Binding of Rat Liver and Hepatoma Polyribosomes to Stripped Rough Endoplasmic Reticulum *in vitro*

BIOLOGICAL OR AN ARTIFACT?

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Exposed thiol groups do not appear to be related to the binding of ^{32}P -labelled polyribosomes to stripped rough endoplasmic reticulum *in vitro*. Treating stripped rough endoplasmic reticulum with GSSG did not diminish binding of polyribosomes, suggesting that binding *in vitro* has no correlation with the inhibition of protein synthesis *in vitro* reported by Kosower *et al.* (1971). Thiol reagents, which are known to dissociate ribosomes, did not significantly decrease binding of ^{32}P -labelled polyribosomes to stripped rough endoplasmic reticulum. Denaturing the protein of ^{32}P -labelled polyribosomes or stripped rough endoplasmic reticulum of liver or hepatoma with heat, trichloroacetic acid, or HClO_4 did not alter the binding *in vitro*. Therefore, the practice of measuring the binding of ^{32}P -labelled polyribosomes to stripped rough endoplasmic reticulum *in vitro* (Shires *et al.*, 1971*b*) is an unsuitable indicator of biological significance in the intact cell.

The binding of polyribosomes to stripped endoplasmic reticulum *in vitro* as measured by ultracentrifugation has been described as being relevant to biological function *in vivo* in eukaryotic cells (Suss *et al.*, 1966; Campbell, 1970; Ragland *et al.*, 1971; Shires *et al.*, 1971*a,b*). The disulphide-interchange enzyme activity has also been utilized as an indirect measurement of polyribosome binding to endoplasmic reticulum (James *et al.*, 1969; Williams & Rabin, 1969; Sunshine *et al.*, 1971; Blyth *et al.*, 1971). Shires *et al.* (1971*b*) have speculated that the affinity binding of polyribosomes to degranulated or 'stripped' rough endoplasmic reticulum and the binding via disulphide formation are similar and that these binding interactions observed *in vitro* are functional *in vivo*. Blobel & Sabatini (1971) have utilized dissociated polyribosomes stripped from rough endoplasmic reticulum by puromycin and KCl to study amino acid incorporation and poly(U)-directed polyphenylalanine synthesis. Further, it has been suggested that thiol groups are important for protein synthesis (Furano & Harris, 1971; Bermek *et al.*, 1971; Kosower *et al.*, 1971; Zehavi-Willner *et al.*, 1971) and in maintaining the integrity of the polyribosome and ribosome structure (Traut & Haenni, 1967; Dunn, 1970; Moore, 1971; Ranu & Kaji, 1971).

The binding assay using polyribosomes and stripped rough endoplasmic reticulum membrane, as used in Pitot's laboratory (Ragland *et al.*, 1971),

was chosen as the preferred procedure in an attempt to study hormonal factors affecting protein synthesis. The present paper deals with the problems involved in utilizing results derived from this assay in making interpretations that can be related to biological function *in vivo* (Shires *et al.*, 1971*b*).

Experimental

Liver cell fractionation

Fed female Sprague–Dawley rats (180–200g), or Buffalo strain rats (weight dependent on kind of tumour) with Morris hepatomas, were used except as noted for polyribosomes. Rats were decapitated and the livers and/or hepatomas were removed, minced and homogenized in 3 vol. of ice-cold 0.25M-sucrose–TKM medium (50mM-tris–chloride, 25mM-KCl, 10mM-MgCl₂, pH7.5). The homogenate was centrifuged at 17000g for 15min to obtain the post-mitochondrial supernatant fraction.

Preparation of endoplasmic reticulum

Endoplasmic reticulum was prepared from the postmitochondrial supernatant fraction as described by Sunshine *et al.* (1971), except that MgCl₂ content of the TKM medium was increased from 5 to 10mM (Dice & Schimke, 1972).

