[2] Caspari, E., *Advan. Genetics*, **2**, 2 (1948).
[3] Granick, S., in *Cytodifferentiation and Macromolecular Synthesis*, ed. M. Locke (New York: Academic Press, 1963), p. 144.
[4] Chun, E. H. L., N. H. Vaughan, Jr., and A. Rich, *J. Mol. Biol.*, **7**, 130 (1963).
[5] Sager, R., and M. R. Ishida, these PROCEEDINGS, **50**, 725 (1963).
[6] Edelman, M., C. A. Cowan, H. T. Epstein, and J. A. Schiff, these PROCEEDINGS, **52**, 1214 (1964).
[7] Kislev, N., H. Swift, and L. Bogorad, *J. Cell. Biol.*, **25**, 327 (1965).
[8] Lyttleton, J. W., and G. B. Petersen, *Biochim. Biophys. Acta*, **80**, 391 (1964).
[9] Jagendorf, A. T., and M. Avron, *J. Biol. Chem.*, **231**, 277 (1958).
[10] Marmur, J., *J. Mol. Biol.*, **3**, 208 (1961).
[11] Bolton, E. T., and B. J. McCarthy, these PROCEEDINGS, **48**, 1390 (1962).
[12] McCarthy, B. J., and E. T. Bolton, these PROCEEDINGS, **50**, 156 (1963).
[13] Hoyer, B. H., B. J. McCarthy, and E. T. Bolton, *Science*, **144**, 959 (1964).
[14] Shipp, W., and R. Haselkorn, these PROCEEDINGS, **52**, 401 (1964).
[15] Similar backgrounds were obtained when analogous material was annealed with *E. coli* DNA-agar. This result also indicates that the labeled DNA is not of bacterial origin. McCarthy and Bolton[12] have detected homology between *E. coli* DNA and many other bacterial species. The failure to observe annealing cited above does not, however, rule out the possibility that the minor components are derived from microbial species having little or no homology with *E. coli*.
[16] Gibor, A., and S. Granick, *Science*, **145**, 890 (1964).

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*SPECIFIC BINDING OF sRNA TO RIBOSOMES: EFFECT OF STREPTOMYCIN**

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Communicated by Louis B. Flexner, May 11, 1965

Although it is still questionable whether the action of streptomycin (SM) on ribosomes is related to the bactericidal activity of this antibiotic,[1] it has been generally accepted that at least one aspect of streptomycin action is on protein biosynthesis.[2–11] A recent report that streptomycin can upset the genetic code[11] prompted us to study the effect of streptomycin on the specific binding of sRNA to the ribosome template complex. In preceding communications we reported that specific sRNA attaches to the ribosome-messenger RNA complex.[12–14] The binding was specific in that only the amino acid sRNA coded for in the messenger RNA was induced to bind to the ribosomes. It was therefore of interest to examine the effect of streptomycin on this binding reaction. In this communication we report that streptomycin causes partial inhibition of binding of some sRNA to polynucleotide-ribosome complex, while it stimulates the binding of other sRNA. A preliminary account of this work has appeared.[15]

Materials and Methods.—Washed ribosomes, aminoacyl sRNA synthetase, *E. coli*-sRNA, and C^{14}-aminoacyl sRNA were prepared as described previously.[13] Polyuridylic acid (poly U); C^{14}-poly U; and copolymers of uridylic and adenylic acid (poly UA, 4:1); uridylic and guanylic (poly UG, 4:1); uridylic and cytidylic (poly UC, 5:1); uridylic, adenylic, and cytidylic (poly UAC, 8:1:1); and uridylic,

TABLE 1

EFFECT OF STREPTOMYCIN ON BINDING OF FREE PHENYLALANINE sRNA AND POLY U TO RIBOSOMES

Streptomycin	Phenylalanine sRNA (H^3) (cpm)	Poly U (C^{14}) (cpm)
0	77,100	4,039
20 μg/ml	46,000	4,355
100 μg/ml	35,550	4,579

