Table 2. Cross adsorption of antiserums to whole cells and cell walls of *Bacillus megaterium*. Titers were determined by complement fixation.

Test antigens	Antiserum to whole cells adsorbed with			Antiserum to cell walls adsorbed with		
	Whole cells	Cell walls	Control	Whole cells	Cell walls	Control
Whole cells	< 20	< 20	20,480	< 20	< 20	2560
Cell walls	1280	< 20	2560	1280	< 20	5120
Protoplast membranes	1280	1280	1280	< 20	< 20	< 20

identify the structural locus of such surface antigens with the cell wall alone. It was observed that antiserum to protoplasts or protoplast membranes did not react significantly with intact cells or cell-wall antigens, but that antiserum to cell walls did react with whole-cell antigens. Moreover, intact cells adsorbed only a portion of the antibodies to cell walls (Table 2).

4) Antibody globulin does not penetrate the cell wall. The literature on immunology and data on the apparent impermeability of bacteria to globulin molecules (*11*) have implied that antibodies to bacterial cells react only with antigens on the exterior of the intact cell and do not penetrate to deeper lying structures. However, experiments with fixed animal cells and tagged antibodies have indicated that penetration of antibody molecules into such cells may occur (*12*). In our experiment, antiserum to protoplasts or protoplast membranes in reactions with whole-cell antigens fixed complement to only a minimal degree (Table 1), suggesting that the cell wall is impermeable to antibody (if the assumption is valid that complement is at least as capable of penetration as antibody). The parallel agglutination tests appear to be inapplicable because of the necessity of spatial proximity for bonding of the reactants; this may not be realized because of the thickness of the cell walls. The conclusion is further substantiated by the observation that adsorption of whole-cell antiserum with whole cells or cell walls failed to change the titer against protoplast membranes, and that adsorption of cell-wall antiserum with whole cells removed only a portion of the antibodies to cell walls (Table 2).

Similarly, it was observed that lipase, although lytic to protoplasts or protoplast membranes, had no apparent effect on whole cells or on protoplast formation from lipase-treated, washed cells. Thus, both antibody- and enzyme-protein molecules, each having a specific affinity for the protoplast membranes, apparently are unable to penetrate the cell wall of this bacterium (*13*).

JOHN W. VENNES
PHILIPP GERHARDT
Department of Bacteriology, University of Michigan Medical School, Ann Arbor

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Algae as Sources of Lysine and Threonine in Supplementing Wheat and Bread Diets

A shortage of protein in many parts of the world has stimulated studies of possible additional resources. Algae have received considerable attention because they can be grown with simple inorganic nutrients in mass culture, and many have a high nitrogen content (*1*). However, their nutritional value has received scant study. Combs (*2*) found that supplements of *Chlorella pyrenoidosa* improved growth and feed efficiency in chicks, an effect attributed to riboflavin, carotene, and perhaps other vitamins contributed by *Chlorella*. Henry (*3*) fed *Chlorella* to young rats and reported a protein efficiency ratio somewhat superior to peanut meal, about equal to brewers' yeast, but considerably inferior to dried skim milk. Bender *et al.* (*4*) found a variable but generally low protein value for several marine algae in rat studies.

Published values on the amino-acid composition of algae indicate a surprisingly good spectrum of essential amino acids, except perhaps for cystine-methionine (*1, 2, 5–7*). The threonine and lysine content of *Chlorella* is about the same as that of hen eggs (*6*). These facts prompted us to test algae as supplements in wheat diets, for lysine, threonine, and valine have been shown to improve wheat flour (*8*), and lysine (*9*) or lysine and threonine improve the nutritional value of enriched white bread.

