lated)/(μmoles oxygen uptake in salt respiration) ranges from 1.7 to 3.1 and is within the requirements of the hypothesis that the maximum possible would be 4. In addition, the two observations (1) that salt reduces the ATP level, probably by preventing its oxidative formation, and (2) that oligomycin prevents ATP formation without inhibiting ion accumulation are also consistent with the hypothesis that salt accumulation and oxidative phosphorylation are alternative consequences of the charge separation of electron transport.

Summary.—The effects of anaerobic conditions and of inhibitors (sodium arsenite, iodoacetamide, mesoxalonitrile 3-chlorophenylhydrazone, and oligomycin) on ATP content, respiration rate, and salt accumulation rate of carrot tissue were investigated. The best hypothesis to explain the observations is that the ion transport mechanism in this tissue is directly coupled to the electron transport system, does not require the intervention of ATP, and may be an alternative to ATP formation.

Help with microbiological tests by Dr. A. Rovira and information from Dr. N. Good on the advantages of *N*-tris(hydroxymethyl)methyl-2-aminoethane sulfonic acid as a buffer are gratefully acknowledged.

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*ALTERATION OF VALYL-SRNA DURING SPORULATION OF BACILLUS SUBTILIS**

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During sporulation of *Bacillus subtilis* an active, vegetative cell is converted to a dormant spore by a complex series of biochemical events.[1] This is a case of unicellular morphogenesis in which differential expression of the genome is evident.[2, 3] The control factors involved in these events are still unknown. In an analysis of the RNA of vegetative cells and spores, it was observed that the sRNA from these two forms had different elution patterns from a MAK column.[4] The role of sRNA in protein synthesis is well established;[5] more recently sRNA has been proposed as having a regulatory function in the translation of messenger RNA.[6, 7] Differential synthesis of proteins, as during morphogenesis, may depend on the functional con-

centration of specific aminoacyl-sRNA. To test whether the functional capacity of sRNA changes during sporulation, the sRNA from vegetative cells and sporulating cells was analyzed by comparing aminoacyl-sRNA elution patterns from a MAK column by the method of Sueoka and Yamane.[8] The elution patterns revealed that six of the sixteen amino acids tested clearly had two or more aminoacyl-sRNA peaks. When the aminoacyl-sRNA's of sporulating cells were analyzed, it was found that the valyl-sRNA pattern was definitely altered; the other aminoacyl-sRNA profiles were essentially unchanged.

Methods.—Organism and medium: Bacillus subtilis W23 was used as the source of sRNA and enzymes. The SCM medium, which was used for all the growth experiments, has been described.[4] Cells used for the preparation of aminoacyl synthetase and vegetative cell sRNA were grown to a density of 1–2 $\times$ 10^8 cells per ml. Sporulating cell sRNA was obtained from cells 0, 1, and 4 hr after sporulation was initiated.

Preparation of sRNA: The sRNA was extracted from cells as described by von Ehrenstein and Lipmann,[9] except for two additional phenol extractions. The sRNA was treated with 0.5 *M* Tris-HCl buffer, pH 8.8, for 45 min at 35°C to remove bound amino acids. The solution was made 1 *M* in NaCl and the sRNA was precipitated by the addition of 2 vol of ethanol. The precipitate was dissolved in water, and after dialysis against distilled water the sRNA was lyophilized and kept at 4°C.

Preparation of aminoacyl-sRNA synthetase: The synthetase was prepared generally as described by Yamane and Sueoka.[10] A frozen pellet of log phase cells (from 1 liter) was ground with half volume of alumina and suspended in 6 ml of 0.01 *M* Tris-HCl buffer, pH 7.3, containing 0.01 *M* $MgCl_2$ and 0.006 *M* 2-mercaptoethanol. The extract was added to a DEAE-cellulose column (0.9 $\times$ 5 cm) which was equilibrated with 0.02 *M* phosphate buffer, pH 7.7, containing 0.006 *M* mercaptoethanol (PM buffer). After the extract was added to the column, 36 ml of PM buffer was passed through the column. The enzyme was then eluted from the column in 2-ml fractions with 0.35 *M* NaCl dissolved in PM buffer. The fractions with high absorbancy at 280 mμ were pooled and used as the enzyme preparation.

