Table 2. Effect of incubation on the state of cystine or cysteine in fractions from cystinotic leukocytes. Approximately 50 mg (wet weight) of leukocytes were lysed (as in Table 1) in a solution which was 0.25*M* in sucrose, 0.1*M* in tris buffer, *p*H 7.4, and $10^{-4}M$ in ethylenediaminetetraacetate. The fractions were prepared as described in Table 1, and samples were assayed for acid phosphatase. The remainder of the granular and supernatant fractions, to which no detergent had been added, were incubated in 4 ml of the buffer at 37°C for 30 minutes, and then an excess of NEM was added. The samples were then prepared for the amino acid analyzer (*8*). The smallest amount that could have been detected in the granular fraction is 0.1 μmole of cysteine per gram of protein. Acid phosphatase is expressed in micromoles of *p*-nitrophenol liberated per 30 minutes per milligram of protein; cystine is expressed in micromoles of ½ cystine per gram of protein; cysteine-NEM is expressed in micromoles per gram of protein. Numbers in parentheses represent percent of total.

Fraction	Acid phosphatase	Cystine	Cysteine-NEM	Total of cystine and cysteine-NEM (%)
Nuclear	1.7 (4.4)	14.0		12.2
Granular	8.5 (70.8)	22.3	< 0.1	62.7
Supernatant	1.0 (24.8)	0.53	2.59	25.1

phosphatase from lysosomes, we performed an experiment in which this compound was added to only a portion of each fraction. In the presence of this detergent, the cystine content increased from 33.5 to 56.6 μmole of 1/2 cystine per gram of protein in the granular fraction, but only from 2.10 to 2.25 μmole of 1/2 cystine per gram of protein in the supernatant fraction. When NEM was added after Triton X-100, no cysteine-NEM was found; hence, the new cystine uncovered had been in the oxidized state.

This evidence suggests that the cystine stores in these leukocytes are separated from the soluble portion of the cytoplasm by a lipid-containing structure. This would explain why therapeutic measures which lower the cystine concentration in the plasma fail to control this disease. Treatment with D-penicillamine for 3 years or a low cystine diet for over 1 year lowered the cystine concentration in the plasma of children with cystinosis by more than 50 percent but failed to alter either the appearance of crystalline deposits in bone marrow or the clinical course of the disease (*3*). In addition, the cystine content of leukocyte was not influenced by either form of therapy (*12*).

These data suggest, but do not prove, that the primary defect in cystinosis is an abnormal subcellular compartmentalization of cystine. Another explanation of these observations could be that microcrystals of cystine form in these leukocytes and are subsequently surrounded by a lipid membrane. More exact fractionations combined with electron microscopy in leukocytes and other tissues, as well as a better understanding of the fate of cystine in normal tissues, is necessary to confirm this hypothesis. The presence of significantly higher concentrations of cystine in leukocytes from the parents of these children is compatible with a recessive mode of inheritance of this disease, as suggested by family pedigrees (*1*), and may allow the detection of the heterozygous carriers.

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Growth of a Thermophilic Bacterium on Hydrocarbons: A New Source of Single-Cell Protein

Abstract. *A species of the genus* Bacillus, *capable of growth on normal alkanes at 70°C, was isolated by the method known as enrichment culture. Preliminary analyses of its amino acid composition indicate that it would be a good source of protein for human nutrition. Possible advantages of the use of such organisms for the production of single-cell protein include simplification of fermentor cooling and asepsis.*

Recent reports (*1*) have dealt with the possibility of producing single-cell protein (SCP) by culturing yeasts or bacteria on hydrocarbon substrates. One of the technological problems to be overcome, if such production is to be economic, is the relatively large amount of heat evolved per pound of cells produced—a consequence of the large amounts of oxygen required for oxidation of hydrocarbons. It has been estimated (*2*) that, with a hydrocarbon substrate, heat production per unit mass of cells is about double what would be evolved if a carbohydrate substrate were used. Heat of fermentation is normally removed by circulating water for cooling through coils or the fermentor jacket. For this method to be effective, the water must be substantially cooler than the operating temperature of the fermentor, and this gradient must be larger when the rate of heat evolution is greater. Previous proposals for the production of SCP by growth on hydrocarbons have described growth temperatures in the range of 25° to 37°C, which would require cooling water of a temperature lower than 30°C. However, in many parts of the world for many months of the year, water temperatures exceed 30°C, and even evaporative cooling towers are ineffective because of high atmospheric humidity. Therefore, mechanical refrigeration, with consequent higher operating costs, would have to be considered.