The reaction mixture for binding of sRNA contained the following in μmole/0.6 ml: 50 Tris (pH 7.8); 11.2 magnesium acetate; 3.5 KCl; 2.0 β-mercaptoethanol; 2.0 ATP (K salt); 0.2 GTP; 4 phosphoenolpyruvate (Na salt). In addition it contained 10 μg of pyruvate kinase, 0.5 mg of sRNA, 0.26 mg of C^{14}-poly U, 22 mg of sucrose, and 1.5 mg of *E. coli* ribosomes (washed three times). The mixture was made at 0°C and 0.45 ml was placed on top of 4.7 ml of a linear sucrose gradient (5–20%) containing 0.1 *M* Tris-HCl (pH 7.8), 0.02 *M* magnesium acetate, and 0.05 *M* KCl. When streptomycin was added to the reaction mixture for the binding, the sucrose density gradient contained the same concentration of streptomycin. The tube was centrifuged for 1.5 hr at 37,000 rpm in the SW-39 rotor of the Spinco model L centrifuge at 2–5°C. After centrifugation, two-drop fractions were collected in test tubes. One-tenth ml was taken from each fraction and the amount of C^{14}-poly U was measured in each fraction as described.[13] The amount of phenylalanine sRNA in each fraction was determined by measuring the incorporation of H^3-phenylalanine into the fraction that was insoluble in cold TCA (trichloroacetic acid), but soluble in hot TCA. The reaction mixture for the assay of sRNA contained the following in μmole/0.5 ml: 50 Tris, pH 7.5; 0.25 puromycin; 2.4 magnesium acetate; 2 β-mercaptoethanol; 2 ATP (K salt); 4 phosphoenolpyruvate (Na salt). It also contained 50 μg of crystalline pyruvate kinase; 0.1 ml of each fraction from the density gradient centrifugation; 0.5 mg of aminoacyl sRNA synthetase; and H^3-phenylalanine. The reaction mixture was incubated for 10 min and the aminoacyl sRNA formed was counted.[13] The phenylalanine sRNA and C^{14}-poly U which sedimented faster than 70*S* particles were measured and regarded as ribosome-bound.

guanylic, and cytidylic (poly UGC, 8:1:1 were purchased from Miles Laboratory. A strain of *E. coli* which is resistant to streptomycin (B/SrII) was obtained from Dr. J. Flaks. Reaction mixtures for binding of free sRNA (discharged sRNA) as well as of aminoacyl sRNA were described previously.[13] The binding of aminoacyl sRNA was studied in some cases using the recently described method of Nirenberg and Leder.[16] Specific activities of radioactive materials used in this paper were as follows: C^{14}-poly U, 0.08 μc/mg; C^{14}-phenylalanine, 200 μc/μmole; C^{14}-isoleucine, 240 μc/mole; C^{14}-histidine, 132 μc/μmole; C^{14}-leucine, 246 μc/μmole; C^{14}-lysine, 222 μc/μmole; C^{14}-serine, 105 μc/μmole; C^{14}-tyrosine, 150 μc/μmole; C^{14}-valine, 166 μc/μmole; H^3-phenylalanine, 2450 μc/μmole. Counting efficiency was 10^6 cpm/μc for C^{14}, and 3×10^5 cpm/μc for H^3.

Results.—Effects of streptomycin on the binding of free sRNA and C^{14}-poly U to ribosomes: In the experiment shown in Table 1, binding of free phenylalanine sRNA to the poly U-ribosome complex was studied. Ribosomes, C^{14}-poly U, sRNA, and other factors were mixed, and the mixture was immediately centrifuged in a linear sucrose gradient. The ribosomes in this experiment were free from detectable amounts of aminoacyl sRNA synthetase and free amino acid. The sRNA used was deacylated as described previously.[13] The amount of bound phenylalanine sRNA in the absence of streptomycin was at least 50 per cent of the total phenylalanine sRNA added to the reaction mixture. The sRNA bound to