Stripped rough endoplasmic reticulum

The procedures of Roobol & Rabin (1971) (KCl and EDTA, method C) and Ragland *et al.* (1971) (pyrophosphate and citric acid, method B) were used to strip polyribosomes from the rough endoplasmic reticulum that had been prepared as described by Sunshine *et al.* (1971).

Polyribosome preparation

Polyribosomes were prepared as described by Ragland *et al.* (1971) (method B) and by Sunshine *et al.* (1971) (method A) from rats that had been injected intraperitoneally with 1 mCi of carrier-free [32 P]P_i (New England Nuclear Corp., Boston, Mass., U.S.A.)/100 g body wt. and then starved for 20 h. Tumour-bearing rats were not starved. Electron micrographs (not shown here) demonstrated that there was no significant membrane contamination of polyribosomes.

Determination of protein, RNA and radioactivity

Protein was determined by the method of Lowry *et al.* (1951), with bovine albumin as standard. RNA was determined by the method of Bloemendal *et al.* (1967) where 1 mg/ml corresponds to E_{260} 22 rather than the E_{260} 32 of Ragland *et al.* (1971). Therefore, the RNA/protein numerical values reported in this paper are greater than those of Ragland *et al.* (1971) and Shires *et al.* (1971a,b). For measuring radioactivity associated with free or membrane-bound polyribosomes, the centrifuged pellets were dissolved first in 0.05 ml of Triton X-100, followed by 0.1 ml of 1 M-NaOH. Duplicate samples (50 μ l) on paper were counted for radioactivity as described by Hochberg *et al.* (1972). Efficiency of radioactivity counting on paper was 60%.

Denaturation of proteins of polyribosomes and stripped rough endoplasmic reticulum

Polyribosomes or stripped rough endoplasmic reticulum (approx. 3 mg of protein) in a volume of 0.5 ml were denatured with 5 ml of 10% (w/v) trichloroacetic acid or HClO₄ or placed in a boiling-water bath for 5 min. The denatured protein was pelleted at 1700g for 20 min, resuspended in the original volume of 0.25 M-sucrose-TKM medium by homogenization and adjusted to pH 7.5 with 6 M-NaOH.

Reaction mixtures

All reaction mixtures and conditions are described in the Table and Figure legends. Unbound polyribosomes in the reaction mixture were separated from

rough endoplasmic reticulum in a no. 40 Spinco rotor as noted in the legends to the Tables or Figures, by using the gradient shown in Fig. 1.

Special chemicals

Chemicals were obtained as follows: 2-mercaptoethanol, Eastman Organic Chemicals, Rochester, N.Y., U.S.A.; dithiothreitol, Aldrich Chemical Co., Milwaukee, Wis., U.S.A.; GSH, GSSG, *N*-ethylmaleimide, *p*-chloromercuribenzoic acid, 5,5'-dithio-bis-(2-nitrobenzoic acid), Sigma Chemical Co., St. Louis, Mo., U.S.A.; iodoacetamide, iodoacetic acid, Schwarz/Mann, Orangeburg, N.Y., U.S.A.; diazine-dicarboxylic acid bis-dimethylamide (diamide), Calbiochem, Los Angeles, Calif., U.S.A.; diphenyliodonium chloride, Professor R. B. Sandin, University of Alberta, Alberta, Canada.

Calculations

In the Tables of the present paper, the percentage of the total 32 P-labelled polyribosomes bound to stripped rough endoplasmic reticulum was calculated on the basis of the total polyribosomes added. For comparison, β in Table 4 was calculated as described by Shires *et al.* (1971b), where $\beta = 100 (M - C)/(M + P)$, M being the membrane-associated radioactivity on the 2.0 M-sucrose layer, P the radioactivity of the unbound ribosomal pellet, and C the radioactivity in a control tube containing polyribosomes only, at the exact position of the membrane-associated radioactivity (Fig. 1). The values of C amounted to 1.6–3.0%, 0.3–5.0%, 4.0–6.1%, and 0.1–0.2% for polyribosomes, heat-denatured polyribosomes, trichloroacetic acid-denatured polyribosomes and HClO₄-denatured polyribosomes, respectively, in Table 4.