In the first experiment, a green algae *Scenedesmus obliquus* WH-50 was mass cultured as described elsewhere (*10*), harvested, dried, ground to a powder and fed to weanling rats as indicated in Table 1. The results show that *Scenedesmus* improved growth significantly with both flour and bread diets. These effects can be attributed almost certainly to amino-acid supplementation. The flour diets were adequately fortified with all known required minerals and vitamins except vitamin B_{12}, which had no growth-promoting effect in parallel experiments. Although the bread diet was not so fortified, other experiments showed that vitamin and mineral supplements produced only a slight growth response with such diets. It is also clear that *Scenedesmus* was an excellent source of threonine (group 4 versus groups 7 and 3). A comparison of group 2 versus groups 1 and 3 suggests that *Scenedesmus* was contributing considerable lysine. However, a comparison of group 6 with groups 7, 2, and 3 indicates that the lysine content of *Scenedesmus* was not sufficient for a complete growth response. This may be due to the fact that lysine probably is more deficient than threonine in these diets. The supply of *Scenedesmus* was insufficient to test it at levels higher than 4 percent. The nitrogen content of this lot of *Scenedesmus* was not determined but was assumed to be equivalent to 50 percent protein, based on experience with other lots that were cultured under the same conditions (*11*). The growth effects produced by *Scenedesmus* cannot be explained as due simply to the addition of a source of amino nitrogen, for group 5 showed no growth response. Furthermore, in parallel experiments, supplementation with amino acids other than lysine and threonine did not produce a growth response with these diets.

Another green algae, *Chlorella pyrenoidosa*, which was cultured and prepared in the same way as *Scenedesmus*, and a different strain of weanling rat (Osborne and Mendel) were used in a second series of experiments. The nitrogen content of two different lots of *Chlorella* was 7.38 and 7.92 percent (dry basis). One test (five rats per group and

19 days of growth) was conducted, using the flour diet (Table 1) and increasing supplements of *Chlorella* to obtain some estimate of effective amounts. *Chlorella* at levels of 1, 2, 4, and 6 percent produced growth 1.3, 2.1, 2.3, and 3.8 times greater than that of animals receiving no *Chlorella*. An additional group in this series received the flour diet plus lysine and threonine and attained a weight 5.5 times greater than the groups that received no algae or amino acid. This suggests that *Chlorella* at the 6-percent level did not fully provide the lysine and threonine that was required under these conditions. A limited supply prevented the testing of higher concentrations of *Chlorella*. A similar experiment, in which all the flour diets were supplemented with lysine, also showed increasing growth with increasing levels of *Chlorella*. However the diets with 4- and 6-percent levels of *Chlorella* produced almost the same growth, the difference not being significant statistically. This result suggests that 4-percent *Chlorella* supplied about as much threonine as the rat could use under these conditions (with lysine added).

Lysine can be produced synthetically at modest cost. A lysine-enriched bread is being marketed experimentally. Threonine, however, remains very expensive. Therefore, additional experiments were conducted to evaluate *Chlorella* as a source of threonine in supplementing enriched white bread, and to compare algae with other sources of protein in this respect. These diets were supplemented with vitamins, minerals, fat, and lysine (except for one control group) to remove possible limitations on growth other than threonine. The first four groups in Table 2 indicate that *Chlorella* is an effective source of threonine in supplementing enriched bread, as judged by both growth and food efficiency. The experiment represented by the last seven groups was conducted at a different time than that represented by the preceding groups. The results are comparable, however, as indicated by the similar growth of the group in each series (96 and 94 g) that received a lysine supplement. The results indicate that *Chlorella* is a better source of threonine than purified soya protein and is equal to several animal-protein foods of high biological value when used as food supplements isonitrogenous to *Chlorella*.

These data indicate that algae protein may have considerable potential application as a source of amino acids that are generally low in cereals.