TABLE 2

COMPARISON OF STREPTOMYCIN EFFECT ON PHENYLALANINE sRNA AND ISOLEUCINE sRNA BINDING

Streptomycin	Phenylalanine sRNA Bound (cpm) Normal ribosomes	Phenylalanine sRNA Bound (cpm) Resistant ribosomes	Isoleucine sRNA Bound (cpm) Normal ribosomes	Isoleucine sRNA Bound (cpm) Resistant ribosomes
0	30,295	30,610	1,965	2,055
20 μg/ml	22,700	28,345	4,190	2,170

The experiment was carried out as in Table 1, except that nonlabeled poly U was used. Isoleucine sRNA and phenylalanine sRNA contents were measured in the fractions obtained after the density gradient centrifugation. Final magnesium concentration in the binding mixture in this experiment was 3×10^{-3} *M*. The bound sRNA sedimenting faster than 70*S* particle was measured.

the ribosomes was measured by determining the amino acid acceptor activity of each fraction after sucrose gradient centrifugation. The results show that streptomycin inhibits the binding of free phenylalanine sRNA to the poly U-ribosome complex. The attachment of C^{14}-poly U to ribosomes was not influenced by the presence of streptomycin. It can therefore be concluded that streptomycin acts on the binding process of sRNA, and not on the attachment of messenger RNA to ribosomes. In Table 2, streptomycin is shown to stimulate the binding of isoleucine sRNA to the poly U-ribosome complex. The inhibitory effect of streptomycin on phenylalanine sRNA attachment in this experiment was less than that in Table 1. This is perhaps due to the difference in the concentration of magnesium ion. It should be recalled that the effect of streptomycin on polypeptide synthesis is greatly dependent on the concentration of magnesium.[11] The present observation that streptomycin stimulated the binding of isoleucine sRNA parallels the report[11] that incorporation of isoleucine into the hot TCA- (trichloroacetic acid) insoluble fraction in the presence of poly U is stimulated by streptomycin. As shown in Table 2, streptomycin had very little effect on the binding of phenylalanine sRNA (8% inhibition) and even less effect on the binding of isoleucine sRNA when ribosomes prepared from a streptomycin-resistant strain were used.

Effect of streptomycin on the binding of aminoacyl sRNA: The data in Table 3 indicate that streptomycin has a similar effect on the binding of aminoacyl sRNA as it does on the binding of free sRNA. Streptomycin inhibited the binding of phenylalanyl sRNA, whereas the antibiotic stimulated the binding of isoleucyl sRNA to the poly U-ribosome complex. The effect was observed both at 0.02 *M* and 0.03 *M* Mg^{++} concentrations. It should be pointed out that neither stimulation nor inhibition was observed when ribosomes from a SM-resistant strain were used.

A similar result was obtained, as shown in Figure 1, when the effect of streptomycin on the binding of phenylalanyl sRNA was studied using the sucrose density gradient centrifugation technique. The amount of phenylalanyl sRNA bound to the particles sedimenting faster than 70*S* ribosomes was markedly decreased in the presence of streptomycin. It is clear that the position of the peak of bound phenylalanyl sRNA was not changed appreciably by the presence of streptomycin, indicating that ribosomal aggregates were still formed even in the presence of streptomycin.

TABLE 3

EFFECT OF STREPTOMYCIN ON THE AMINOACYL sRNA ATTACHMENT TO THE POLY U-RIBOSOME COMPLEX

Additions and deletions	Magnesium acetate	Normal Ribosomes (cpm)		Resistant Ribosomes (cpm)	
		C^{14}-Phe-sRNA	C^{14}-Isoleu-sRNA	C^{14}-Phe-sRNA	C^{14}-Isoleu-sRNA
Complete	0.02 *M*	2,775	454*	5,122	655
−Poly U	0.02 *M*	178	58*	309	99
+SM	0.02 *M*	1,830	650*	5,715	600
Complete	0.03 *M*	3,317	215	5,251	404
−Poly U	0.03 *M*	404	155	255	89
+SM	0.03 *M*	2,529	370	5,342	374