Preparation of aminoacyl-sRNA: The reaction mixture of 0.6 ml contained in μmoles: Tris-HCl buffer, pH 7.3, 40; ATP, 1; KCl, 5; reduced glutathione, 2; $MgCl_2$, 5; 1 μmole each of 19 unlabeled amino acids; phosphoenolpyruvate, 5; 0.1–0.15 mg of enzyme; 0.5–1 mg sRNA; 10 μg pyruvate kinase; 2.5 μc of labeled amino acid. The reaction was incubated at 37°C for 10 min with gentle shaking. The reaction was stopped by adding an equal volume of water-saturated phenol to the reaction mixture in an ice bath, and extracted by the method of Gierer and Schramm.[11] The final ethanol precipitate was dissolved in 0.05 *M* citrate-phosphate buffer, pH 6.2, and kept at −20°C. Some preparations of aminoacyl-sRNA were stable for 2 months under these conditions.

Preparation of methylated albumin kieselguhr (MAK) column: The MAK column was made as described by Sueoka and Yamane.[8] The column contained 6 gm of kieselguhr and 1.5 ml of 1% methylated albumin. A shallow linear elution gradient was obtained by using 160 ml each of 0.3 *M* and 1.1 *M* NaCl in 0.05 *M* phosphate buffer, pH 6.8; 2-ml fractions were collected and each fraction was analyzed for its optical density at 260 mμ. The radioactivity of each fraction was determined by use of a Packard scintillation spectrometer,[4] as reported previously.

Materials: Reagents were obtained from the following sources: New England Nuclear Co.—L-alanine-C^{14}, 123 mc/mmole; DL-alanine-H^3, 236 mc/mmole; L-glutamate-H^3, 5.0 c/mmole; L-glycine-C^{14}, 74 mc/mmole; L-leucine-H^3, 5.0 c/mmole; L-methionine-C^{14}, 13 mc/mmole; DL-tryptophane-C^{14}, 8.95 mc/mmole; Schwarz BioResearch, Inc.—L-arginine-C^{14}, 240 mc/mmole; L-araginine-H^3, 330 mc/mmole; L-aspartate-C^{14}, 160 mc/mmole; L-aspartate-H^3, 300 mc/mmole; L-glutamate-C^{14}, 200 mc/mmole; L-glycine-H^3, 1.0 c/mmole; L-histidine-C^{14}, 201 mc/mmole; L-histidine-H^3, 5.0 c/mmole; L-isoleucine-C^{14}, 201 mc/mmole; L-isoleucine-H^3, 1.5 c/mmole; L-leucine-C^{14}, 170 mc/mmole; L-lysine-C^{14}, 240 mc/mmole; L-lysine-H^3, 800 mc/mmole; L-methionine-H^3, 2.0 c/mmole; L-phenylalanine-C^{14}, 302 mc/mmole; L-phenylalanine-H^3, 1.5 c/mmole; L-serine-C^{14}, 120 mc/mmole; L-serine-H^3, 870 mc/mmole; L-threonine-C^{14}, 160 mc/mmole; L-threonine-H^3, 2.4 c/mmole; L-tryptophan-H^3, 600 mc/mmole; L-tyrosine-

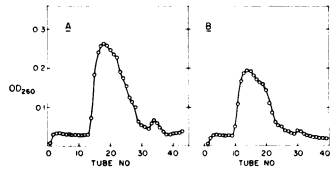

FIG. 1.—MAK column chromatography of sRNA from log phase cells and sporulating cells of *B. subtilis;* 1,000 μg of log phase sRNA and 800 μg of sporulation sRNA were adsorbed onto the respective columns. A linear gradient of 0.3 *M*–1.2 *M* NaCl was used to elute the sRNA; 5.0-ml fractions were collected and analyzed for absorbancy at 260 mμ. (*A*) Log phase cell sRNA. (*B*) Sporulating cell sRNA.