Table 1. Effect of *Scenedesmus obliquus*, lysine, and threonine in white flour or bread diets. Each group consisted of 9 or 10 National Institutes of Health black male rats. Each rat was housed separately in a raised-bottom, wire-mesh cage. Diets were fed *ad libitum*. The flour diet consisted of commercial unenriched white wheat flour (92 g), starch (1 g), cottonseed oil (3 g), and HMW salts (4 g) (*12*). Each gram of starch contained added thiamine (0.2 mg), riboflavin (0.3 mg), pyridoxine (0.25 mg), pantothenate (2 mg), niacin (2 mg), folic acid (0.1 mg), biotin (0.01 mg), 2-methyl naphthoquinone (0.1 mg), inositol (10 mg), and choline chloride (100 mg). The 3 g of cottonseed oil contained added vitamins A and D (550 and 110 U.S.P. units, respectively) and α-tocopherol (5 mg). The bread diet consisted of commercial white enriched bread (3 percent dry skim milk solids) that was air dried and ground to a powder. No other vitamins, minerals, or fat were added.

Group	Diet	Weight gain in 27 days (g/rat)*
1	Flour	6.4 ± 0.6
2	Flour with 4% *Scenedesmus*†	20.3 ± 1.2
3	Flour with 0.75% lysine · HCl	25.0 ± 1.2
4	Flour with 0.75% lysine and 4% *Scenedesmus*†	53.2 ± 3.1
5	Flour with 1.2% DL-threonine	7.3 ± 1.0
6	Flour with 1.2% DL-threonine and 4% *Scenedesmus*†	18.6 ± 1.8
7	Flour with 1.2% DL-threonine and 0. 75% lysine · HCl	46.5 ± 2.9
8	Bread	22.0 ± 1.2
9	Bread with 4% *Scenedesmus*†	40.8 ± 2.6

* Standard error = range divided by number of animals (*13*).
† The algae replaced an equal weight of flour or bread.

Table 2. *Chlorella pyrenoidosa*, lysine, threonine, and various proteins as supplements in enriched white-bread diets. The rats tested were Osborne-Mendel male weanling rats. Each group contained ten rats, except the group that received *Chlorella*, which contained 5 rats. The bread diet contained air-dried commercial white enriched bread (3 percent dry milk solids) (92 g), HMW salts (4 g) (*12*), starch (1 g), and cottonseed oil (3 g). The starch and cottonseed oil provided vitamins in the same amounts as those listed in Table 1. The amino-acid and protein supplements replaced an equal weight of bread in the diets.

Diet	Weight gain in 28 days (g/rat)	Food efficiency*
Bread	14.8 ± 1.6	11.7
Bread with 0.75% lysine · HCl	96.3 ± 10.0	3.2
Bread with 0.75% lysine · HCl and 4% *Chlorella*	133.0 ± 8.4	2.9
Bread with 0.75% lysine · HCl and 1.2% DL-threonine	139.3 ± 5.7	2.6
Bread with 0.75% lysine · HCl	94.0 ± 1.7	3.6
Bread with 0.75% lysine · HCl and 2.1% casein	116.1 ± 4.0	3.1
Bread with 0.75% lysine · HCl and 2.1% soya protein	99.1 ± 5.9	3.5
Bread with 0.75% lysine · HCl and 2.9% dried liver	117.6 ± 4.3	3.2
Bread with 0.75% lysine · HCl and 5.4% dry skim milk	127.3 ± 3.8	3.0
Bread with 0.75% lysine · HCl and 4.9% whole dried egg	123.2 ± 8.4	2.9
Bread with 0.75% lysine · HCl and 1.2% DL-threonine	125.4 ± 4.2	3.0

* Food efficiency = grams of food eaten per gram of weight gained.

J. M. HUNDLEY
R. B. ING
Laboratory of Nutrition and Endocrinology, National Institutes of Health, Bethesda, Maryland
R. W. KRAUSS
Department of Botany, University of Maryland, College Park

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San Augustin Plains—Pleistocene Climatic Changes

The sediments of the former Lake San Augustin, Catron County, western New Mexico, afford a long and apparently continuous record of vegetation and climate (*1*). These deposits form the San Augustin Plains below the well-known Bat Cave, within a closed basin some 7000 feet above sea level and in a region of definite though irregular altitudinal belts of vegetation. The flora of this basin is now alkaline semidesert, with chenopods (*Atriplex* and *Sarcoba-*