Results

Liver polyribosomes and stripped rough endoplasmic reticulum were isolated by two different procedures, i.e. as described from Rabin's laboratory (Roobol & Rabin, 1971, method A; Sunshine *et al.*, 1971, method C) and Pitot's laboratory (Ragland *et al.*, 1971, method B). There was no apparent difference in binding of liver polyribosomes with stripped liver rough endoplasmic reticulum isolated by either procedure (Table 1) in 0.25 M-sucrose-TKM medium, pH 7.5. In the presence of an amino acid mixture and ATP-regenerating system (Bloemendal *et al.*, 1967), liver polyribosomes from either source were bound to a greater extent by the liver rough endoplasmic reticulum prepared by method C than by that prepared by method B. On the basis of these results, only polyribosomes and rough endoplasmic reticulum prepared by methods

Table 1. *Binding and cross-binding of polyribosomes to stripped endoplasmic reticulum isolated from livers of normal rats by two different methods*

Polyribosomes were prepared by either method A (Sunshine *et al.*, 1971) or method B (Ragland *et al.*, 1971) and stripped rough endoplasmic reticulum by either method C (Roobol & Rabin, 1971) or method B (Ragland *et al.*, 1971) from the livers of normal Sprague-Dawley female rats. All reaction mixtures contained 0.5 mg of stripped rough endoplasmic reticulum protein, ^{32}P -labelled polyribosomes equivalent to 100 μg of RNA (specific radioactivity; method A, 193 000; method B, 165 000 c.p.m./mg of protein) and 0.5 mg of postmicrosomal supernatant protein. Single incubations in Reaction I and duplicate incubations in Reaction II were done in 2 ml of 0.25 M-sucrose-TKM medium, pH 7.5, for 1 min at 37°C. In addition, Reaction II contained the amino acid mixture and ATP-regenerating system of Bloemendal *et al.* (1967). RNA/protein ratios were: smooth endoplasmic reticulum, 0.21; rough endoplasmic reticulum, 0.51; stripped rough endoplasmic reticulum (method C), 0.19; stripped rough endoplasmic reticulum (method B), 0.18. For polyribosomes the E_{260}/E_{280} ratio was: method A, 1.8; method B, 1.8. Unbound polyribosomes in the reaction mixture were separated from rough endoplasmic reticulum by centrifugation at 105 000g for 90 min.

Source of polyribosomes ... Source of stripped rough endoplasmic reticulum	Percentage of total ^{32}P -labelled polyribosomes bound to stripped endoplasmic reticulum			
	Method A		Method B	
	Single value or mean	Range	Single value or mean	Range
Reaction I				
Method B	18.9	—	13.4	—
Method C	14.5	0.5	15.3	1.0
Reaction II				
Method B	9.9	—	11.7	—
Method C	15.5	3.7	15.3	0.7

A and C respectively was used in subsequent experiments.

Since the binding of polyribosomes to stripped rough endoplasmic reticulum seemed to be a protein-protein interaction (Frieden, 1971), polyribosomes were added to the reaction mixture in the experiments described below on the basis of protein rather than RNA, as was done by Ragland *et al.* (1971).

The exposure of stripped rough endoplasmic reticulum from liver to GSSG or GSH, thiol-oxidizing and thiol reagents for 2 h at 37°C before the addition of liver polyribosomes did not significantly affect binding except that diazinedicarboxylic acid bis-dimethylamide significantly decreased binding (Table 2). The apparent ineffectiveness of these treatments to change the percentages of polyribosomes bound might have resulted from incomplete separation of the reaction mixture. Therefore, the total centrifugal field-time used to separate unbound polyribosomes from liver stripped rough endoplasmic reticulum in the reaction mixture was increased from 157 000g-h in Tables 1 and 2 to 2 100 000g-h for the subsequent experiments. The decrease in binding shown in Table 3 either is due to the starved condition of the liver from hepatoma-bearing rats or reflects the increase in centrifugal force, as reported by Lowe *et al.* (1970).