C^{14}, 360 mc/mmole; L-tyrosine-H^3, 0.5 c/mmole; L-valine-C^{14}, 200 mc/mmole; L-valine-H^3, 1.2 c/mmole; Calbiochem —2 phosphoenolpyruvic acid, pyruvate kinase (rabbit muscle), ATP.

Results.—A meaningful comparison of the sRNA of vegetative and sporulating cells of *B. subtilis* required an isolation procedure which yielded undegraded sRNA. The phenol extraction procedure of von Ehrenstein and Lipmann[9] was used to prepare sRNA from all stages of growth. The isolated sRNA preparations had elution patterns from a MAK column which were very similar and completely reproducible. Figure 1 represents typical elution profiles of sRNA from MAK columns. Analysis by sedimentation velocity determinations in the analytical centrifuge revealed a homogeneous 4*S* peak and no evidence of degradation into smaller components.

A thorough analysis of *B. subtilis* aminoacyl-sRNA by MAK column chromatography had not been undertaken previously. Therefore, initially, the sRNA of vegatative cells was analyzed by charging it with specific radioactive amino acids and by obtaining specific aminoacyl-sRNA elution patterns from the MAK column as described by Sueoka and Yamane.[8] A series of distinct and reproducible profiles for alanine, arginine, aspartate, glutamate, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, serine, threonine, tryptophan, tyrosine, and valine were obtained. According to the earlier studies of Sueoka and Yamane,[8] the presence of more than one aminoacyl-sRNA peak for an amino acid suggested that more than one sRNA species existed for the amino acid. Five such cases are illustrated in Figure 2. For arginine and isoleucine there were three peaks; for valine (not illustrated), glutamate, serine, and tyrosine there were two peaks. These were the only amino acids for which good separation of peaks was obtained. The number of peaks obtained represents the minimum number of sRNA species, since the resolving power of the MAK column is limited.

To determine whether the pattern of sRNA remained constant or changed during sporulation, cells were harvested in the log phase of growth, at the initial stages of sporulation, at mid-sporulation, and at late sporulation preceding the appearance of endospores. The sRNA's from all these phases of growth were compared for specific aminoacyl-sRNA patterns. The aminoacyl-sRNA synthetase for these experiments was always obtained from early log phase vegetative cells. The vegetative cell sRNA present in the enzyme fraction was removed by adsorbing the crude extract to a DEAE-cellulose column and eluting only the enzyme with 0.35 *M* NaCl. When radioactive sRNA was purposely mixed with the enzyme prior to adsorption to the DEAE-cellulose column, no radioactivity was found in the enzyme fractions eluted from the column. All the radioactive sRNA was still adsorbed to the column. In kinetic studies, it was found that saturation of most sRNA with an amino acid occurred within 10 min (see reaction conditions in *Methods*). Valine-specific sRNA was saturated within 5–10 min and was very stable in the presence of the enzyme. The counts for valyl-C^{14}-sRNA remained constant for over 20 min in the reaction

