PROTEIN BIOSYNTHESIS BY A CELL-FREE BACTERIAL SYSTEM

By

MIRKO BELJANSKI AND SEVERO OCHOA

Department of Biochemistry, New York University College of Medicine, New York, New York

Reprinted from the Proceedings of the National Academy of Sciences
Vol. 44, No. 6, pp. 494–501. June, 1958.

Reprinted from the Proceedings of the NATIONAL ACADEMY OF SCIENCES,
Vol. 44, No. 6, pp. 494–501. June, 1958.

*PROTEIN BIOSYNTHESIS BY A CELL-FREE BACTERIAL SYSTEM**

BY MIRKO BELJANSKI† AND SEVERO OCHOA

DEPARTMENT OF BIOCHEMISTRY, NEW YORK UNIVERSITY COLLEGE OF MEDICINE, NEW YORK, NEW YORK

Read before the Academy, April 28, 1958

INTRODUCTION

The mechanism of protein biosynthesis has been extensively investigated during the last few years. Work by Zamecnik and his collaborators[1] has led to the view that, in animal tissues, enzymes present in the cytoplasmic fluid[2] catalyze a carboxyl activation of the amino acids by ATP,[3] followed by transfer of the amino acid residues to RNA and their incorporation into the protein of microsomal ribonucleoprotein particles. This view has been strengthened by recent work from various laboratories.[4,5,6] Amino acid-activating enzymes have also been found in yeast,[7] bacterial,[8,9] and plant[10] cells. There appear to be as many activating enzymes as there are amino acids, and enzymes strictly specific for methionine[7] and tryptophan[11] have been isolated in highly purified form. In the presence of ATP, radioactive pyrophosphate (PP^{32}), Mg^{++}, and amino acid, the activating enzymes catalyze a rapid incorporation or exchange of PP^{32} into ATP, which affords a sensitive method of assay. The reaction has been formulated[2] as in equation (1):

$$\text{ATP} + \text{amino acid} + \text{enzyme} \rightleftharpoons \text{aminoacyl-AMP-enzyme} + \text{PP}. \qquad (1)$$

Because of their high rate of multiplication, bacterial cells should be particularly appropriate for studies of protein biosynthesis and have been used by several investigators.[12,13,14] Gale and Folkes[13] have described a cell-free system from *Staphylococcus aureus* which actively incorporates amino acids into protein and appears to bring about the synthesis of certain enzymes.

We have found, in agreement with Gale, that in *Alicaligenes faecalis* the amino acid–incorporating activity is mainly associated with a particulate fraction, consisting largely of cell membrane fragments, sedimented by centrifugation at relatively low speed (20,000 $\times g$) after disruption of the cells. The same is true of *Azotobacter vinelandii*.[15] It is the purpose of this paper to report on a hitherto unrecognized enzyme which stimulates the incorporation of amino acids into protein of the *Alcaligenes* particles. This enzyme, which is present in the supernatant extract, has been isolated in highly purified form. The purified enzyme does not catalyze the amino acid–dependent exchange of PP^{32} with ATP and this activity also appears to be absent from the particles.

METHODS

Preparation of Particles.—*A. faecalis* (8750) was grown as described by Pinchot[16] with vigorous shaking for 16 hours at 32°. The cells were harvested by centrifugation and washed twice at 4° with 0.2 *M* potassium chloride. Five to 6 gm. of cells, suspended in 25 ml. of 0.05 *M* Tris buffer, pH 7.0, were disintegrated for 4–8 minutes at 70 volts and about 7° in a 10-Kc Raytheon sonic oscillator. The resulting suspension was centrifuged for 10 minutes at 2000 $\times$ *g*, to remove intact cells and large cell debris, and the sediment discarded. After repeating this centri-