The reaction mixture for binding was the same as in Table 2, except that total volume was 50 μl and contained C^{14}-aminoacyl sRNA instead of free sRNA. The reaction was carried out at 0°C and started by addition of 5,700 cpm of C^{14}-phenylalanyl sRNA or 4,000 cpm of C^{14}-isoleucyl sRNA. Normal ribosomes (0.3 mg) or SM-resistant ribosomes (0.53 mg) were used per 50 μl of the reaction mixture. One minute after addition of aminoacyl sRNA, 3 ml of buffer (0.1 *M* Tris-HCl, pH 7.1, 0.02 *M* or 0.03 *M* magnesium acetate, 0.05 *M* KCl) was added to the reaction mixture. The mixture was passed through a cellulose nitrate Millipore filter (pore size 0.45 μ) and ribosome-bound aminoacyl sRNA was counted as described.[16]

* Experimental conditions of these experiments were as described in Table 4 (incubation for 10 min).

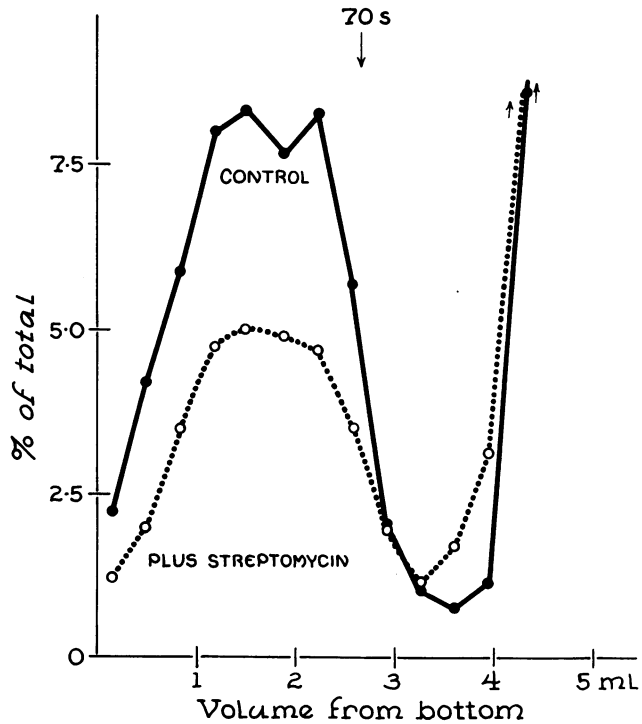

FIG. 1.—Effect of streptomycin on the binding of C^{14}-phenylalanyl sRNA to poly U-ribosome complex. Experimental conditions were identical to those in Table 2 except the following: 64,000 cpm of C^{14}-phenylalanyl sRNA (instead of free sRNA), and 3 mg of ribosomes were used; the pH and magnesium ion concentration for both the reaction mixture and the sucrose density gradient were 7.1 and 0.02 *M*, respectively. When streptomycin was added to the reaction mixture for binding, the sucrose gradient contained the same concentration of antibiotic (20 μg/ml). The bound phenylalanyl sRNA was measured by counting the radioactivity of each fraction which was soluble in hot TCA but insolbule in cold TCA. The values are expressed as percentage of total phenylalanyl sRNA recovered.

Effect of streptomycin on the binding of phenylalanyl and isoleucyl sRNA to the complex of ribosomes and U-containing copolymers: It has been shown that phenylalanine is incorporated into the polypeptide chain under the direction not only of poly U, but also of many U-containing copolymers.[17, 18] It was of interest, therefore, to see if the effect of streptomycin would be manifested when a copolymer was used. As shown in Table 4, the binding of phenylalanyl sRNA induced by a U-containing copolymer was inhibited in all cases, and the binding of isoleucyl sRNA was likewise stimulated under these conditions, indicating that the presence of nucleotides other than uridylic acid in the polynucleotide chain did not alter the action of streptomycin.