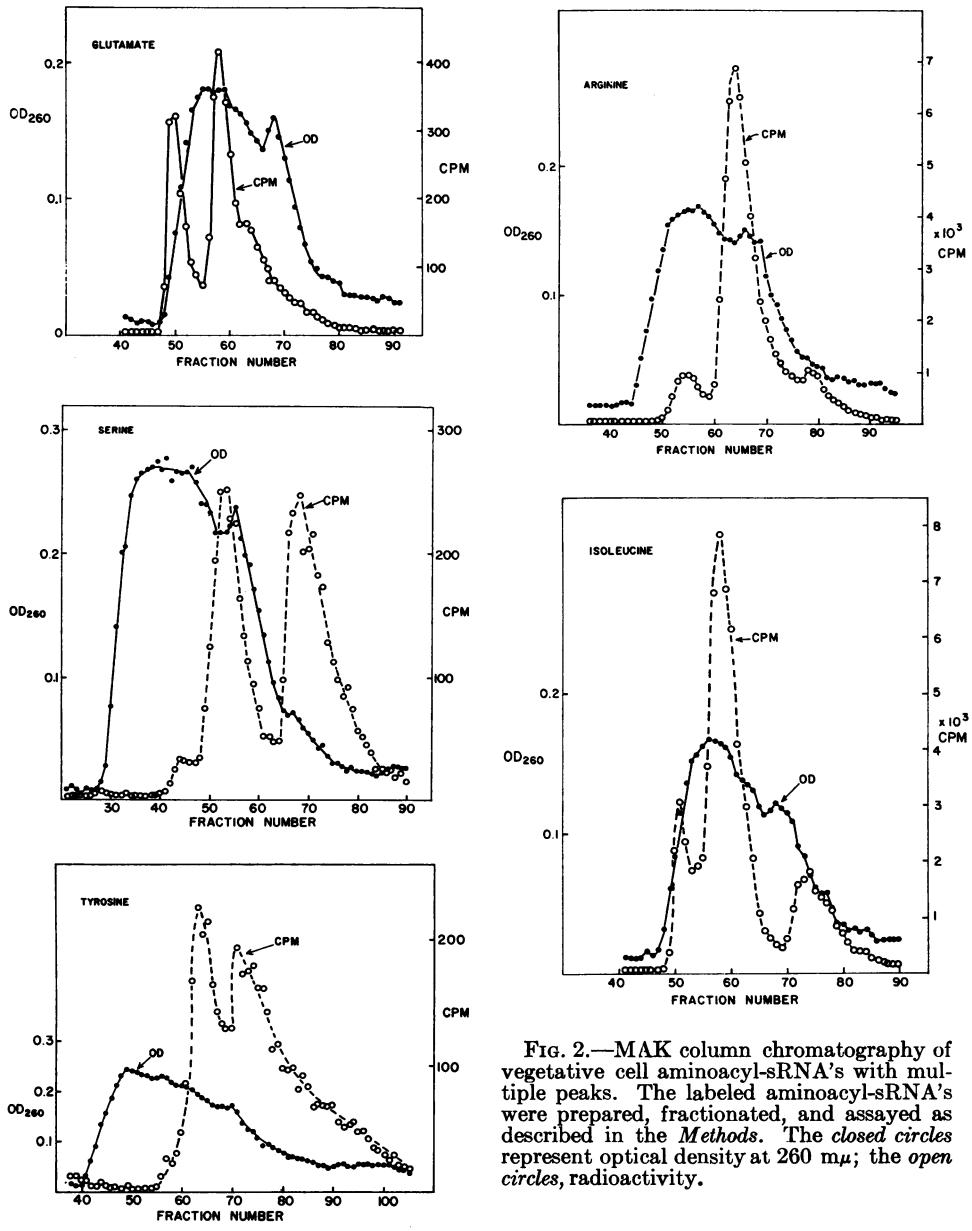

FIG. 2.—MAK column chromatography of vegetative cell aminoacyl-sRNA's with multiple peaks. The labeled aminoacyl-sRNA's were prepared, fractionated, and assayed as described in the *Methods*. The *closed circles* represent optical density at 260 mμ; the *open circles*, radioactivity.

mixture. The incubation time for the reactions was therefore set at 10 min to avoid complications arising from any possible side reactions. The sRNA's were compared by labeling one sRNA with tritiated amino acid, the other with C^{14}-labeled amino acid, and by cochromatography of the two aminoacyl-sRNA's through a MAK column.

A comparison of all the aminoacyl-sRNA's by this method revealed that a specific alteration of the valyl-sRNA pattern occurred during the stages of early sporulation. In the experiments illustrated in Figure 3, the vegetative cell sRNA was charged