fugation once more, the supernatant fluid was centrifuged at top speed of the Servall, Model SS1 centrifuge in the cold room for 20 minutes, and the sediment suspended evenly in 15 ml. of ice-cold 0.05 *M* Tris buffer. This suspension was centrifuged once more for 3 minutes at 2000 × *g*, to insure removal of unbroken cells, and the supernatant fluid dialyzed for 1 hour at 4° against 500 ml. of Tris buffer. The particles were then collected by centrifugation in the Servall angle centrifuge as above and resuspended in Tris buffer, either without further treatment (unwashed particles) or after washing three times at room temperature, each with 5 ml. of 1.0 *M* sodium chloride and once with 5 ml. of water (washed particles), to give a suspension containing 12 mg. of particles (dry weight) per ml. Washing of the particles, whether preceded or not by ribonuclease, only reduced their nucleic acid content by 40–50 per cent. Electron micrographs showed that the particles consisted mainly of irregularly shaped cell-membrane, and probably wall, fragments of widely differing sizes. Particle preparations lose about half of their activity if kept overnight at 0°. For this reason, only freshly prepared particles were used in this work.

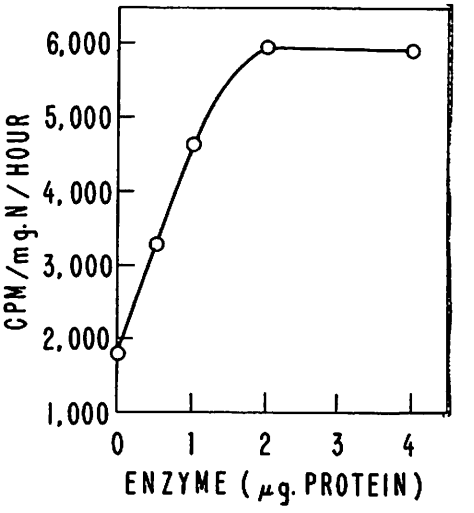

FIG. 1.—Stimulation of glycine incorporation into protein of *Alcaligenes* particles as a function of the concentration of amino acid incorporation enzyme. *Ordinate*, C^{14}-glycine incorporation in cpm per mg. protein nitrogen of particles per hour. The samples contained: Tris buffer, pH 7.0, 50 μmoles; $MgCl_2$, 5.0 μmoles; K_2HPO_4, 5.0 μmoles; C^{14}-glycine (specific radioactivity, 120,000 cpm per μmole), 0.25 μmole; washed particles with 300–400 μg. of protein; and amino acid incorporation enzyme, specific activity (glycine) 24, as indicated. Final volume, 1.0 ml. Incubation in air, 1 hour at 30°.

Measurement of Amino Acid Incorporation.—The composition of the reaction mixtures and incubation conditions were as described for Figure 1. The reaction was stopped by addition of 5 ml. of 10 per cent trichloroacetic acid. After cooling in ice for 10–15 minutes, the precipitate was centrifuged off, washed twice with 5 ml. of 10 per cent trichloroacetic acid, twice with hot (100°) 5 per cent trichloroacetic acid, each time for 15 minutes, and then with acetone. The precipitate was collected by filtration on a filter-paper disk and its radioactivity determined with a thin-window counter. Results are expressed as cpm, or millimicromoles of amino acid, incorporated, per milligram of protein nitrogen of particles (determined by the micro-Kjeldahl method) per hour.

PP^{32}-ATP Exchange Assay.—The assay conditions were as described for Table 2. The reaction was stopped with 2 ml. of 10 per cent trichloroacetic acid and the mixture centrifuged. The precipitate was washed once with 2 ml. of 10 per cent trichloroacetic acid and the supernatant fluid combined with the first. Absorption of the ATP on charcoal, washing, and counting were done essentially as described by DeMoss and Novelli.[8]

RESULTS

Amino Acid Incorporation into Protein of Particles.—All of the (D,L-, or L-) C^{14}-labeled amino acids so far investigated are incorporated to a greater or lesser extent into protein of *Alcaligenes* particles. Some unlabeled D-amino acids (D-threonine, D-leucine) did not depress the incorporation of their labeled L-enantiomorphs, suggesting that they were not utilized by this system, but others (D-glutamic acid, D-phenylalanine) caused a significant decrease. The incorporation is linear with time for acout 1 hour and continues at a decreased rate for another 2 or 3 hours.

There is no incorporation when the incubation is carried out in nitrogen, and that observed in air is inhibited by 2,4-dinitrophenol, an uncoupler of oxidative phosphorylation. Addition of ATP has little effect, presumably because of failure to reach the active sites. Thus, as in the case of isolated nuclei,[17] the system seems to depend on oxidative phosphorylation for generation of the ATP required for activation and subsequent incorporation into protein of the amino acids. It should be mentioned in this connection that the particles exhibit a small oxygen uptake, apparently at the expense of endogenous substrate.

