*NAF INHIBITION OF THE INITIAL BINDING OF AMINOACYL-SRNA TO RETICULOCYTE RIBOSOMES**

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NaF has been found to produce a nearly complete but reversible inhibition of hemoglobin biosynthesis and brings about a conversion of reticulocyte polysomes to monomeric ribosomes.[1-3] In earlier work with the complete cell-free reticulocyte systems, it was found that NaF has a direct inhibitory effect on protein synthesis,[4] in addition to possible indirect effects related to its inhibition of ATP formation in intact cells.[3] A portion of the cell-free peptide synthesis with regular reticulocyte ribosomes was observed to be resistant to NaF inhibition.[2, 4] Peptides synthesized with labeled valine under these inhibited conditions contained very little valine in their N-terminal position. This was interpreted to indicate that the incorporation observed in the presence of the inhibitor was primarily into the C-terminal portion of previously initiated chains and that NaF had blocked the initiation of new chains. Studies of the initial rate of incorporation with regular ribosomes in the cell-free system indicated that the inhibitor had little or no effect on reactions involved in chain completion.

Ribosomes isolated from reticulocytes previously incubated with inhibitory concentrations of NaF were primarily monomeric and lacked nascent peptide chains. These "NaF-treated ribosomes" were found to be active for cell-free incorporation of valine into natural peptides. The peptides formed were found to contain N-terminal valine in the approximate amount expected for randomly labeled globin. This indicated that NaF-treated ribosomes were strongly dependent on chain initiation for cell-free synthesis. In contrast to the results with regular ribosomes, cell-free synthesis with NaF-treated ribosomes was almost completely inhibited by NaF. The poly U-directed synthesis of polyphenylalanine, using either regular or NaF-treated ribosomes, was also found to be very sensitive to inhibition by NaF. It was concluded that the sensitivity to NaF inhibition reflects the dependence of the systems on the initiation of new peptide chains.

The experiments described here, using the reticulocyte transfer system, offer evidence that NaF inhibits the poly U-directed, nonenzymatic binding of phenylalanyl-sRNA to ribosomes and the poly U-directed, GTP-dependent enzymatic binding thought to be the first enzymatic step in peptide bond formation. Resistance to NaF inhibition was established by preincubation of poly U, phenylalanyl-sRNA, and ribosomes in salt solution containing adequate concentrations of $MgCl_2$ to promote peptide bond formation in the complete transfer system. A preliminary report of this work has been made.[5]

Materials and Methods.—Preparation of rabbit liver sRNA: Frozen young rabbit livers, Type 1, were obtained from Pel-Freez Biologicals, Inc., Rogers, Arkansas. The livers (300 gm) were allowed to defrost slightly in 850 ml of 0.1 *M* Tris, pH 7.5, containing 0.347 *M* sucrose, 0.003 *M* $MgCl_2$, and 0.024 *M* KCl, and were then homogenized in a Waring Blendor at 0°. After centrifugation of the homogenate at 9,000 × *g* for 20 min, followed by centrifugation at 78,000 × *g* for 90 min, the supernatant solution was collected, and the pH adjusted to 4.5 by the addition

of 1 *M* HAc. The precipitate was collected by centrifugation and was resuspended in 250 ml of 0.1 *M* Tris, pH 7.5. An equal volume of 90% phenol (freshly distilled) was added and the suspension was shaken at room temperature for 45 min. The phases were separated by centrifugation and the aqueous phase was collected. The sRNA was then precipitated from the aqueous phase and stripped of bound amino acids by the procedure of von Ehrenstein and Lipmann.[6] The yield of sRNA was approximately 120 mg.

Preparation of charged sRNA: To prepare aminoacyl-sRNA, the reaction mixture contained in a total volume of 10 ml: stripped rabbit liver sRNA, 10 mg; Tris, pH 7.5, 0.1 *M;* $MgCl_2$, 0.01 *M;* GSH, 0.02 *M;* ATP, 0.002 *M;* 40–70% ammonium sulfate fraction,[4] 7 mg of protein; 19 C^{12}-amino acids, 0.02 mM each; and C^{14}-valine, 0.02 mM (specific activity 20 $\mu c/\mu$mole). The reaction mixture was incubated for 15 min at 37° and then extracted with an equal volume of 90% phenol. The aminoacyl-sRNA was recovered from the aqueous phase by precipitation with ethanol, washed by two additional ethanol precipitations, and dissolved in a small volume of 0.01 *M* KAc, pH 5. To prepare C^{14}-phenylalanyl-sRNA, 0.02 mM C^{14}-phenylalanine was substituted for C^{14}-valine and the C^{12}-amino acids were omitted from the reaction mixture. The C^{14}-phenylalanyl-sRNA prepared in this manner was passed through a Sephadex G-25 column in order to remove contaminating ATP and GTP.