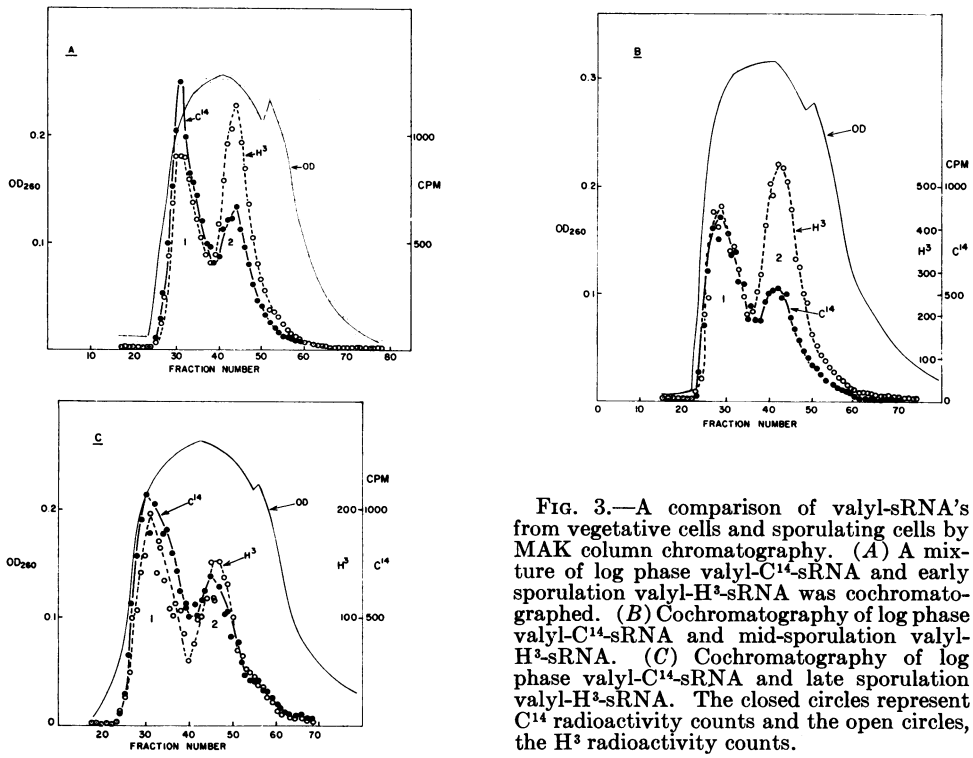

FIG. 3.—A comparison of valyl-sRNA's from vegetative cells and sporulating cells by MAK column chromatography. (*A*) A mixture of log phase valyl-C^{14}-sRNA and early sporulation valyl-H^3-sRNA was cochromatographed. (*B*) Cochromatography of log phase valyl-C^{14}-sRNA and mid-sporulation valyl-H^3-sRNA. (*C*) Cochromatography of log phase valyl-C^{14}-sRNA and late sporulation valyl-H^3-sRNA. The closed circles represent C^{14} radioactivity counts and the open circles, the H^3 radioactivity counts.

with C^{14}-valine and the sporulation phase sRNA was charged with H^3-valine before cochromatography. Valyl-sRNA of log phase cells eluted from the column in two peaks. The first peak was larger than the second (Fig. 3*A* and Table 1). However, a dramatic change in the proportion of valyl-sRNA in the two peaks was observed during early sporulation (Fig. 3*A*). The ratio of peaks 1 to 2 which was 1.2 in vegetative cells shifted to 0.59 in early sporulation cells. This ratio remained at 0.58 during mid-sporulation (Fig. 3*B*). However, late in sporulation (Fig. 3*C*) the ratio rose to 1.1, approaching that found in log phase cells. This pattern was consistently reproduced. The ratio changes are summarized in Table 1. That the pattern was not the function of the labeled amino acid was shown by labeling the vegetative cell sRNA with H^3-valine and sporulating cell sRNA with C^{14}-valine before cochromatography. Results essentially identical to that of Figure 3 were obtained; the peak 1 to peak 2 ratio was 1.4 for the vegetative cell sRNA and 0.6 for early sporulation sRNA.

The results with all the other aminoacyl-sRNA's tested have not revealed any striking differences in the ratios or the profiles of the peaks during sporulation. The ratios of the multiple peaks of several aminoacyl-sRNA's are given in Table 2. Small differences in the ratios were noted which can be attributed to slight variations in chromatography. No other aminoacyl-sRNA was modified to the extent of valyl-sRNA. A significant aspect of these results was the fact that the elution pattern of all but one of the sporulation cell aminoacyl-sRNA's tested remained constant; these results militate against the possibility that the sporulation sRNA was altered by degradative enzymes during the isolation procedures.