As observed by Gale and Folkes[13] with *Staphyloccocus*, washing of the *Alcaligenes* particles with 1.0 *M* NaCl results in a more or less marked reduction of their amino acid–incorporating activity. Their activity can be increased to levels higher than observed with unwashed particles by addition of the supernatant fluid obtained after removing the particles by centrifugation of the bacterial extract. Most of the activity of the supernatant fluid was thermostable, but a small fraction was destroyed by heating and was presumably enzymatic in nature. A similar thermolabile activity was found in extracts of *A. vinelandii* and *Escherichia coli*.

On assaying enzyme fractions obtained in the course of purification of polynucleotide phosphorylase of *A. vinelandii*[18] for stimulation of amino acid incorporation by washed particles of *A. faecalis*, the two activities (phosphorylase and stimulation of amino acid incorporation) were found to increase together through several purification steps but were eventually separated from each other. Highly purified preparations of the enzyme stimulating amino acid incorporation have now been obtained from *A. faecalis* extracts. These preparations are free of activating enzymes catalyzing the amino acid–dependent exchange of PP^{32} with ATP. Pending elucidation of its nature and mode of action, the new enzyme will be provisionally referred to as the amino acid incorporation enzyme.

Amino Acid Incorporation Enzyme.—The enzyme assay was based on the stimulation of C^{14}-glycine incorporation by NaCl-washed *Alcaligenes* particles. As shown in Figure 1, with highly purified enzyme, this stimulation is proportional to the concentration of enzyme within rather narrow limits, since, as the concentration of incorporation enzyme is increased, a plateau is soon reached. At this point, further increase of the enzyme concentration (up to ten-fold) is without effect. One unit was defined as the amount of enzyme catalyzing the incorporation of 1 μmole of glycine/mg of protein nitrogen of particles per hour at 30° over a blank without enzyme, and the specific activity expressed as units per mg. of protein. Protein was determined by the biuret method[19] or spectrophotometrically.[20]

For purification of the incorporation enzyme the combined extract (from 70 gm. of cells) obtained after removing the particles by centrifugation at top speed of the Servall angle centrifuge, as already described, was adjusted from a protein con-

centration of 33 mg/ml to 10 mg/ml by dilution with ice-cold 0.01 *M* phosphate buffer, pH 7.4, and chilled in ice. Solid ammonium sulfate was added with mechanical stirring at 0° to 0.25 saturation, and the precipitate was removed by centrifugation and discarded. The enzyme was precipitated by raising the ammonium sulfate concentration of the supernatant solution to 0.55 saturation. The precipitate was dissolved in ice-cold 0.01 *M* phosphate buffer, pH 7.4, and dialyzed overnight, at 4°, against 2 liters of the same buffer. The dialyzed solution, containing 26 mg. of protein/ml, was adjusted to 10 mg/ml with the above phosphate buffer and to pH 5.1 with 0.1 *M* acetate buffer, pH 4.2. This solution was stirred for 10 minutes at 0° with calcium phosphate gel (prepared by the Sigma Chemical Company, St. Louis, Missouri) in the proportion of 7 ml. of gel suspension (40 mg. of $Ca_3(PO_4)_2$ per ml.) per 25 ml. of enzyme solution. The gel was collected by centrifugation, eluted with 5 ml. of 0.1 *M* phosphate buffer, pH 6.0, for every 7 ml. of gel suspension used, and the eluate dialyzed for 2 hours at 4° against 500 ml. of 0.01 *M* phosphate buffer, pH 7.4. To 5-ml. aliquots of the dialyzed eluate (containing 9 mg. of protein/ml) was added, with stirring for 2 minutes at 0°, 0.7 ml. of 1 per cent protamine sulfate adjusted to pH 5.0. The precipitate was removed by centrifugation and discarded, and the supernatant solution dialyzed against phosphate buffer as above. The resulting solution was refractionated with ammonium sulfate and the fraction at 0.55 saturation discarded. The supernatant solution, containing the enzyme, was freed of ammonium sulfate by dialysis against 0.01 *M* phosphate buffer, pH 7.4, and stored at −18°. The results of a typical fractionation are summarized in Table 1.