Preparation of ribosomes: Regular reticulocyte ribosomes and ribosomes from NaF-treated cells were prepared as previously described.[4] To remove transfer enzymes from the ribosomes, 0.2 ml of 5% potassium deoxycholate, pH 7.5, was added per ml of ribosomes (approximately 20 mg/ml) and the mixture was incubated for 10 min at 37°. The mixture was then diluted tenfold with 0.05 *M* Tris, pH 7.5, containing 0.05 *M* KCl and 0.001 *M* $MgCl_2$, and the ribosomes were sedimented by centrifugation at 150,000 × *g* for 90 min. The washed ribosomes were resuspended in 0.01 *M* Tris, pH 7.5, containing 0.001 *M* KCl and 0.0001 *M* $MgCl_2$, and stored at −90°.

Preparation of enzyme fractions: Transfer enzyme fractions were prepared by a modification of the procedure described by Bishop and Schweet.[7] The amount of calcium phosphate gel used to absorb the transfer factors from the 40–70% ammonium sulfate fraction was increased to 1.5 mg of gel per mg of protein. The gel was eluted as described, and eluates 2, 3, and 4 were obtained. Eluate 2 will be referred to below as Fraction II, and eluate 4 will be referred to as Fraction I. Fraction I (20 mg of protein) was placed on a 1-gm DEAE-cellulose column which had been equilibrated with 0.01 *M* Tris, pH 7.5, containing 0.005 *M* GSH. The column was washed with the same buffer and the absorbed protein eluted stepwise with 0.1 *M* KCl and 0.2 *M* KCl in 0.01 *M* Tris, pH 7.5, and 0.005 *M* GSH. The 0.2 *M* KCl elution is referred to below as Fraction I*a*. All enzyme fractions were stored at −90°.

Assays: Bound C^{14}-phenylalanyl-sRNA was measured by a modification of the procedure of Nirenberg and Leder.[8] The reaction mixture contained in a volume of 0.5 ml: 0.08 *M* Tris, pH 7.5; 0.07 *M* KCl; $MgCl_2$, as indicated; 0.1 mg of poly U; 1 mg of washed NaF-treated ribosomes; and C^{14}-phenylalanyl-sRNA which had been passed through a Sephadex G-25 column. After 5 min of incubation at 37°, the assay tubes were placed in ice and the reaction mixture diluted tenfold with 0.05 *M* Tris, pH 7.5, containing 0.05 *M* KCl and 0.008 *M* $MgCl_2$. The diluted reaction mixture was filtered immediately through Schleicher and Schuell B-6 membrane filters, and the filters were washed with three 5-ml portions of cold buffer. The filters were counted, as previously described,[4] in a Packard Tri-Carb liquid scintillation spectrometer. To measure enzymatically bound C^{14}-phenylalanyl-sRNA, 0.2 mM GTP and Fraction I*a* (14 μg protein) were added to the reaction mixture.

Polyphenylalanine synthesis was measured by the incorporation of radioactivity into hot TCA-insoluble material. The reaction mixture contained, in addition to the components listed above, 0.2 mM GTP, 0.01 *M* GSH, 0.008 *M* $MgCl_2$, and transfer factors I and II in a total volume of 0.5 ml. After 5 min of incubation at 37°, 5% TCA was added and the suspension was heated at 90° for 15 min. The precipitates were collected on membrane filters and the filters were washed three times with 5% TCA. The filters were counted as described above.

Results.—Globin synthesis with regular ribosomes in the reticulocyte complete cell-free system was found to be less sensitive to NaF inhibition than synthesis with NaF-treated ribosomes.[4] This difference apparently reflects the effect of NaF on a reaction unique to the initiation of new native peptides.