There is a possibility that angle-rotor centrifugation

might trap unbound ^{32}P -labelled polyribosomes within the endoplasmic reticulum, whereas Ragland *et al.* (1971) and Shires *et al.* (1971a,b) used swinging-bucket rotor centrifugation, allowing possibly more complete separation. However, T. K. Shires (personal communication) reports that the no. 50 Ti Spinco angle rotor was also used successfully for separation of polyribosomes and endoplasmic reticulum.

Heat-denatured liver polyribosomes or stripped rough endoplasmic reticulum from liver of hepatoma-bearing rats (Table 3) gave results similar to those from normal rats. Heat-denaturing the protein of hepatoma polyribosomes increased significantly the binding to stripped rough endoplasmic reticulum from hepatoma. Treating stripped rough endoplasmic reticulum from liver of hepatoma-bearing rats or from the hepatoma with diazinedicarboxylic acid bis-dimethylamide failed to affect significantly the binding of the corresponding polyribosomes as it did with the membranes from normal rats (Table 2). The inability of diazinedicarboxylic acid bis-dimethylamide to decrease binding, as shown in Table 2, may be due to differences in stripped rough endoplasmic reticulum from liver of normal rats and hepatoma-bearing rats, since we observed lower yields of rough endoplasmic reticulum from livers of hepatoma-bearing rats. Liver polyribosomes were

Table 2. *Effect of glutathione, thiol-oxidizing and thiol reagents on the ability of stripped endoplasmic reticulum to bind polyribosomes*

Polyribosomes and stripped rough endoplasmic reticulum were prepared from livers of normal Sprague-Dawley rats as described by Roobol & Rabin (1971) (method C) and Sunshine *et al.* (1971) (method A). Triplicate reaction mixtures of 2ml of 0.25M-sucrose-TKM medium, pH7.5, containing 0.5mg of stripped rough endoplasmic reticulum protein, 2.84mg of postmicrosomal supernatant protein and 5mM-thiol reagent were incubated for 2h at 37°C. Immediately, ^{32}P -labelled polyribosomes equivalent to 210 μg of protein (specific radioactivity 202000 c.p.m./mg of protein) were added, mixed and allowed to incubate for an additional 1 min. RNA/protein ratios were: smooth endoplasmic reticulum, 0.12; rough endoplasmic reticulum, 0.43; stripped rough endoplasmic reticulum, 0.16. For polyribosomes the E_{260}/E_{280} ratio was 1.65. Unbound polyribosomes in the reaction mixture were separated from rough endoplasmic reticulum by centrifugation at 105 000g for 90 min. *Significantly different from control, $P < 0.01$.

Treatment	Percentage of total ^{32}P -labelled polyribosomes bound to stripped rough endoplasmic reticulum (mean $\pm$ S.D.)
Control	24.4 $\pm$ 1.6
2-Mercaptoethanol	25.4 $\pm$ 0.5
GSH	24.4 $\pm$ 3.2
GSSG	23.6 $\pm$ 2.1
Dithiothreitol	27.2 $\pm$ 2.7
Iodoacetamide	23.2 $\pm$ 2.5
Iodoacetic acid	25.9 $\pm$ 1.2
N-Ethylmaleimide	22.4 $\pm$ 4.8
p-Chloromercuribenzoic acid	22.2 $\pm$ 2.7
5,5'-Dithiobis-(2-nitrobenzoic acid)	27.0 $\pm$ 1.3
Diphenyliodonium chloride	21.3 $\pm$ 2.0
Diazinedicarboxylic acid bis-dimethylamide	18.5 $\pm$ 1.4*

bound to stripped rough endoplasmic reticulum from hepatoma to a significantly lesser extent than were the hepatoma polyribosomes.