TABLE 1

PURIFICATION OF AMINO ACID INCORPORATION ENZYME OF *Alcaligenes faecalis**

Step	Volume (Ml.)	Units†	Protein (Mg.)	Specific Activity‡	280§/260	Yield (Per Cent)
1. Extract	300	338	9900	0.03	0.58	100
2. 1st. $(NH_4)_2SO_4$ fractionation (0.25–0.55)	120	292	5520	0.05	0.60	86
3. $Ca_3(PO_4)_2$ gel eluate	24	236	255	0.92	0.63	70
4. Protamine supernatant	25	200	60	3.7	1.00	59
5. 2nd $(NH_2)SO_4$ fractionation (0.55 supernatant)	25	192	8	24.0	1.00	57

* 70 gm. of cells.

† Assayed with C^{14}-glycine. The assay of fractions at steps 1 through 4, with high nucleic acid content, refers to heat-labile activity; at steps 4 and 5, all the activity of the fractions is heat-labile.

‡ Units per mg. of protein.

§ Ratio of absorption of light at wave length 280 mμ to that at 260 mμ.

When assayed for incorporation of C^{14}-valine, the initial extract contained 220 units of specific activity 0.02 and the final fraction 41 units of specific activity 5.1. It is not possible to decide at this time whether the difference in yield of glycine and valine activity is due to partial separation of individual activities or to the low accuracy of the assay method particularly with crude enzyme fractions. The fractions of specific activity (glycine) 24 are free of polynucleotide phosphorylase and of enzymes catalyzing the amino acid–dependent exchange of PP^{32} with ATP, although these enzymes are quite active in the initial extract.[21] Table 2 illustrates the removal of amino acid activating enzymes on purification of the incorporation enzyme. Similar results have been obtained with each of the remaining amino

acids not shown in the table. The activating enzymes still present at step 4 (Table 2) are found in the 0–0.55 ammonium sulfate fraction discarded at step 5.

The activity of the incorporation enzyme is completely destroyed by heating for 3 minutes at 100°. Preparations up to step 3 keep their activity unchanged for several weeks when stored at −18°, but the purest preparations are exceedingly unstable. They lose about half of the activity on storage at −18° for 8–10 days and most of the activity after about 2 weeks, even if repeated freezing and thawing are avoided.

TABLE 2

REMOVAL OF AMINO ACID–ACTIVATING ENZYMES ON PURIFICATION OF INCORPORATION ENZYME*

	Purification Step†			
L-Amino Acid Substrate	1. Extract	3. Gel eluate	4. Protamine	5. 2d $(NH_4)_2SO_4$
Mixture of 19	36.4	34.2	17.0	0
Glycine	1.6	2.3	1.1	0
Threonine	2.7	0.1	0.1	0
Valine	14.4	18.2	15.0	0
Leucine	13.9	12.5	4.1	0
Lysine	5.6	0	0	0
Proline	15.4	0.5	0.5	0

* The assay samples for amino acid activation (exchange of P^{32}-labeled pyrophosphate with ATP in the presence of amino acid) contained per ml.: Tris buffer, pH 7.1, 100 μmoles; ATP, 3 μmoles; PP^{32}, 3 μmoles (60,000 to 200,000 cpm); $MgCl_2$, 10.0 μmoles; KF, 5 μmoles; L-amino acid, 2–3 μmoles; and enzyme fraction with 0.1 mg. of protein. Incubation, 20 minutes at 37°. Results expressed as per cent exchange over a blank without added amino acid. The blank varied from 0.6 to 0.8, with fractions from steps 1, 3, and 4, and was 0.3 with fraction from step 5. The blank without enzyme was 0.3 throughout.

† Cf. Table 1.

