the courses; others never finish the dissertations; and most take much longer than the program is planned to require. Apparently the transition from the rigidity of a set lecture program to the flexibility and freedom of independent scholarship and research is difficult.

In the Soviet Union scientists and professors have both high status and high relative income. Based on a tourist rate of exchange of 10 rubles to the dollar, professors who are department heads receive $7,000 per year. Farmers on the collective farm I visted near Rostov received last year $700, or just one-tenth as much. Half of the farmer's income was paid in grain, butter, or hay for his own use. Young workers in a shoe factory I visited in Kiev received $840 a year for a 46-hour week. Some laborers are reported to receive as little as $350 a year. Relative to other individuals in Soviet society, the professor and the scientist occupy an extremely favorable position.

Summary.—The evidence seems clear that the Soviet Union has succeeded admirably in training and productively utilizing a very large number of scientists, that it has been able to achieve high levels of scientific effort in many fields, and that it has been able strongly to motivate scientists by a system of high financial rewards, high social status, and appeals to patriotism and social responsibility as well as to scientific curiosity.

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*METABOLIC ACTIVITIES OF SUBCELLULAR PARTICLES FROM AZOTOBACTER VINELANDII**

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The subcellular fraction of bacteria referred to as "protoplasts" retains a large amount of the activity of the cell.† For nitrogen-fixing species, the ability to incorporate nitrogen by this fraction would seem to be a prerequisite for its appearance in further purified fractions. Merely diluting such protoplasts yields a par-

ticulate preparation which may answer the need stated by Magee and Burris:[1] "A more gentle (and yet complete) method for cell rupture would be highly desirable, and it would be valuable to test cellular fragments more extensively for their ability to fix nitrogen, as the enzyme system may be closely associated with the cellular membrane..."

The object of this study is to investigate the enzymatic properties associated with nitrogen fixation of protoplasts prepared from *Azotobacter vinelandii* (*Azotobacter agile* var. *vinelandii*). One of the difficulties foreseen was that the reagents used for preparation of the protoplasts, especially the chelating agents such as Versene (sodium ethylenedinitrilotetraacetate), might inhibit fixation through binding of ions essential for fixation (calcium, iron, molybdenum). In an attempt to circumvent such inhibition two variations in the technique were introduced: (1) the substitution of other metal binding agents; (2) use of penicillin.

Methods.—Culture of Microorganisms: *Azotobacter vinelandii* strain O was grown as described by Esposito and Wilson.[2] Cells intended for protoplast preparations were incubated at 30°C until they had reached the early logarithmic phase of growth, usually 14–16 hr. Such a culture had reached a turbidity of 125–145 Klett-Sumerson units (600 mμ) at this age. Suspensions diluted in Burk's medium, 2 per cent sucrose, were counted in a Spencer Bright Line Improved Neubauer counting chamber as described by Wilson and Knight.[3] Viable counts were made after 48 hr with the aid of a Quebec counter. All suspensions were diluted in Burk's medium, 2 per cent sucrose. Spread plates were made on the same medium and, at times, on agar containing 0.5 per cent sucrose and 0.5 per cent yeast extract.

Preparation of Protoplasts: Protoplasts were prepared by procedures based on the techniques described by Lederberg,[4] Repaske,[5] and Rotman.[6] Since most of the research reported in this paper is based on the protoplasts made by variations in the method of Repaske, details of only these will be supplied. Cells were in the early logarithmic phase of growth when harvested. The incubation mixture consisted of: 2 ml of tris (hydroxymethyl) aminomethane buffer containing sucrose, 1 ml of sodium (diethylenedinitrilo) tetraacetate (Versene), 2 ml of lysozyme, water washed cells and deionized water to a total volume of 6 ml. Such mixtures had an initial turbidity of 300–310 Klett-Sumerson units and a pH of 7.8. The final concentrations per milliliter were: 33 μM tris, 1.4 μM Versene, 13.3 μg lysozyme, 200–250 μg cell nitrogen, 0.06 mM sucrose. Under these conditions, the rods changed to osmotically sensitive spheres in 15 to 30 minutes at room temperature. Five-tenths ml of 1.2 M magnesium sulfate (92 μM/ml) was added when microscopic examination indicated that the reaction was complete. After five minutes further incubation, the protoplasts were centrifuged at 400 × G and washed in sucrose or lactose solutions buffered at pH 7. For fixation experiments, protoplasts were resuspended in Burk's nitrogen-free medium in 0.06 M sucrose. Lysozyme and versene were omitted consecutively from the incubation mixture to establish the effect of these agents. In some experiments potassium citrate replaced versene.