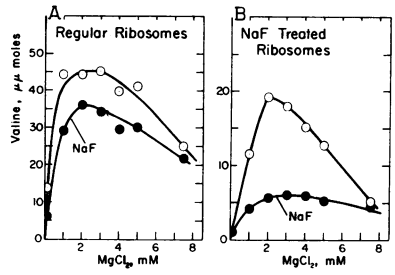


FIG. 1.—NaF inhibition of valine incorporation at varying concentrations of $MgCl_2$. The reaction mixture contained in a total volume of 0.5 ml: 0.08 *M* Tris, pH 7.5; 0.07 *M* KCl; $MgCl_2$, as indicated; 1 mg of unwashed regular ribosomes or 1 mg of unwashed NaF-treated ribosomes; 0.2 mM GTP; 0.01 *M* GSH; 0.12 mg of charged rabbit liver sRNA containing 122 $\mu\mu$moles of C^{14}-valine; and, when indicated, 0.02 *M* NaF. After 5 min of incubation at 37°, the reaction was stopped by the addition of 5% TCA, and the amount of radioactivity incorporated into hot TCA-insoluble material was determined as described in *Methods*. O—O, No NaF; ●—●, 0.02 *M* NaF.

A similar relation of NaF-treated and regular ribosomes to NaF inhibition is shown in Figure 1 for the reticulocyte transfer system using the transfer of valine from valyl-sRNA into naturally occurring peptides, most of which are related to the α and β chains of rabbit globin. Valine transfer by regular ribosomes containing a high proportion of polysomes and nascent globin chains was only inhibited about 20 per cent by 0.02 *M* NaF at the optimal magnesium concentration. In contrast, valine transfer with NaF-treated ribosomes was inhibited about 70 per cent under similar conditions. The inhibition observed for regular ribosomes apparently reflects some chain initiation that occurs with this system. Incorporation with NaF-treated ribosomes in the presence of NaF probably reflects incorporation into some remaining nascent chains and to incomplete inhibition of chain initiation.

Figure 1 emphasizes the critical magnesium dependence of amino acid transfer into natural peptides. Both regular ribosomes (Fig. 1*A*) and NaF-treated ribosomes (Fig. 1*B*) have critical optima at 2 mM $MgCl_2$ under the conditions employed here. The low solubility of MgF_2 suggests that NaF inhibition might be related to an effective removal of available magnesium ions from the system in an insoluble form. The data of Figure 1 indicate that this is not the case. Magnesium optima are seen at about 2 mM magnesium, either in the presence or absence of NaF. If NaF were effective only by removing magnesium ions from the system, it would be expected that inhibition might be overcome at higher concentrations of $MgCl_2$, and that the magnesium optimum for the NaF-inhibited system would be shifted to a higher concentration of $MgCl_2$. These results have led us to believe that a reduction in magnesium ion concentration is not a primary factor in the fluoride inhibition of amino acid incorporation, although it may be of greater significance at the higher concentration of $MgCl_2$ used for the poly U-directed incorporation of phenylalanine.

A fractionated transfer system similar to the one previously described by Arlinghaus *et al.*[8] has been employed to investigate the specific NaF-sensitive site. One of the fractions, designated I*a*, is capable of stimulating the binding of phenylalanyl-sRNA to ribosomes measured by a slightly modified form of the membrane filter assay reported by Nirenberg and Leder.[9] The GTP-dependent binding promoted by this fraction is apparently similar or identical to the "enzymatic binding" reported by Arlinghaus *et al.*[10] However, we prefer the cautious designation of this fraction as "I*a*" rather than "binding enzyme" or "transferase I" due to the apparently complex nature of the reaction involved and the, as yet, poorly understood function of the fraction. Some requirements for enzymatic binding and for formation of hot TCA-insoluble phenylalanine peptides are presented in Table 1. The

TABLE 1

GTP AND SOLUBLE FRACTIONS REQUIRED FOR ENZYMATIC BINDING AND POLYMERIZATION

Addition to incubation mixture	Phenylalanine bound to ribosomes ($\mu\mu$moles)	Polyphenylalanine ($\mu\mu$moles)
None	3.0	0.6
Fraction I*a*	3.1	0.7
GTP	3.2	0.7
Fraction I*a* + GTP	17.1	1.1
Fractions I and II	—	1.3
Fractions I and II + GTP	—	75.9