Effect of streptomycin on the binding of other aminoacyl sRNA's in the presence of various polynucleotides: In the hope of obtaining a general rule as to stimulation or inhibition by streptomycin, experiments were carried out on the effect of this antibiotic on the binding of aminoacyl sRNA other than isoleucyl or phenylalanyl sRNA to the complex of ribosomes and various polynucleotides. Table 5 summarizes results of such studies. Bindings of leucyl and seryl sRNA to U-con-

TABLE 4

EFFECT OF STREPTOMYCIN ON THE BINDING OF PHENYLALANYL OR ISOLEUCYL sRNA IN THE PRESENCE OF U-CONTAINING COPOLYMERS

Polynucleotide	Aminoacyl sRNA tested	Bound Aminoacyl sRNA (cpm) SM 0 μg	SM 20 μg/m
None	Phenylalanyl sRNA	183	—*
None	Isoleucyl sRNA	60	—*
UA	Phenylalanyl sRNA	2,070	1,490
UA	Isoleucyl sRNA	369	480
UC	Phenylalanyl sRNA	2,892	1,998
UC	Isoleucyl sRNA	295	429
UGC	Phenylalanyl sRNA	2,237	1,192
UGC	Isoleucyl sRNA	253	380
UAC	Phenylalanyl sRNA	2,441	1,594
UAC	Isoleucyl sRNA	436	524

The reaction mixture contained 0.1 *M* Tris-HCl, pH 7.1, 0.02 *M* magnesium acetate, 0.05 *M* KCl, 20 μg of polynucleotide, 0.2 mg of washed ribosomes, and 5,700 cpm of C^{14}-phenylalanyl sRNA, or 4,000 cpm of isoleucyl sRNA. The reaction mixture was incubated for 10 min at 0°C and analyzed by the Millipore filter method for the ribosome-bound aminoacyl sRNA.[16]

* Binding of sRNA in these experiments was not measured.

taining copolymers were significantly stimulated by streptomycin when an appropriate magnesium concentration was present. There was a slight stimulation of lysyl sRNA to poly A-ribosome complex, but this effect could not be observed when the concentration of magnesium ion was 0.01 *M* where the poly A-dependent binding of lysyl sRNA was at maximum. The magnesium concentration in general has a large influence on the effect of streptomycin. For example, the stimulatory effect of SM on seryl sRNA binding to poly U-ribosome complex could not be observed in 0.01 *M* magnesium acetate. Streptomycin inhibited the binding of valyl sRNA to poly UG-ribosome complex significantly in 0.01 *M* magnesium acetate, whereas it had no effect in 0.03 *M* magnesium acetate. There was no effect of SM under the same conditions as described in Table 5, when the following combinations of aminoacyl sRNA and polynucleotides were studied: histidyl sRNA and poly U or poly UAC; leucyl sRNA and poly A or poly C; lysyl sRNA and poly U, or poly UA,

TABLE 5

EFFECT OF STREPTOMYCIN ON THE BINDING OF AMINOACYL sRNA OTHER THAN PHENYLALANYL AND ISOLEUCYL sRNA

C^{14}-Aminoacyl sRNA	Polynucleotide added	Streptomycin (20 μg/ml)	Bound sRNA (cpm) Mg^{++} 0.01 *M*	0.02 *M*	0.03 *M*
Leu	None	−	430	440	485
Leu	None	+	380	428	516
Leu	U	−	979	1,291	1,176
Leu	U	+	1,286	1,662	1,379
Leu	UC	−	1,023	1,763	1,525
Leu	UC	+	1,412	2,259	1,750
Lys	None	−	429	—*	440
Lys	None	+	387	—*	443
Lys	A	−	5,772	1,967	668
Lys	A	+	5,511	2,392	743
Ser	None	−	76	—*	243
Ser	None	+	93	—*	232
Ser	U	−	223	343	288
Ser	U	+	236	398	417
Val	None	−	62	—*	251
Val	None	+	39	—*	269
Val	UG	−	786	—*	1,096
Val	UG	+	354	—*	1,123

The binding of aminoacyl sRNA was studied as in Table 4 except that the reaction mixture was incubated for 20 min at 24°C. The following amounts of aminoacyl sRNA were added to 50 μl of the reaction mixture: 13,416 cpm of leucyl sRNA, 10,000 cpm of lysyl sRNA, 4,468 cpm of seryl sRNA, and 7,557 cpm of valyl sRNA.