Denaturing the protein of stripped rough endoplasmic reticulum from liver by heat, trichloroacetic acid or HClO_4 did not significantly decrease the binding of normal liver polyribosomes (Table 4). Likewise, denatured liver polyribosomes bound untreated stripped rough endoplasmic reticulum from liver as effectively as did undenatured liver polyribosomes (Table 4).

In fact, trichloroacetic acid denaturation of polyribosomes and/or stripped rough endoplasmic reticulum appears to be an effective method to increase the percentage of the total polyribosomes bound. However, when the binding assay was carried out with the same preparations on the next day, trichloroacetic acid-denatured polyribosomes or rough endoplasmic reticulum were bound to a lesser extent to the corresponding untreated component than were those components denatured by other means. The reason for this phenomenon is unknown. Similar results were obtained when binding values (β) were calculated as described by Shires *et al.* (1971b), except that HClO_4 treatment of liver polyribosomes or both liver polyribosomes and stripped endoplasmic reticulum from liver slightly enhanced binding. In

other experiments, increasing the protein concentration of stripped rough endoplasmic reticulum from liver from 140 μg to 700 μg resulted in a linear relationship of binding of liver polyribosomes up to 500 μg , which was similar to that reported by Ragland *et al.* (1971).

The distribution of radioactivity on the gradient and the overall recovery of radioactivity were similar when either normal or heat-treated stripped rough endoplasmic reticulum from liver was allowed to bind to liver polyribosomes (Fig. 1). Our inability to recover more than 70–75% of the total radioactivity added may result from radioactivity remaining bound to the polycarbonate tube, since only the radioactivity of the fractions and pellet was determined. Suss *et al.* (1966) reported $90 \pm 10\%$ recovery. However, neither Ragland *et al.* (1971) nor Shires *et al.* (1971b) report recovery values.

Discussion

The results of our experiments cast doubt as to the biological significance of experiments in which ^{32}P -labelled polyribosome binding to stripped rough endoplasmic reticulum is measured. Binding as measured by the use of cell organelles prepared by the methods of Ragland *et al.* (1971) or Rabin and

Table 3. *Effect of heat denaturation, glutathione and thiol reagents on the binding of liver and hepatoma polyribosomes to stripped rough endoplasmic reticulum*

Polyribosomes and stripped rough endoplasmic reticulum were prepared from the host liver and Morris hepatoma 5123tc of Buffalo rats as described in Table 2. Duplicate reactions with 0.5mg of stripped rough endoplasmic reticulum protein, ^{32}P -labelled polyribosomes equivalent to $150\mu\text{g}$ of protein, and 1.3mg of postmicrosomal supernatant protein were carried out as described in Table 2. The specific radioactivities of liver and hepatoma polyribosomes were 366000 and 494000 c.p.m./mg of protein respectively. RNA/protein ratios were: liver rough endoplasmic reticulum, 0.45; hepatoma rough endoplasmic reticulum, 0.51; liver stripped rough endoplasmic reticulum, 0.16; hepatoma stripped rough endoplasmic reticulum, 0.15. For liver and hepatoma polyribosomes the E_{260}/E_{280} ratios were 1.46 and 1.74 respectively. Unbound polyribosomes in the reaction mixture were separated from rough endoplasmic reticulum by centrifugation at 105000g for 20h. 'Range' is the difference between the highest and lowest values.

Source and treatment		Percentage of total ^{32}P -labelled polyribosomes bound to stripped rough endoplasmic reticulum	
Stripped rough endoplasmic reticulum	Polyribosomes	Mean	Range
None	Liver	0.7	0.2
Liver	Liver	14.9	3.1
Liver	Hepatoma	17.2	1.2
Liver (heat-denatured)	Liver	14.4	0.3
Liver	Liver (heat-denatured)	15.8	1.3
Liver (GSSG)	Liver	14.0	2.2
Liver (dithiothreitol)	Liver	14.3	5.4
Liver (<i>N</i> -ethylmaleimide)	Liver	16.5	0.9
Liver (diazinedicarboxylic acid bis-dimethylamide)	Liver	17.6	2.6
None	Hepatoma	0.6	0.3
Hepatoma	Hepatoma	16.5	1.8
Hepatoma	Liver	12.8	0.0
Hepatoma (heat-denatured)	Hepatoma	18.5	0.4
Hepatoma	Hepatoma (heat-denatured)	22.3	2.8
Hepatoma (GSSG)	Hepatoma	14.8	2.2
Hepatoma (dithiothreitol)	Hepatoma	13.9	2.6
Hepatoma (<i>N</i> -ethylmaleimide)	Hepatoma	12.9	3.2
Hepatoma (diazinedicarboxylic acid bis-dimethylamide)	Hepatoma	15.5	5.2

