled water was boiled. The boiled cells had a halogen-like taste which was removed by successive treatment in the cold (4° C) with cationic and anionic exchange resins ('Dowex 50-4*X*' and 'Dowex 21*K*'). The bacterial cells were then filtered through nylon cloth and lyophilized.

Preliminary tests included feeding this lot of bacteria to albino mice at twice the maximum anticipated intake for humans (60 per cent of mouse diet). As in earlier, more extensive rodent experiments with other lots of *H. eutropha* (refs. 2 and 3 and our unpublished results), the preparation did not produce any adverse effects. The mice continued to gain weight over a period of 3 weeks, at a slower rate than the chow-fed animals but not below the usual rate of mice fed purified diets of this approximate composition.

Bacteriological examination of the unprocessed cells received revealed very minor contaminants: two *Streptococcus* spp., one of which was haemolytic, and a Gram-positive bacillus. No species of *Staphylococcus* or *Clostridia* were present. In the fully processed cell preparation only the Gram-positive bacillus remained viable, indicating that it was a spore-forming type.

Six adult male volunteers were confined in a metabolic ward. Four were fed 15 to 25 g of *H. eutropha* and two control subjects were given isonitrogenous amounts of casein. The bacterial cells and casein were served with specially processed low-protein wheat products in sauces which masked the identity of the test substances. The untoward symptoms that ensued are summarized (Table 1). Another volunteer (*G*), a laboratory worker,

Table 1. HUMAN RESPONSE TO INGESTION OF AUTOTROPHICALLY GROWN *Hydrogenomonas eutropha*

Subject	Time of feeding (h) (g dry *H. eutropha* fed) 0830	1230	1730	2130	Symptoms (time of first occurrence) Vertigo	Nausea	Vomiting	Diarrhoea	Stool weight (g/24 h)
A	8·6	17·2	—	—	None	1000	1300	1500	No record
B	8·6	8·6	—	—	0930	1000	1500	1315	921
C	—	—	9·6	5·1	1900	1930	None	2230	423
D	—	—	17·2	—	1930	2030	2100	Evening	1,265
E	—	—	—	—	None	None	None	None	0
F	—	—	—	—	None	None	None	None	277

was given two 6 g doses of bacteria with different foods. His reactions were identical to those of the confined subjects.

A second lot of *H. eutropha*, grown and collected as the first but which had no off-flavour, was washed, boiled and lyophilized before blind administration to two of six volunteers in the metabolic unit. Subject *H* was fed 12 g at 0830 and 6 g at 1230 h. At 1100 h he complained of abdominal discomfort and thereafter he had thirteen bowel movements weighing a total of 955 g. He also complained of headache, weakness and, later, of pain in the extremities. These symptoms persisted for 12 h. The second man (*D*) had been fed the first lot of bacteria 2 weeks earlier (Table 1). On the second occasion he did not become nauseated but complained of feeling less fit than usual during 5 days of feeding graduated doses (12, 18, 21·7, 21·7 and 21·7 g/day). He also passed large volumes of soft to liquid stools. His temperature, pulse and respiration rate were normal throughout this period, and blood samples taken on the last day of feeding showed normal indices of hepatic and renal function.

Another lot of *H. eutropha* and one of *Aerobacter aerogenes* were purchased from a commercial supplier (Grain Processing Company, Cedar Rapids, Iowa. The *A. aerogenes* was marketed as *E. coli*). Both species were grown on media containing sucrose and casein hydrolysate. The cells were washed, boiled and lyophilized before feeding to subjects in the metabolic unit. *Aerobacter* was pale grey in colour and became unpleasantly slimy on wetting, whereas *Hydrogenomonas* was the usual light tan colour and powdery or granular in texture. To mask the identity of treatments, all men were fed a starch based formula with a small amount of herbs added, with or without bacteria.

A summary of the feeding schedule and symptoms (Table 2) shows that response to this lot of *Hydrogenomonas* was less severe than previously. The *Aerobacter* produced the same symptoms as in the first tests of *H. eutropha* except that subject *J* also developed a rash on his arms and trunk the second day of feeding which disappeared when he returned to his normal diet. In all cases, blood pressure was unaffected and there was no

Table 2. HUMAN RESPONSE TO INGESTION OF HETEROTROPHICALLY GROWN *Hydrogenomonas eutropha* AND *Aerobacter aerogenes*

Subject	Day	*H. eutropha*, time of feeding (h) 0830 (g fed)	1230 (g fed)	1730 (g fed)	*A. aerogenes*, time of feeding (h) 0830 (g fed)	1230 (g fed)	1730 (g fed)	Symptoms (time of first occurrence) Asthenia	Nausea	Vomiting	Diarrhoea	Stool weight (g/24 h)
F	1	6	—	6	—	—	—	None	1000	None	None	77
	2	12	—	12	—	—	—	1000	1000	None	None	74
H	1	—	—	—	—	6	6	1230	1230	None	2100	251
	2	—	—	—	12	—	—	0900	0900	None	1800	206
I	1	6	—	6	—	—	—	None	None	None	None	88
	2	12	—	12	—	—	—	None	1630	2300	None	160
J	1	—	—	—	—	6	6	1230	1300	None	2200	213
	2	—	—	—	12	—	—	1030	1030	None	None	162
K	1	—	—	—	—	—	—	None	None	None	None	164
	2	—	—	—	—	—	—	None	None	None	None	183
L	1	—	—	—	—	—	—	None	None	None	None	112
	2	—	—	—	—	—	—	None	None	None	None	78

Table 3. SUMMARY OF ORAL DOSAGES OF *Hydrogenomas eutropha*

Species	No. of animals	Mode of introduction	Amount given in one day (g/kg body wt.)
Man	8	Mixed with diet	0·14–0·38
Chimpanzee	2	Mixed with diet	0·5
Chimpanzee	4	Gastric tube	0·5 and 1·0
Dog	2	Mixed with diet	0·5–0·7
Miniature swine	1	Mixed with diet	0·7
Monkey	2	Mixed with diet	0·8 and 1·1
Mouse	6	Gastric tube	2·5

elevation of body temperature. One day later concentrations of blood cells, glucose, serum uric acid and key enzymes were the same as values before the test. No blood was detected in stools at any time.

None of a variety of animal species tested has shown any evidence of gastrointestinal disturbance after peroral administration of *H. eutropha* at dosages far in excess of that which affected men (Table 3). *E. coli* is also well tolerated by rats[5] and chicks[6].

The bacterial cells had been washed free of medium before feeding, so the material responsible for gastrointestinal disturbances in man must be within or bound to the cell. The time at which symptoms occur suggests that digestion of the cell is necessary for release of the toxicant. Variation in response with different lots of *H. eutropha* may represent the development of tolerance to the organism with chronic or slow introduction, or it may simply reflect subject variation. It is also possible that with different growth conditions varying amounts of toxicant are produced.

This research was supported by NASA and NIH grants. Special protein-free wheat products used as a diet base were supplied by Diet-Erba Division of Carlo Erba, Milan, Italy. We thank the men who volunteered for these studies, the staff of our metabolic unit, and colleagues who participated in the animal tests: Drs B. F. Rahlmann, M. Redfearn, G. D. Smith, T. Butler, M. D. Fetters and D. T.-L. Lau, and Mr Delroy Brown.

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