* Not measured.

or poly C; seryl sRNA and poly UC, or poly UCG; tyrosyl sRNA and poly U, or poly UA, or poly A; valyl sRNA and poly U. These results show that even the bindings of aminoacyl sRNA induced by polynucleotide of the corresponding codewords may not necessarily be influenced by streptomycin.

Comments.—It has been shown that the 30*S* portion of ribosomes is responsible for binding of messenger RNA[3, 21] and the 50*S* unit of ribosomes is responsible for binding of sRNA.[19] Therefore, for specific binding of sRNA to ribosomes, both 50 *S* and 30 S particles are necessary.[20] It has been established that the 30*S* part of the ribosome is the site of action of streptomycin.[3, 10] The present observations therefore suggest that streptomycin binds to the 30*S* subunits together with messenger RNA in such a way that binding of sRNA is altered and the amount of bound messenger RNA is not.[3, 10] The observation that free sRNA as well as aminoacyl sRNA bindings are similarly influenced strongly supports the concept that streptomycin acts at the level of interaction between sRNA and messenger RNA before the peptide bond synthesis step.

Summary.—Streptomycin inhibits the binding of free phenylalanine sRNA as well as charged phenylalanyl sRNA to the ribosome-poly U complex. In contrast, it stimulates the binding of free isoleucine sRNA and isoleucyl sRNA to the complex. It inhibits the binding of valyl sRNA to poly UG-ribosome complex while it stimulates the binding of leucyl sRNA to the complex of ribosomes and poly U or poly UC. It also stimulated the binding of seryl sRNA to poly U-ribosome complex. The effect of streptomycin was strongly dependent on magnesium concentration. The attachment of C^{14}-poly U to ribosomes was not inhibited by streptomycin.

After this paper was written, similar studies on streptomycin effect were independently reported by Pestka *et al.*[22] The authors wish to thank Drs. J. Flaks and M. W. Nirenberg for reviewing this paper.

* Supported by grant GM-12053 of the U.S. Public Health Service, the National Science Foundation (GB-2253), and American Cancer Society grant (IN-49F).

† Established Investigator of Helen Hay Whitney Foundation.

[1] Stern, J. L., and S. S. Cohen, these PROCEEDINGS, **51**, 883 (1964).

[2] Brownstein, B., these PROCEEDINGS, **52**, 1045 (1964).

[3] Cox, E. C., J. R. White, and J. G. Flaks, these PROCEEDINGS, **48**, 684 (1962).

[4] Flaks, J. G., E. C. Cox, and J. R. White, *Biochem. Biophys. Res. Commun.*, **7**, 385 (1962).

[5] Speyer, J. F., P. Lengyel, and C. Basilio, these PROCEEDINGS, **48**, 684 (1962).

[6] Kirk, J. T. O., *Biochim. Biophys. Acta*, **59**, 476 (1962).

[7] Flaks, J. G., E. C. Cox, M. L. Witting, and J. R. White, *Biochem. Biophys. Res. Commun.*, **7**, 390 (1962).

[8] Mager, J., M. Benedict, and M. Artman, *Biochim. Biophys. Acta*, **62**, 202 (1962).

[9] Erdös, T., and A. Ullman, *Nature*, **183**, 618 (1959).

[10] Davies, J. E., these PROCEEDINGS, **51**, 659 (1964).

[11] Davies, J. E., W. Gilbert, and L. Gorini, these PROCEEDINGS, **51**, 883 (1964).

[12] Kaji, A., and H. Kaji, *Biochem. Biophys. Res. Commun.*, **13**, 186 (1963).

[13] Kaji, H., and A. Kaji, these PROCEEDINGS, **52**, 1541 (1964).

[14] Kaji, A., and H. Kaji, *Federation Proc.*, **23**, 478 (1964).

[15] Kaji, H., and A. Kaji, *Federation Proc.*, **24**, 1540 (1965).