co-workers (Roobol & Rabin, 1971; Sunshine *et al.*, 1971) was not significantly different, except that stripped rough endoplasmic reticulum prepared by Rabin's procedures bound polyribosomes from either source to a greater extent than that prepared by the method of Ragland *et al.* (1971) in the presence of the amino acid mixture and ATP-regenerating system of Bloemendal *et al.* (1967).

Pretreatment of stripped rough endoplasmic reticulum with glutathione, thiol-oxidizing or thiol reagents for 2h at 37°C failed to alter the binding of ^{32}P -labelled polyribosomes. These results suggest that exposed thiol groups are not related to the binding of polyribosomes to stripped rough endoplasmic reticulum under our conditions *in vitro*. The inability of GSSG and GSH to alter binding is not due to the lack of affinity of these agents for exposed

thiol groups, since both GSSG and GSH labelled with $[2\text{-}^{14}\text{C}]\text{glycine}$ were bound to polyribosomes, smooth endoplasmic reticulum and rough endoplasmic reticulum (A. A. Hochberg, B. Binns, R. N. Zahltan, F. W. Stratman & H. A. Lardy, unpublished work). In *Escherichia coli*, GSSG dissociates ribosomes completely into 30S and 50S subunits, which are inactive in polypeptide synthesis (Furano & Harris, 1971). These 30S subunits could bind poly(U) but not phenylalanyl-tRNA. In rat liver, inhibition of the binding of phenylalanyl-tRNA to 60S subunits by *N*-ethylmaleimide suggested that the most sensitive functional thiol group(s) involved reside in this subunit (Bermek *et al.*, 1971). Under conditions where protein synthesis would be impaired (Kosower *et al.*, 1971; Zehavi-Willner *et al.*, 1971) we still observed binding of ^{32}P -labelled polyribosomes to

Table 4. *Binding of polyribosomes to stripped rough endoplasmic reticulum derived from normal rat liver as influenced by various protein-denaturation methods*

Polyribosomes and stripped rough endoplasmic reticulum were prepared as described in Table 2. Duplicate reaction mixtures of 1 ml of 0.25M-sucrose-TKM medium, pH7.5, containing 0.5mg of stripped rough endoplasmic reticulum protein, ^{32}P -labelled polyribosomes equivalent to 100 μg of protein (specific radioactivity 240000 c.p.m./mg of protein) and 2mg of postmicrosomal supernatant protein were incubated for 1 min at 37°C. RNA/protein ratios were: rough endoplasmic reticulum, 0.44; stripped rough endoplasmic reticulum, 0.20. For polyribosomes the E_{260}/E_{280} ratio was 1.7. Unbound polyribosomes in the reaction mixture were separated from rough endoplasmic reticulum by centrifugation at 105000g for 20h. Values in parentheses are from experiments with the same preparations carried out the next day. β was calculated as described in the Experimental section.