TABLE 3

EFFECT OF AMINO ACID–INCORPORATION ENZYME ON INCORPORATION OF C^{14}-LABELED AMINO ACIDS INTO PROTEIN OF *Alcaligenes* PARTICLES*

	Labeled Amino Acid Incorporated into Particles (Millimicromoles per Mg. Protein N of Particles per Hour)				
			Washed Particles		
Added Labeled Amino Acid	Unwashed Particles (*a*)	No Addition (*b*)	Amino Acids (*c*)	Enzyme (*d*)	Amino Acids + Enzyme (*e*)
Glycine	57	17	47	49	59
D,L-Alanine	40	16	...	30	...
L-Threonine	310	116	166	245	270
D,L-Valine	13	7	13	12	17
D,L-Leucine	24	12	19	20	22
L-Lysine	45	23	...	50	...
L-Proline	458	338	...	415	...
D,L-Phenylalanine	42	10	...	25	...
L-Tyrosine	40	18	...	36	...
D,L-Tryptophan	45	15	30	28	38

* Conditions as in Fig. 1 with 0.25–0.5 μmole (6,000–30,000 cpm) of labeled amino acid. Unwashed particles (*a*). Washed particles with no further additions (*b*). Washed particles with addition of either a mixture of non-labeled 18 L-amino acids (making a total of 19 with the labeled amino acid present in a given experiment), each 0.3 μg. (*c*). amino acid incorporation enzyme, specific activity (glycine) 24, with 10 μg. of protein (*d*), or both (*e*). The L-amino acids used were glycine, alanine, serine, threonine, valine, leucine, isoleucine, cystine, cystine, methionine, arginine, lysine, proline, phenylalanine, tyrosine, tryptophan, histidine, aspartic acid, and glutamic acid.

Activity of Incorporation Enzyme.—The incorporation enzyme stimulates to a varying degree the incorporation of all the labeled amino acids, so far tested, into protein of washed *A. faecalis* particles. Typical results with highly purified enzyme are shown in Table 3. Similar stimulation of the incorporation of some labeled amino acids is produced by a mixture containing small amounts of the non-labeled remaining 18 amino acids. The stimulation due to the joint addition of amino acids and incorporation enzyme is somewhat higher than that produced by either alone, and, under these conditions, the incorporation of labeled amino acid

approaches that obtained with unsupplemented, unwashed particles (Table 3). The results suggest that washing with NaCl makes the particles partially deficient in both free amino acids and incorporation enzyme. It has already been mentioned that the original supernatant extract increases the incorporation of amino acids to levels higher than obtained with unwashed particles alone and that most of this effect is due to heat-stable compounds. This suggests that the extract contains factors (possibly RNA), other than free amino acids and incorporation enzyme, involved in amino acid incorporation by the particles.

Incubation of the washed particles with an amino acid mixture leads to a significant increase in protein, which, as shown in Table 4, is larger in the presence of added incorporation enzyme.

TABLE 4

INCREASE OF PROTEIN ON INCUBATION OF *Alcaligenes* PARTICLES WITH AMINO ACIDS

SYSTEM	PROTEIN (μG.) Initial	Final	Increase
Complete*	474	575	101
No incorporation enzyme	435	510	75
No amino acids	475	525	50
No incorporation enzyme, no amino acids	435	465	30

* The complete system contained Tris buffer, pH 7.0, 50 μmoles; $MgCl_2$, 10 μmoles; K_2HPO_4, 40 μmoles; mixture of 19 L-amino acids, each 26 μg.; washed particles with 430–440 μg. of protein; and amino acid incorporation enzyme, specific activity (glycine) 24, with 40 μg. of protein. Final volume, 1.0 ml. Incubation in air. 1 hour at 30°. The amino acid mixture contained the amino acids listed in the legend to Table 3.

It appeared possible that the incorporation enzyme might be involved in oxidative phosphorylation and thus indirectly stimulate protein synthesis in the particles. This was not unlikely in view of Pinchot's work[22] implicating a soluble enzyme (or enzymes) in the oxidative phosphorylation system of *A. faecalis*. This possibility was ruled out in joint experiments with Dr. Pinchot, showing that the purified incorporation enzyme could not substitute for his soluble, heat-labile component, nor did it have any effect on the phosphorylation coupled to the oxidation of reduced diphosphopyridine nucleotide by the washed *Alcaligenes* particles used in this work. It would, therefore, appear that the incorporation enzyme is more or less directly concerned with some phase of protein biosynthesis.