TABLE 1

RELATIVE AMOUNTS OF TWO VALYL-sRNA PEAKS ELUTED FROM A MAK COLUMN

Source of sRNA		Peak 1, cpm*	Peak 2, cpm*	Peak 1/peak 2, ratio of cpm
Log phase cells	Fig. 3*A*	9,287	7,755	1.2
Early sporulation		5,995	10,145	0.6
Log phase cells	Fig. 3*B*	8,101	5,943	1.3
Mid-sporulation		3,686	6,320	0.6
Log phase cells	Fig. 3*C*	11,692	8,172	1.4
Late sporulation		1,847	1,740	1.1

* These numbers were obtained from experiments described in Fig. 3*A–C*. Log phase cell sRNA was labeled with C^{14}-valine. The various sporulation phase sRNA's were labeled with H^3-valine. The double labeled log phase and sporulation phase sRNA were cochromatographed through a MAK column, and each fraction was assayed for radioactivity. The C^{14} and H^3 counts were counted separately for each peak as follows: Peaks 1 and 2 in Fig. 3*A* are total counts of fractions 23–39 and 40–65, respectively. Peaks 1 and 2 in Fig. 3*B* are total counts of fractions 22–37 and 38–65, respectively. Peaks 1 and 2 in Fig. 3*C* are total counts of fractions 22–40 and 41–63, respectively.

Discussion.—The analysis of aminoacyl-sRNA from *B. subtilis* by MAK column chromatography has revealed the following: (*a*) Multiple peaks are obtained for several of the amino acids; the size of the peaks for a specific amino acid reveals that a considerable difference in the amount of each sRNA exists (see arginyl-sRNA and isoleucyl-sRNA in Fig. 2); and (*b*) the elution pattern for valyl-sRNA changes dramatically during the initial stages of sporulation while the patterns for the other 15 amino acids examined remain constant.

The constant ratios of the aminoacyl-sRNA peak sizes (see Table 2) of several of the amino acids illustrate that a regulated concentration of sRNA species for a particular amino acid exists. Even under the stresses of medium depletion and sporulation, the sRNA pattern remains unchanged. The single exception occurs with valyl-sRNA peaks. This shift in ratio is first evident during the initial stages of sporulation and continues until the later stages when the ratio again approaches that of the vegetative cell. The alteration of the valyl-sRNA pattern does not appear to be an all-or-none modification of the sRNA species. However, each of the two valyl-sRNA peaks may actually be comprised of one or more valine-specific sRNA's. In this connection, it should be noted that valine has been assigned four coding triplets.[12] It is possible that a minor component is being completely inactivated or activated resulting in an alteration of the ratios of the two peaks.

The actual mechanism of this alteration could depend on a modification of either the aminoacyl-sRNA synthetase or of the sRNA. Since the change in profiles is observed when the two sRNA populations are charged with the aminoacyl-sRNA synthetase from log phase cells, the alteration must affect the accepting ability of

TABLE 2

RATIOS OF VARIOUS AMINOACYL-sRNA PEAKS ELUTED FROM A MAK COLUMN*

	Vegetative Cells		Early Sporulating Cells	
Aminoacyl-sRNA	Peak 1/ peak 2	Peak 1/ peak 3†	Peak 1/ peak 2	Peak 1/ peak 3†
Arginyl-sRNA	0.30	1.60	0.31	1.80
Glutamyl-sRNA	0.49	—	0.43	—
Isoleucyl-sRNA	0.33	3.66	0.32	3.86
Seryl-sRNA	0.81	—	0.77	—
Tyrosyl-sRNA	0.53	—	0.63	—

* In these experiments the vegetative cell sRNA's were charged with C^{14}-amino acids and the early sporulating cell sRNA's with H^3-amino acids. They were mixed and eluted from the MAK column as described in the *Methods*. The counts in the peaks for each aminoacyl-sRNA were determined in a manner similar to that described for valyl-sRNA in Table 1. The ratios represent the ratios of the total counts in the peaks.