The assay for bound phenylalanyl-sRNA was carried out as described in *Methods*. The reaction mixture contained in a total volume of 0.5 ml: 0.08 *M* Tris, pH 7.5; 0.07 *M* KCl; 0.008 *M* $MgCl_2$; 0.1 mg of poly U; 1 mg of washed NaF-treated ribosomes; and 86 $\mu\mu$moles of C^{14}-phenylalanyl-sRNA. To assay for polyphenylalanine, the reaction mixture was supplemented with 0.01 *M* GSH and the concentration of C^{14}-phenylalanyl-sRNA was increased to 150 $\mu\mu$moles. The amount of radioactivity incorporated into hot TCA-insoluble material was determined as described in *Methods*. Additions to the reaction mixtures of Fraction I*a* (14 μg protein), 0.2 mM GTP, Fraction I (80 μg protein), and Fraction II (200 μg protein) were made as indicated.

Fraction I*a*-dependent binding of phenylalanyl-sRNA to ribosomes is critically dependent on GTP, as is the formation as hot TCA-insoluble peptides. Since small amounts of GTP or ATP remaining in the system would lower the requirement for added GTP,[10] these data indicate very low levels of residual ATP and GTP in the fractionated components of the system. Phenylalanine, with no more than trace amounts of diphenylalanine or longer phenylalanine peptides, has been recovered from the product of the enzymatic binding reaction and recognized by paper chromatography.

The effects of NaF on enzymatic and nonenzymatic binding are shown in Figure 2. Nonenzymatic binding is very dependent on magnesium ion concentration, as is shown in the top figure. Addition of Fraction I*a* and GTP increases binding as indicated. This Fraction I*a*- and GTP-dependent increase in binding, referred to as enzymatic binding below, has a magnesium concentration optima near 8–10 mM, as indicated by the bottom curves of Figure 2. The curves representing enzymatic binding in the presence of NaF exhibit some apparent displacement toward higher concentrations of magnesium. This may reflect the formation of significant amounts of insoluble material involving magnesium and fluoride ions at higher concentrations of $MgCl_2$. However, NaF effectively inhibits both enzymatic binding and nonenzymatic binding as they are measured in these experiments.

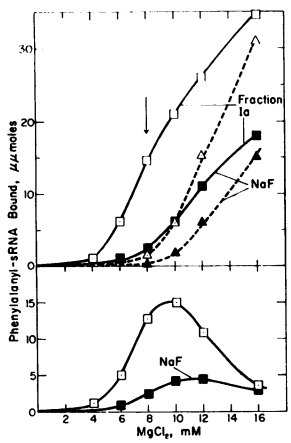


FIG. 2.—The effect of NaF on the nonenzymatic and enzymatic binding of phenylalanyl-sRNA. For nonenzymatic binding, the reaction mixture contained in a total volume of 0.5 ml: 0.08 *M* Tris, pH 7.5; 0.07 *M* KCl; 0.1 mg of poly U; 1 mg of washed NaF-treated ribosomes; 0.18 mg of rabbit liver sRNA containing 70 $\mu\mu$moles of C^{14}-phenylalanyl-sRNA; and $MgCl_2$, as indicated. For enzymatic binding, 0.2 mM GTP and Fraction I*a* (14 μg protein) were added to the reaction mixture. The reaction mixtures were incubated at 37° for 5 min and the amount of bound C^{14}-phenylalanyl-sRNA was determined as described in *Methods*. △—△, Nonenzymatic binding in the absence of NaF; ▲—▲, nonenzymatic binding in the presence of 0.02 *M* NaF; □—□, enzymatic binding in the absence of NaF; ■—■, enzymatic binding in the presence of 0.02 *M* NaF.

TABLE 2

FACTORS REQUIRED FOR ESTABLISHING RESISTANCE DURING PREINCUBATION TO INHIBITION BY NaF

Preincubation mixture	Polyphenylalanine ($\mu\mu$moles) −NaF	+NaF	Per cent inhibition
No preincubation	87.5	12.5	86
Ribosomes	77.4	7.6	91
Phe-sRNA + ribosomes	73.1	7.3	90
Poly U + ribosomes	91.7	18.7	80
Phe-sRNA + poly U	71.1	11.4	84
Phe-sRNA + poly U + ribosomes	89.6	63.9	29
Phe-sRNA + poly U + ribosomes*	89.1	61.5	31