Denaturant		Percentage of total ^{32}P -labelled polyribosomes bound to stripped rough endoplasmic reticulum							
Stripped rough endoplasmic reticulum	Polyribosomes	Mean	Range	Mean	Range	β	Range	β	Range
None	None (control)	25.1	1.5	(22.3)	(5.7)	84.9	0.0	(73.0)	(6.2)
Heat	None	24.8	0.9	(25.7)	(0.3)	80.5	2.9	(72.1)	(5.1)
Trichloroacetic acid	None	27.8	0.3	(18.4)	(0.2)	79.5	2.0	(63.5)	(3.6)
HClO_4	None	23.9	1.8	(21.0)	(10.2)	71.0	11.9	(67.4)	(17.3)
None	Heat	26.0	6.4	(20.8)	(5.5)	78.9	5.4	(80.5)	(3.5)
None	Trichloroacetic acid	34.0	4.4	(17.9)	(3.5)	71.9	7.9	(49.0)	(6.7)
None	HClO_4	26.9	2.3	(24.9)	(2.9)	89.6	2.4	(89.1)	(3.9)
Heat	Heat	22.9	2.4			79.2	2.7		
Trichloroacetic acid	Trichloroacetic acid	31.6	4.8			77.7	8.3		
HClO_4	HClO_4	27.3	6.2			91.7	0.0

Fig. 1. *Distribution and recovery of radioactivity from ^{32}P -labelled polyribosomes after binding to stripped rough endoplasmic reticulum*

Polyribosomes (312000 c.p.m./reaction mixture), stripped rough endoplasmic reticulum and heat-denatured stripped rough endoplasmic reticulum were identical with those in Table 4. ■, Pellet of unbound polyribosomes; ■, endoplasmic reticulum with bound polyribosomes. Gradients were centrifuged at 105000g for 20h in a Spinco polycarbonate tube no. 335170.

stripped rough endoplasmic reticulum (Tables 2 and 3).

Thiol reagents, such as *p*-chloromercuribenzoate, are known to have three effects on ribosomes of *Escherichia coli*: (1) conversion of 100S ribosomes into 70S particles (Wang & Matheson, 1966); (2) conversion of 70S ribosomes into subunits (Tamaoki & Miyazawa, 1967); and (3) a 40–80% loss of amino acid incorporation activity and a loss of messenger-directed tRNA binding (Traut & Haenni, 1967). *N*-Ethylmaleimide inactivated *Escherichia coli* ribosomes, whereas iodoacetamide had little effect (Moore, 1971). Depending on the thiol reagent used, similar effects were observed by Ranu & Kaji (1971) on ribosomes of *Bacillus subtilis*. Diazinedicarboxylic acid bis-dimethylamide has been shown to stop protein synthesis in the intact rabbit reticulocyte (Kosower *et al.*, 1969). Dunn (1970) has also reported that thiol reagents dissociate polyribosomes in rat liver by release of RNA. Although we did not incubate ³²P-labelled polyribosomes with thiol reagents before incubation, they were exposed during the 1 min incubation for binding and for at least 5–10 min during transfer to centrifuge tubes. Thus, one could assume that with the stronger reagents some dissociation of the ribosomes might take place. Under our conditions, inhibition of protein synthesis as reported by Kosower *et al.* (1969) would have occurred; yet we did not observe a significant decrease in binding of ³²P-labelled polyribosomes to stripped rough endoplasmic reticulum.

Denaturing the protein of the stripped rough endoplasmic reticulum or the ³²P-labelled polyribosomes by heat, trichloroacetic acid or HClO₄ did not alter the ability to combine with untreated ³²P-labelled polyribosomes or stripped rough endoplasmic reticulum, respectively. Denaturing the corresponding organelles from Morris hepatoma did not alter their binding properties. The decreased binding of liver polyribosomes to stripped rough endoplasmic reticulum from hepatoma when compared with hepatoma polyribosomes is noteworthy.

The binding of ³²P-labelled polyribosomes to stripped rough endoplasmic reticulum as a criterion of biological function (Shires *et al.*, 1971b) in the intact cell must be re-evaluated in the light of the foregoing experiments. The interactions observed appear to have no significant relationship to the reactivity of exposed thiol groups on the membrane, binding *in vitro*, and protein synthesis. Burke & Redman (1972) have also reported that polyribosome-membrane association *in vitro* is different from that occurring *in vivo* on the basis of puromycin-peptide release.

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