If thermophilic organisms could be obtained, the problem of fermentor cooling would be simplified, since a large gradient between the operating temperature of the fermentor and the temperature of the cooling water would be available. Unfortunately, reports of the growth of thermophiles on hydrocarbons are scanty. Allen (*3*) failed to obtain organisms from paraffin en-

richment cultures at 55° to 65°C. More recently, molds have been reported to grow on paraffin at 50°C (*4*), and there has been a brief mention of the growth of bacteria on normal paraffins at 45° to 50°C (*5*). It appeared possible that enrichment cultures would be successful, and these were attempted.

Samples of water or soil (about 0.1 g) were mixed with 50 ml of a mineral salts–hydrocarbon medium (*6*) and incubated without shaking at 57°C until turbidity developed. This usually took 4 to 6 days. Samples of the turbid medium were then streaked on agar of the same composition (*7*) and incubated at 57°C until colonies developed; typical colonies were selected and purified. An indication of the variety of environments that yielded organisms is given in Table 1.

A number of different isolates were examined for their morphological and biochemical characteristics, which seemed to indicate that the isolates were identical. The gram-positive bacterial rods formed terminal spores, hydrolyzed starch, were weakly proteolytic, reduced nitrate to nitrite, failed to produce indole from tryptone, and grew on normal alkanes ranging from C_{12} to C_{20}, as well as on glucose, sucrose, lactose, citrate, arabinose, and glycerol. They failed to produce acetylmethylcarbinol. Studies conducted with the use of the basal medium with 2 percent sucrose indicated that the optimum temperature for growth was about 55° to 60°C (see Table 2). Preliminary observations indicate that the isolates fall within the *Bacillus stearothermophilus* group of Allen (*3*), and more detailed studies are in progress to confirm this identification.

To evaluate the suitability of these organisms as a source of SCP, one isolate was grown in shake-flasks with 0.5 percent hexadecane as the carbon source (Table 2). The growth rate was estimated by following the disappearance of ammonium nitrogen from the medium by means of a nesslerization procedure (*8*). At 65°C the doubling time was in the range of 2 to 4 hours with considerable variation from run to run. These variations are not uncommon in determinations of the growth rates of microorganisms on hydrocarbon substrates (*9*). Cells from the late log phase of growth at 65°C were collected by centrifugation and washed once with distilled water; the paste was freeze-dried. Nitrogen was determined by a Kjeldahl method (*10*); lysine and methionine, by microbiological assay of an acid hydrolyzate with the use of *Leuconostoc mesenteroides* (*11*); and total amino acids, by a column chromatographic technique (*12*). The results of these analyses are shown in Table 3; the protein contents (value for nitrogen × 6.25) for the different batches varied from 73 to 79 percent.

While the results presented here are preliminary, they clearly indicate that thermophilic organisms capable of growth on hydrocarbons can be obtained. The amino acid composition of their protein suggests that their nutritional value would be better than that of previously described sources of SCP. One possible advantage of the use of such organisms for the production of SCP is that problems of fermentor cooling are mitigated. A further advantage may be that the fermentation need not be carried out under aseptic conditions, since the number of organisms capable of growth, on hydrocarbons, above 60°C is probably restricted. Considerable work remains to be done before the utility of these organisms for the production of SCP is confirmed, but these organisms represent a group that is worthy of further investigation.

Table 1. Environments that yielded isolates of thermophilic bacteria.

Source	Temperature of source (°C)
Steam condensate	30
Asphalt emulsion	90
Waste refinery water	45
Oil-water mixture	8
Ditch water	22
M.I.T. lawn soil*	20

* Initially no turbidity developed. However, after 50 g of soil was mixed with 1 ml of kerosene and incubated for 1 month at 57°C, isolations were made by the standard technique (see text).

Table 2. Growth rates of a species of the genus *Bacillus* on sucrose medium. Specific growth rate refers to the slope of a semilogarithmic plot of growth at a given temperature.

Temperature (°C)	Specific growth rate (hr^{-1})
37	0.25
45	.49
55	1.16
60	1.14
65	1.06
70	0.85

Table 3. Amino acid composition of hydrocarbon-utilizing thermophile. Values for microbiological assays are means and standard deviations calculated for three separate batches of cells that were hydrolyzed and analyzed separately. Values for chromatographic determinations are means of two batches of cells.

Amino acid	Microbiological assay (grams per 16 g of N)	Chromatographic determination (grams per 100 g of amino acids)
Cysteine		0.5
Isoleucine		6.1
Leucine		8.9
Lysine	7.4 ± 1.0	6.9
Methionine	2.7 ± 0.2	2.7
Phenylalanine		5.6
Threonine		4.3
Tyrosine		4.2
Valine		6.7

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