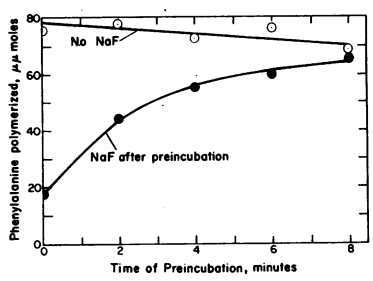
The preincubation mixture contained in a total volume of 0.5 ml: 0.08 *M* Tris, pH 7.5; 0.07 *M* KCl; 0.008 *M* $MgCl_2$; and when indicated, 1 mg of washed NaF-treated ribosomes, 0.1 mg poly U, and 0.42 mg of charged rabbit liver sRNA containing 140 $\mu\mu$moles of C^{14}-phenylalanyl-sRNA. The preincubation mixture was incubated for 5 min at 37°. The assay tubes were cooled in ice and the additional components of the final reaction mixture were added. The final reaction mixture contained in a total volume of 1 ml: 0.08 *M* Tris, pH 7.5; 0.07 *M* KCl; 8 mM $MgCl_2$; 1 mg of washed NaF-treated ribosomes; 0.1 mg poly U; 0.42 mg of charged rabbit liver sRNA containing 140 $\mu\mu$moles of C^{14}-phenylalanyl-sRNA; 0.01 *M* GSH; 0.1 mM GTP; Fraction I (80 μg protein); Fraction II (200 μg protein); and 0.02 *M* NaF when indicated. The final reaction mixture was incubated at 37° for 5 min, after which time the reaction was stopped by the addition of 5% TCA and the amount of radioactivity incorporated into hot TCA-insoluble material was determined as described in *Methods*.

* After 5 min of preincubation at 37°, NaF was added and the preincubation mixture allowed to incubate for an additional 5 min at 37° before the addition of the components of the final incubation mixture.

The data presented in Figure 2 indicate that NaF effectively inhibits the enzymatic binding reaction that has been suggested to be required for the formation of each peptide bond.[11] A direct NaF inhibition of this reaction would suggest that it had special characteristics for the formation of the first peptide bond of a nascent peptide, since NaF has been found to have little or no effect on the extension of previously initiated chains.[4] An alternate hypothesis is that NaF inhibits a reaction related to the initiation of new chains and this reaction must precede enzymatic binding in establishing nascent phenylalanine peptides. The data presented in Table 2 support the latter hypothesis. Resistance to inhibition by NaF was formed in the system by preincubation of ribosomes, poly U, and phenylalanyl-sRNA in a solution containing the salts used for the poly U-directed transfer system. Preincubation of any one or any pair of the three reactants involved failed to establish resistance to inhibition by NaF. After resistance to NaF had been established during preincubation, as described above, additional preincubation in the presence of NaF, but in the absence of the transfer factors and GTP, was found not to lower appreciably the initial resistance that had been established. After resistance had been established, the poly U-directed transfer system seemed, in its insensitivity to inhibition by NaF, quite similar to valine transfer with regular ribosomes bearing nascent globin chains.

Figure 3 presents the effect of the time of preincubation in establishing resistance

FIG. 3.—The effect of time of preincubation on the establishment of resistance to inhibition by NaF. The composition of the preincubation mixture was the same as the preincubation mixture described in Table 2 containing phenylalanyl-sRNA, poly U, and ribosomes. The preincubation mixture was incubated at 37° for the times indicated. The assay tubes were cooled in ice and the additional components of the final reaction mixture added as described in Table 2. The final reaction mixture was incubated for 5 min at 37° and the amount of radioactivity incorporated into hot TCA-insoluble material was determined as described in *Methods*. O—O, No NaF; ●—●, 0.02 *M* NaF added after preincubation.



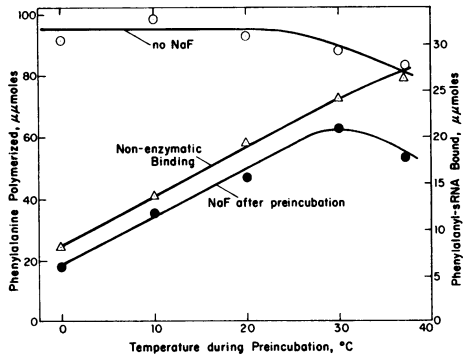


FIG. 4.—The effect of temperature on the establishment of resistance to inhibition by NaF. The procedure was the same as described in Fig. 3. The preincubation mixture was incubated for 5 min at the temperatures indicated. O—O, No NaF; ●—●, 0.02 *M* NaF added after preincubation; △—△, nonenzymatic binding measured in the presence of 16 mM $MgCl_2$ as described in Fig. 2.