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

[17] Nirenberg, M. W., J. H. Matthaei, O. W. Jones, R. G. Martin, and S. H. Barondes, *Federation Proc.*, **22**, 55 (1963).

[18] Ochoa, S., *Federation Proc.*, **22**, 62 (1963).

[19] Cannon, M., R. Krug, and W. Gilbert, *J. Mol. Biol*, **7**, 360 (1963)

[20] Kaji, A., and H. Kaji, *Biochim. Biophys. Acta*, **87**, 519 (1964).

[21] Okamoto, T., and M. Takanami, *Biochim. Biophys. Acta*, **68**, 325 (1963).
[22] Pestka, S., R. Marshall, and M. W. Nirenberg, these PROCEEDINGS, **53**, 639 (1965).

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*MEMBRANE SUBUNITS OF MYCOPLASMA LAIDLAWII AND THEIR ASSEMBLY TO MEMBRANELIKE STRUCTURES**

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Communicated by G. Evelyn Hutchinson, June 1, 1965

Mycoplasma (pleuropneumonialike organisms, PPLO) are bounded by a thin lipoprotein "unit membrane."[1] The high sensitivity of *Mycoplasma laidlawii* to osmotic lysis[2, 3] allows the isolation of cell membranes in large quantities and in a rather pure state. Previous analysis of *M. laidlawii* membranes[4] showed them to be composed of 47–60 per cent protein, 35–37 per cent lipid, 4–7 per cent carbohydrate and small amounts of RNA and DNA. Subsequent improvements in isolation and washing procedures of the membranes yielded material composed almost exclusively of protein and lipid. These lipids contain yellow-colored carotenoid pigments, which serve as a convenient marker for membrane material.[4, 5] The high purity of the isolated membranes of *M. laidlawii* prompted us to use them as models for the study of the "unit membrane" substructure.

The sensitivity of *M. laidlawii* membranes to dissolution by detergents, such as sodium lauryl sulfate,[1] suggested the use of detergents for disaggregation of the lipoprotein membrane to smaller units. It was found that detergents disaggregated *M. laidlawii* membranes into rather uniform subunits composed of lipid and protein, and that these subunits could be reaggregated in the presence of di- or multivalent cations to form structures which by the criteria of electron microscopy were very similar to the original membranes.

Materials and Methods.—Organism and growth conditions: Mycoplasma laidlawii strain B was used throughout the experiments reported here. The organism was grown in tryptose broth consisting (per liter) of: bacto-tryptose, 20 gm; NaCl, 5 gm; Tris (hydroxymethyl) aminomethane, 5 gm; bacto-PPLO serum fraction, 10 ml; penicillin G (crystalline) 50,000 units. The final pH of the medium was about 8.3 without adjustment. Growth was carried out in 1-liter vol of medium dispensed in 2-liter flasks incubated statically at 37°. The organisms were harvested at the end of the logarithmic phase of growth (16–24 hr incubation) by centrifugation at 9000 × *g* for 15 min. The sedimented cells were then washed twice in β-buffer[6] (NaCl, 0.156 *M;* Tris, 0.05 *M;* 2-mercaptoethanol, 0.010 *M* in deionized water, adjusted to pH 7.4 with HCl).

Isolation of cell membranes: The sedimented washed organisms (about 0.5 gm wet weight) were osmotically lysed by resuspension in 40 ml of β-buffer diluted 1:20 in deionized water.[6] The suspension was incubated at 37° for 30 min and then centrifuged at 37,000 × *g* for 40 min to sediment the membranes. The membranes were washed twice in 1:20 β-buffer, resuspended in a small volume of the dilute buffer, and kept at −20° until used. Storage of the membrane suspensions at −20° for several days did not affect any of their properties studied in the present investigation.

Assessment of membrane dissolution by detergents: Various amounts of the tested detergents were added to 3 ml membrane suspension in 1:20 β-buffer. The degree of membrane dissolution was estimated by measuring the decrease in optical density of the membrane suspension at wavelengths 400–700 mμ, using a Bausch and Lomb Spectronic 505 spectrophotometer.