DISCUSSION

The washed *Alcaligenes* particles seem to be devoid of activating enzymes catalyzing the amino acid–dependent exchange of PP^{32} with ATP, even if further disintegrated by prolonged sonic oscillation. Suspensions of disintegrated particles (2 mg. of protein per ml. of reaction mixture), when assayed under the conditions of Table 2, gave a blank value, i.e., 0.3 per cent exchange, in the absence as well as in the presence of an amino acid mixture. Thus the system described in this paper is capable of active incorporation of amino acids into particle protein in the apparent absence of conventional amino acid–activating enzymes. However, the occurrence of some kind of amino acid activation is indicated by the inactivity of the system under conditions in which oxidative phosphorylation cannot take place.

Although it has as yet not been ascertained whether the incorporated amino acids are present in peptide linkage in the particle proteins, the stimulation of the

approaches that obtained with unsupplemented, unwashed particles (Table 3). The results suggest that washing with NaCl makes the particles partially deficient in both free amino acids and incorporation enzyme. It has already been mentioned that the original supernatant extract increases the incorporation of amino acids to levels higher than obtained with unwashed particles alone and that most of this effect is due to heat-stable compounds. This suggests that the extract contains factors (possibly RNA), other than free amino acids and incorporation enzyme, involved in amino acid incorporation by the particles.

Incubation of the washed particles with an amino acid mixture leads to a significant increase in protein, which, as shown in Table 4, is larger in the presence of added incorporation enzyme.

TABLE 4

INCREASE OF PROTEIN ON INCUBATION OF *Alcaligenes* PARTICLES WITH AMINO ACIDS

SYSTEM	PROTEIN (μG.) Initial	Final	Increase
Complete*	474	575	101
No incorporation enzyme	435	510	75
No amino acids	475	525	50
No incorporation enzyme, no amino acids	435	465	30

* The complete system contained Tris buffer, pH 7.0, 50 μmoles; $MgCl_2$, 10 μmoles; K_2HPO_4, 40 μmoles; mixture of 19 L-amino acids, each 26 μg.; washed particles with 430–440 μg. of protein; and amino acid incorporation enzyme, specific activity (glycine) 24, with 40 μg. of protein. Final volume, 1.0 ml. Incubation in air, 1 hour at 30°. The amino acid mixture contained the amino acids listed in the legend to Table 3.

It appeared possible that the incorporation enzyme might be involved in oxidative phosphorylation and thus indirectly stimulate protein synthesis in the particles. This was not unlikely in view of Pinchot's work[22] implicating a soluble enzyme (or enzymes) in the oxidative phosphorylation system of *A. faecalis*. This possibility was ruled out in joint experiments with Dr. Pinchot, showing that the purified incorporation enzyme could not substitute for his soluble, heat-labile component, nor did it have any effect on the phosphorylation coupled to the oxidation of reduced diphosphopyridine nucleotide by the washed *Alcaligenes* particles used in this work. It would, therefore, appear that the incorporation enzyme is more or less directly concerned with some phase of protein biosynthesis.

DISCUSSION

The washed *Alcaligenes* particles seem to be devoid of activating enzymes catalyzing the amino acid–dependent exchange of PP^{32} with ATP, even if further disintegrated by prolonged sonic oscillation. Suspensions of disintegrated particles (2 mg. of protein per ml. of reaction mixture), when assayed under the conditions of Table 2, gave a blank value, i.e., 0.3 per cent exchange, in the absence as well as in the presence of an amino acid mixture. Thus the system described in this paper is capable of active incorporation of amino acids into particle protein in the apparent absence of conventional amino acid–activating enzymes. However, the occurrence of some kind of amino acid activation is indicated by the inactivity of the system under conditions in which oxidative phosphorylation cannot take place.

Although it has as yet not been ascertained whether the incorporated amino acids are present in peptide linkage in the particle proteins, the stimulation of the

incorporation of some labeled amino acids by a mixture of non-labeled amino acids and the observed small but significant net increases of protein are suggestive of the occurrence of true protein synthesis. These suggestions are reinforced by the observation (*a*) that the incorporation of amino acids is not reversible, since a labeled amino acid (valine, leucine, glutamic acid, phenylalanine), once incorporated, is not displaced by reincubation of the particles with a forty fold excess of the same unlabeled amino acid under conditions of active incorporation, and (*b*) the incorporation is inhibited by chloramphenicol, which is generally considered to be an inhibitor of protein biosynthesis.