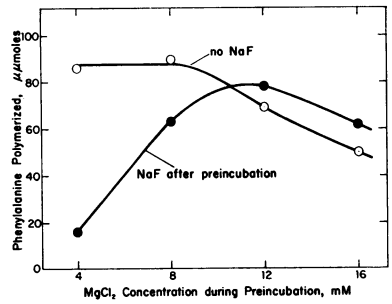


FIG. 5.—The effect of $MgCl_2$ concentration on the establishment of resistance to inhibition by NaF. The procedure was the same as described in Fig. 3. The preincubation mixture was incubated for 5 min at 37° at the concentrations of $MgCl_2$ as indicated. O—O, No NaF; ●—●, 0.02 *M* NaF added after preincubation.

to NaF inhibition. In these experiments, poly U, phenylalanyl-sRNA, and ribosomes were preincubated at 37° for the times indicated. NaF and the other components required for the synthesis of polyphenylalanine were then added before polymerization during the final incubation at 37° for 5 min. The values given represent hot TCA-insoluble polyphenylalanine formed in the presence and absence of NaF during the final 5 min of incubation. Nearly complete resistance to NaF inhibition was established after 8 min of preincubation under these conditions. Figure 4 presents the data of experiments similar to those described above, except that different temperatures were used during a 5-min preincubation.

The effect of temperature on nonenzymatic binding during a 5-min preincubation at 16 mM $MgCl_2$ is plotted on the same graph. Comparable values for nonenzymatic binding at 8 mM $MgCl_2$ are low (see Fig. 2), but exhibit a similar temperature dependence. The parallel effects of preincubation at the lower temperatures on nonenzymatic binding and the development of resistance to NaF inhibition suggest that a common reaction may be involved. Incubation at 37° for 5 min under the conditions used here produces some drop in total polymerization compared with preincubation at 30°. A more pronounced inhibition of total polymerization is seen after preincubation at 12 mM or 16 mM $MgCl_2$, as shown in Figure 5. Although total polymerization is limited by preincubation at higher concentrations of $MgCl_2$ that promote relatively high levels of nonenzymatic binding, it seems clear that magnesium ions are required for the establishment of resistance to NaF during preincubation. This also suggests a relation between NaF resistance and the formation of a magnesium-stabilized complex between poly U, phenylalanyl-sRNA, and ribosomes.

Discussion.—The difference in the NaF inhibition of valine transfer into native peptides with regular and NaF-treated ribosomes is similar to results obtained with the complete cell-free system[4] and apparently reflects differences in the proportion of *in vitro* chain initiation with the two types of ribosomes. The poly U-dependent

synthesis of polyphenylalanine is also inhibited by NaF, but resistance to inhibition could be established by preincubation of phenylalanyl-sRNA, poly U, and ribosomes. Establishment of resistance may be dependent on the formation of a magnesium-stabilized complex involving these components of the transfer system. Once resistance is established in the poly U-directed system, the transfer of phenylalanine is similar to the transfer of valine with regular ribosomes in its insensitivity to inhibition by NaF. These findings strongly support the hypothesis that NaF can specifically inhibit protein synthesis by inhibiting at a point early in the sequence of reactions.

There is no indication that the establishment of resistance to NaF inhibition in the poly U-directed system is dependent on either ATP or GTP. Nonenzymatic binding and the establishment of resistance were not altered by the addition of ATP or GTP under conditions in which enzymatic binding and polymerization were highly dependent on the addition of a soluble fraction and GTP. These results indicate that the establishment of resistance to NaF inhibition involves a step in the series of sequential reactions that precedes the GTP-dependent enzymatic binding, but make it seem unlikely that an energy-dependent formylation of exisiting phenylalanyl-sRNA[12, 13] is involved. The requirement for both ribosomes and poly U in preincubation with phenylalanyl-sRNA that yields NaF-resistant systems supports this conclusion.