† Only arginine and isoleucine had three peaks; the others had two peaks (Fig. 2).

the sRNA and not the activity of the synthetase. The identical elution pattern of 15 other aminoacyl-sRNA's appears to eliminate ribonuclease activity as a significant factor in the alteration of valyl-sRNA. Furthermore, it is the ratio of the two peaks which changes and not the elution positions. Although this study has revealed a significant change of only valyl-sRNA, minor but significant changes may have occurred which were not detected by this method. An alternate method is being used to investigate this possibility.

The role of sRNA in protein synthesis has been well characterized and defined.[5] Ames and Hartman[6] and Stent[7] have proposed that sRNA may also be involved in regulation of protein synthesis at the messenger RNA translation level. Sueoka and Kano-Sueoka,[13] who found that the leucyl-sRNA pattern of *E. coli* was altered after T2 phage infection, have proposed that adaptor modification may play an important role when a dramatic change in metabolic patterns is observed. Assuming degeneracy of the code, they suggested that a modification of one of the multicomponent sRNA's for an amino acid would alter the translation of messenger RNA containing the codon for the altered sRNA; messenger RNA not containing the codon would be translated as usual.

The ramifications of this proposal for regulation at the translation level can be extended to a differentiating system such as the sporulating bacterium. Since sporulation involves the expression of many genes[14, 15] and represents a major shift in metabolic activity for the cell,[1] an alteration of sRNA might be expected under the modification model of regulation. The present results fulfill this expectation. It is interesting that in the case of T2 phage infection and bacterial sporulation, a major alteration of only one aminoacyl-sRNA was observed. These results will imply, if the modification model becomes a reality, that minor changes in the total sRNA population exert a major shift in metabolic activity. The exact function that the change in valyl-sRNA may play in bacterial morphogenesis is unknown. However, since the alteration occurs during the initial stages of sporulation, it suggests that valyl-sRNA modification may be involved in some regulatory role required for transforming an active vegetative cell to a sporulating form.

Summary.—An analysis of the aminoacyl-sRNA patterns of vegetative cells and sporulating cells of *Bacillus subtilis* by methylated albumin column chromatography revealed that the ratio of two valyl-sRNA peaks underwent a significant change during the initial stages of sporulation. The ratio returned to that of vegetative cells during the later sporulation stages. The profiles and patterns of the fifteen other amino acids tested were not altered. The alteration in the valyl-sRNA pattern was a function of the sRNA and not the aminoacyl-sRNA synthetase.

The authors thank Dr. Tetsuo Yamane for helpful technical advice and Richard Igarashi for assistance with the MAK column chromatography experiments.

The following abbreviations are used: RNA, ribonucleic acid; sRNA, soluble RNA; MAK, methylated albumin kieselguhr; Tris buffer, tris (hydroxymethyl) aminomethane buffer; ATP, adenosine triphosphate.

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SUPPRESSION OF THE SHUNT PATHWAY IN PRIMARY GOUT BY AZATHIOPRINE

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An increasing volume of studies[1-3] lends support for a dual etiology of hyperuricemia in primary gout. In this disorder there may be either normal or excessive production of uric acid. Patients with primary gout related to overproduction of uric acid show a pattern of incorporation of a labeled precursor (e.g., glycine) into urinary uric acid which consists of a high initial value followed by a fairly rapid decline in isotope concentration during the succeeding days (Fig. 1). This pattern has been taken as evidence of a shunt pathway whereby precursor is incorporated into uric acid more promptly than in normal man by bypassing nucleic acid purines. This shunt pathway is generally considered to be responsible for overproduction of uric acid in primary gout.

When azathioprine (Imuran) was given to gout patients who displayed excessive excretion of uric acid in the urine, we found a significant reduction in both plasma and urinary uric acid. To evaluate this finding more precisely, the incorporation of glycine into uric acid during treatment with azathioprine was studied in three patients who had previously been shown to possess the shunt pathway.

The administration of azathioprine to patients with primary gout was an indirect result of a joint study with Drs. N. Bricker and R. Rieselbach at Washington University, St. Louis, on the nature of primary gout in a patient who had developed progressive renal failure due to gouty nephropathy which necessitated renal homotransplantation in December 1964.[4] The patient has, since then, been maintained on azathioprine in order to suppress a homograft reaction.