SUMMARY

Particulate preparations of *Alcaligenes faecalis*, consisting largely of cell membrane fragments, incorporate amino acids into their proteins. This incorporation, which appears to reflect protein biosynthesis, is driven by oxidative phosphorylation and is stimulated by an enzyme, present in the supernatant extract, which has been isolated in highly purified form. The purified enzyme is free of activating enzymes catalyzing the amino acid–dependent exchange of PP^{32} with ATP and the same seems to be true of the bacterial particles.

We are indebted to Mrs. Monique Beljanski for skilful technical assistance.

* Aided by grants from the National Institute of Arthritis and Metabolic Diseases (Grant A-1845) and the National Cancer Institute (Grant C-2784) of the National Institutes of Health, United States Public Health Service; the Rockefeller Foundation; the American Cancer Society (recommended by the Committee on Growth, National Research Council); and by a contract (Nonr-285(31), NR 120-490) between the Office of Naval Research and New York University College of Medicine.

† Chargé de Recherches, Centre National de la Recherche Scientifique, on leave of absence from the Service de Biochimie Cellulaire, Institut Pasteur, 28 rue du Docteur Roux, Paris XV, France.

[1] M. B. Hoagland, P. C. Zamecnik, and M. L. Stephenson, *Biochim. et Biophys. Acta*, 24, 215, 1957.

[2] M. B. Hoagland, E. B. Keller, and P. C. Zamecnik, *J. Biol. Chem.*, 218, 345, 1956.

[3] Abbreviations: ATP, adenosine triphosphate; RNA, ribonucleic acid; AMP, adenosine-5′-monophosphate; PP, pyrophosphate; Tris, tris(hydroxymethyl)aminomethane; cpm, counts per minute.

[4] F. Lipmann and others, Symposium on amino acid activation, these PROCEEDINGS, 44, 67, 1958.

[5] R. S. Schweet, F. C. Bovard, E. Allen, and E. Glassman, these PROCEEDINGS, 44, 173, 1958.

[6] S. B. Weiss, G. Acs, and F. Lipmann, these PROCEEDINGS, 44, 189, 1958.

[7] P. Berg, *J. Biol. Chem.*, 222, 1025, 1956.

[8] J. A. De Moss and G. D. Novelli, *Biochim. et Biophys. Acta*, 22, 49, 1956.

[9] B. Nismann, F. H. Bergmann, and P. Berg, *Biochim. et Biophys. Acta*, 26, 639, 1957.

[10] G. C. Webster, *Arch. Biochem. Biophys.*, 70, 622, 1957.

[11] E. W. Davie, V. V. Koningsberger, and F. A. Lipmann, *Arch. Biochem. Biophys.*, 65, 21, 1956.

[12] E. F. Gale and J. P. Folkes, *Biochem. J.*, 59, 661, 675, 1955.

[13] M. Beljanski, *Biochim. et Biophys. Acta*, 15, 425, 1954.

[14] S. Spiegelman, in *The Chemical Basis of Heredity* (Baltimore: Johns Hopkins Press, 1955), p. 232.

[15] G. E. Connell, P. Lengyel, and R. C. Warner, submitted to *Biochim. et Biophys. Acta*.

[16] G. B. Pinchot, *J. Biol. Chem.*, 205, 65, 1953. We are indebted to Dr. Pinchot for a culture of the organism.

[17] V. G. Allfrey and A. E. Mirsky, these PROCEEDINGS, 43, 589, 1957.

[18] S. Mii and S. Ochoa, *Biochim. et Biophys. Acta*, 26, 445, 1957.
[19] A. G. Gornall, C. J. Bardawill, and M. M. David, *J. Biol. Chem.*, 177, 751, 1949.
[20] O. Warburg and W. Christian, *Biochem. Z.*, 310, 384, 1941.
[21] To a greater or lesser extent the extract promoted the PP^{32}-ATP exchange with each of the 19 amino acids tested.
[22] G. B. Pinchot, *J. Biol. Chem.*, 229, 1, 1957.