Characterization of the specific mechanism of NaF inhibition and the process by which resistance to inhibition is established has involved attempts to distinguish resistance from nonenzymatic binding and to establish the enzymatic or nonenzymatic nature of the reaction involved. There is a close parallel between the conditions required for nonenzymatic binding and the development of resistance to inhibition by NaF so that, when considered together, it seems likely that the development of resistance is dependent on, if not identical to, nonenzymatic binding. However, the relatively low nonenzymatic binding obtained at concentrations of $MgCl_2$ that lead to the establishment of nearly complete resistance to inhibition by NaF may indicate that the two are not identical.

Attempts to demonstrate a requirement for an enzyme in the development of resistance to NaF have been unsuccessful. We are familiar with the difficulties in removing trace amounts of the transfer enzymes from ribosomes. It is possible that a relatively stable enzyme tightly bound to the ribosomes might remain in the ribosomal fraction, at least in sufficient amounts to provide the activity noted in the transfer system. However, additional washing of the ribosomes with buffer or deoxycholate have failed to indicate a requirement for a soluble factor, even though a loss in the capacity of the ribosomes to promote polymerization has been noted.

An interesting model for considering the mechanism of NaF inhibition of protein synthesis is found in the NaF inhibition of the reaction catalyzed by phosphoglucomutase. Najjar[14] demonstrated the formation of an inactive magnesium fluorophosphate-enzyme complex in which the phosphate in the complex was part of the substrate, glucose-1-phosphate, and probably its conversion product, glucose-6-phosphate. Similar complexes involving phosphate of one or more of the RNA components of the system might be formed. A 5′ terminal phosphate of mRNA is a particularly interesting possibility. The formation of a complex involving poly U, phenylalanyl-sRNA, and ribosomes, in which some stabilizing phosphate-magne-

sium-phosphate bridges between RNA chains are replaced by complexes of the phosphate-magnesium-fluoride type, might lead to inhibition of nonenzymatic binding due to the formation of a relatively unstable complex, as well as block reactions related to enzymatic binding.

Summary.—Evidence is presented from studies employing the reticulocyte transfer system that support earlier work interpreted to indicate that NaF can act as a specific inhibitor of protein synthesis by interfering with a reaction associated with the initiation of new peptide chains on ribosomes. NaF was found to inhibit the poly U-directed, nonenzymatic binding and the poly U-directed, GTP-dependent enzymatic binding of phenylalanyl-sRNA to ribosomes, thought to be the first enzymatic step in the biosynthesis of polyphenylalanine. Resistance to NaF inhibition could be established by preincubation of poly U, phenylalanyl-sRNA, and ribosomes under conditions that promoted nonenzymatic binding.

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[1] Marks, P. A., E. R. Burka, R. Rifkind, and D. Dannon, in *Cold Spring Harbor Symposia on Quantitative Biology*, vol. 28 (1963), p. 223.

[2] Colombo, B., L. Felicetti, and C. Baglioni, *Biochem. Biophys. Res. Commun.*, **18**, 389 (1965).

[3] Marks, P. A., E. R. Burka, F. M. Conconi, W. Perl, and R. A. Rifkind, these PROCEEDINGS, **53**, 1437 (1965).

[4] Lin, S., R. Mosteller, and B. Hardesty, *J. Mol. Biol.*, in press.

[5] Ravel, J. M., and B. A. Hardesty, *Federation Proc.*, **25**, 215 (1966).

[6] von Ehrenstein, G., and F. Lipmann, these PROCEEDINGS, **47**, 941 (1961).

[7] Bishop, J. O., and R. Schweet, *Biochim. Biophys. Acta*, **54**, 617 (1961).

[8] Arlinghaus, R., J. Shaeffer, and R. Schweet, these PROCEEDINGS, **51**, 1291 (1964).

[9] Nirenberg, M., and P. Leder, *Science*, **145**, 1399 (1964).

[10] Arlinghaus, R., G. Favelukes, and R. Schweet, *Biochem. Biophys. Res. Commun.*, **11**, 92 (1963).

[11] Schweet, R., R. Arlinghaus, R. Heintz, and J. Shaeffer, in *Nineteenth Annual Symposium on Fundamental Cancer Research*, M. D. Anderson Hospital and Tumor Institute (University of Texas Press, in press).

[12] Adams, J., and M. Capecchi, these PROCEEDINGS, **55**, 147 (1966).

[13] Webster, R., D. Engelhardt, and N. Zinder, these PROCEEDINGS, **55**, 155 (1966).

[14] Najjar, V., *J. Biol. Chem.*, **175**, 281 (1948).