Experimental Results.—Hydrogenase: Table 1 lists the distribution of hydrogenase in whole cells, protoplasts, protoplast membranes, and soluble protoplast fractions. When protoplast membranes are broken with sonic oscillation, most of the activity is found in the fraction sedimenting after 4 hr at 29,000 × G. Apparently,

TABLE 1

DISTRIBUTION of HYDROGENASE in *Azotobacter vinelandii* O

Fraction	$Q_{H_2}(N)$*
Cells	8,450
Protoplasts	3,280
Lysed protoplasts	1,300, 1,765
Protoplast membrane	600
Protoplast soluble fraction	0

* $Q_{H_2}(N)$ = μL H_2 taken up/hr/mg N. Hydrogenase activity was determined manometrically using an atmosphere of 100% H_2. Each Warburg vessel held: (*a*) 300 μM of potassium phosphate, pH 8; (*b*) 25 μM methylene blue chloride; (*c*) about 50 μg cell nitrogen, and (*d*) water for a total of 3.2 ml. In all manometric trials (hydrogenase and respiration) the protoplasts were stabilized in 5 per cent lactose–2 μM $MgSO_4$.

hydrogenase in the azotobacter is a particulate system localized exclusively in the membrane.[7]

Oxidative Properties: Typical results of experiments in which the ability of the protoplasts prepared by the lysozyme-Versene technique to oxidize various substrates are shown in Figures 1 and 2. In general the observed rate was not as high as that of intact cells; lysis of the protoplasts lowers the activity further. Versene, citrate, and Amberlite M-B-3 resin have been used as the metal binding agents, but the most satisfactory results were obtained with the lysozyme-Versene preparations. With citrate as a metal binding agent, high endogenous oxidation may result in protoplast preparations; hence, this method is not satisfactory for respiration experiments. The cause of the high endogenous value is undoubtedly oxidation of the citrate reagent which depends upon the magnesium to citrate ratio.[8]

Nitrogen Fixation: Table 2 summarizes the results from numerous experiments on uptake of N_2^{15} by protoplasts of *Azotobacter vinelandii* O. Early in the investigations, 4 hr exposure to N_2^{15} was used, but as this time accentuates the effect of

TABLE 2

N_2^{15} UPTAKE IN ATOM PER CENT EXCESS BY PROTOPLASTS OF *Azotobacter vinelandii* O

A. Lysozyme sensitivity induced by versene

0.050	0.020	0.003	0.446	0.105	0.009
0.000	0.002	0.016	0.464	0.147	0.294
0.031	0.018	0.091	0.089	0.247	0.046
0.037	0.073	0.015	0.020	0.345	0.042
0.013	0.016	0.018	0.092	0.515	
0.032	0.006	0.010			

B. Lysozyme sensitivity induced by Amberlite M-B-3

Protoplasts	0.08, 0.001, 0.030, 0.011
Deionized cells	0.357, 0.028
Cells plus lysozyme only	0.124, 0.142

C. Lysozyme sensitivity induced by citrate

Protoplasts	0.033, 0.014
Citrate treated cells	0.064, 0.048
Whole cell control	0.293

D. Control Preparations

10^5 cells per ml (0.3 μg cell nitrogen per ml)	
Plus:	
180 μg serum albumin per ml	0.011, 0.006
500 μg serum albumin per ml	0.003, 0.000
washed protoplast suspension	0.020
Lysed protoplast preparation containing maximum of 10^5 viable cells per ml	0.000, 0.000

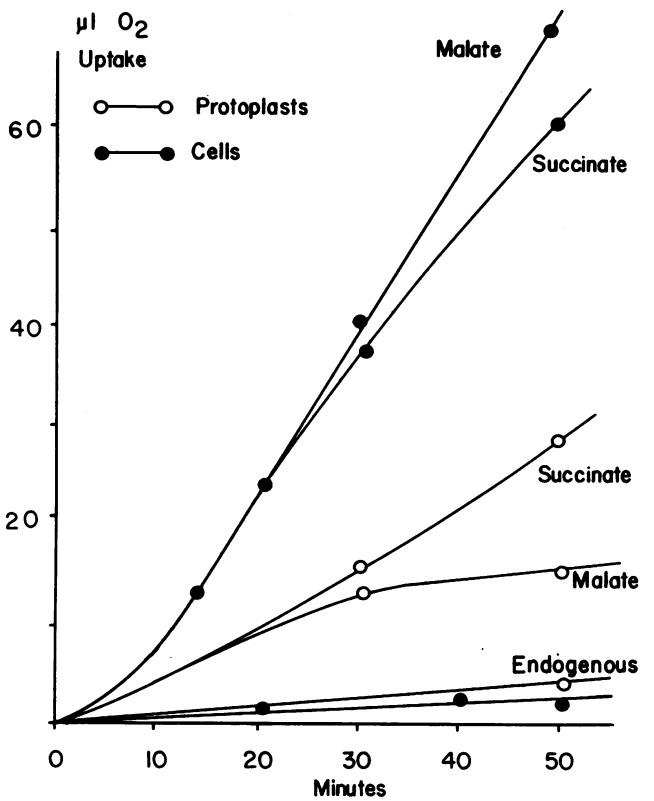

FIG. 1.—Comparison of oxidation of succinate and malate by whole cells and protoplasts of *Azotobacter vinelandii* O. Oxygen uptake was measured in an atmosphere of 50-50 mixture of helium and oxygen by standard manometric techniques. Each flask held: (*a*) 80 µM potassium phosphate pH 7; (*b*) 2 µM magnesium sulfate; (*c*) suspension equivalent to 0.1 mg of cell nitrogen; (*d*) 0.2 ml of 20 per cent KOH in the center well; (*e*) and 40 µM substrate in the side arm; (*f*) total volume, 3.2 ml. With succinate and malate, 1–2 µM of acetate "sparker" was included. To stabilize the protoplasts, lactose to a final concentration of 2.5 per cent was added with magnesium sulfate.

viable cells present, it was shortened to 1–2 hr. The level of whole cells in protoplast preparations is indicated by the viable count.

Although incorporation of N_2^{15} by protoplast preparations is definite, fixation is not as great as with intact cells. The reasons may be the presence of ammonia, action of versene, or high protein content (from lysed protoplasts). Ammonia rapidly and extensively inhibits nitrogen fixation;[9] however, quantitative analysis of a lysed protoplast preparation by the Conway diffusion technique showed ammonia to be absent. The data in Table 3 suggest that versene is responsible for at least part of the impaired fixation of protoplasts. Versene reduces viability of water washed cells below 1 per cent. The function of antibiotics as chelating agents has been discussed by Weinberg,[10] who reported Fe^{++} reversed inhibition of *Azotobacter vinelandii* by tetracycline. In our experiments, Fe^{++} (as Fe^{++} citrate complex) and ammonia enabled growth (measured turbidimetrically) of versene-treated

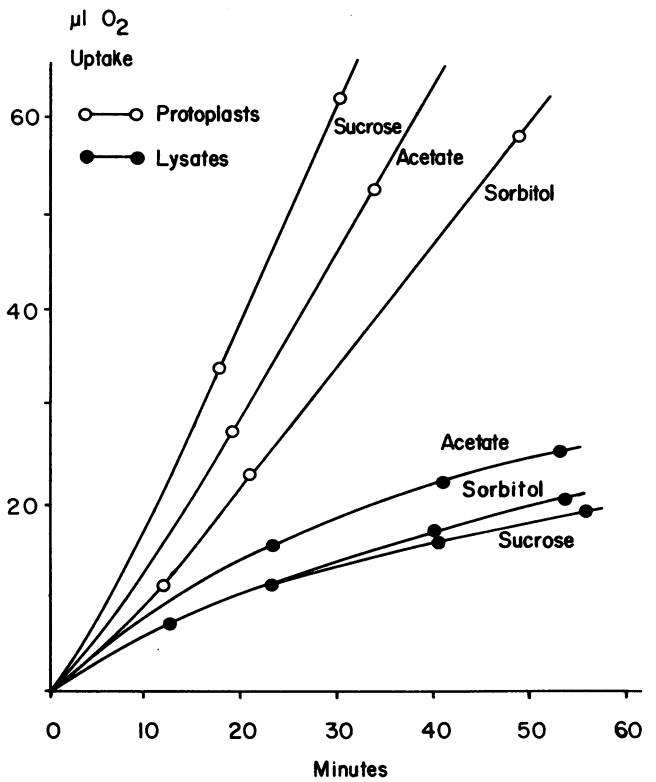

FIG. 2.—Effect of lysis on oxidative properties of protoplasts of *Azotobacter vinelandii* O. Same conditions as in Fig. 1.

cells to resume for about 4 hr. Versene-treated cells assimilated N_2^{15} comparable with that by protoplasts. Cells with versene omitted in the treatment mixture (i.e., exposed only to enzyme) were unaffected in N_2^{15} uptake.

Because ferrous ion partially reversed versene inhibition of cells, its action on N_2^{15} uptake was observed. On both versene-treated cells and protoplasts, Fe^{++} is inhibitory at a level of 50 µg per ml. Atom per cent excess values of 0.01, 0.013 (treated cells) and 0.45, 0.092 (protoplasts) were obtained. A protoplast control showed 0.400 atom per cent excess. The growth constant of whole cells, however, is little changed by 50 µg per ml Fe (as citrate complex).

The high protein content owing to lysed protoplasts is probably not inhibitory to fixation. Nason, Takahashi, Hoch, and Burris[11] state that the 15–40 per cent whole

TABLE 3

THE EFFECT OF REAGENTS USED TO PRODUCE THE PROTOPLAST ON N_2^{15} UPTAKE

	Fraction	µg N/ml	Total Count	Viable Count	Atom % Excess
I.	Protoplasts	418	10×10^8	9×10^4	0.446, 0.464
	Cells, versene treated	405	6×10^8	6×10^6	0.400
	Cells, lysozyme treated	400	...	...	0.530, 0.680
II.	Cells	208	16×10^8	5×10^8	0.949
	Cells, lysozyme treated	200	8×10^8	4×10^8	0.950

Protoplasts were prepared by use of the versene-lysozyme method. In the experiments reported in Tables 2 and 3, uptake of N_2^{15} was determined by the conventional methods on suspensions that were incubated under a gas mixture of: 0.1–0.2 atom N_2 (32 atom per cent N_2^{15}), 0.4 atom O_2, and 0.5–0.4 atom He.

cells remaining in a sonically disintegrated preparation fix with twice the efficiency of comparable whole cell controls.

The crucial question, of course, is not the magnitude of the observed fixation, but is it real or only an experimental artifact arising from the whole cells remaining in the preparation. Routine counting established that intact cells ranged between 10^4–10^5/ml. Controls using 10^5 cells/ml took up no detectable N_2^{15} during a 2-hr exposure. It is recognized, however, that such controls do not accurately reproduce the conditions in the protoplasts preparations. In an effort to simulate these conditions, serum albumin was added to such controls (to correspond to protein in the protoplasts) as well as lysed protoplasts. As is evident from the data in Table 2D, none of these additions induced whole cells at a concentration of 10^5/ml to take up appreciable quantities of N_2^{15} during a 2-hr exposure.

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† Subcellular spherical particles prepared as described in this report have been termed "protoplasts" by most investigators. For the reasons cited by a group of workers in this field in a recent note (*Nature*, **181**, 1713–1714, 1958) this may be a misnomer—such particles often are not analogous to the protoplasts (as originally defined by Weibull) in that the entire cell wall may not be removed by the treatment. No simple replacing term, however, was proposed; one might note this limitation by placing quotation marks around the term, but this device becomes awkward when constantly repeated. This footnote acknowledges that this qualification is understood and accepted even though the term is retained in the text.

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DIHYDROTHIOCTYL DEHYDROGENASE—A FLAVOPROTEIN

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The α-ketoglutaric (KG) dehydrogenase purified from hog heart mitochondrial preparations is a multifunctional enzyme complex of molecular weight 2×10^6 and diameter 120 Å. It contains 8 to 10 per cent lipide, and two tightly bound nondialyzable cofactors, diphosphothiamine (DPT) and thioctic (lipoic) acid.[1] Two other coenzymes, diphosphopyridine nucleotide (DPN) and coenzyme A (CoASH), participate in the physiological oxidation as represented in equation