The Effects of Polyuridylic Acid on Phenylalanine Incorporation by Subcellular Fractions from Carbon Tetrachloride-Poisoned Rat Liver

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1. Ribosomes and microsomes isolated from the livers of rats that had received carbon tetrachloride 1 hr. previously had decreased endogenous capacity to incorporate amino acid. 2. The capacity of the isolated structures to respond to a synthetic messenger, polyuridylic acid, and to incorporate phenylalanine was investigated. 3. It was found that ribosomes from carbon tetrachloride-treated animals, prepared with detergent and at high ionic strength, could be restored to the same specific activity as control particles with polyuridylic acid but that these particles required more Mg^{2+} in the incubation mixture. 4. Microsomes could also be stimulated to control activities with polyuridylic acid, but had a narrow optimum range of Mg^{2+} concentration. 5. Microsomes prepared from poisoned animals could be preprogrammed with polyuridylic acid to a significantly greater degree than could control particles, and this response was greater with increasing Mg^{2+} concentrations. These data suggested that in carbon tetrachloride poisoning the messenger-ribosome interaction had been altered. 6. Attempts to deprogramme particles from control and treated animals resulted in decreased endogenous activity of both particles and a decreased capacity for the treated particles to be restored with the synthetic messenger. 7. It is suggested that two effects are present in carbon tetrachloride poisoning, namely an alteration of the messenger-ribosome interaction and an increased lability of the ribosome, as either separate or related events.

Administration of carbon tetrachloride to intact animals or preincubation of liver slices with carbon tetrachloride results in a decreased capacity for protein synthesis (Smuckler, Iseri & Benditt, 1961, 1962; Smuckler & Benditt, 1963, 1965; Smuckler, 1966; Seakins & Robinson, 1963). This decrease in activity occurs very rapidly, within 1 hr. after intragastric administration of the haloalkane, and is associated with polysome loss and an alteration of ribosomal sedimentation behaviour (Smuckler & Benditt, 1963, 1965). The possible reasons for the decreased functional activity include loss of messenger RNA, alteration in the ribosome or alteration of the interaction between ribosomes and messenger RNA, either as isolated defects or in combination. Changes in the Ca^{2+} content in the liver and liver fractions have focused attention on the possible role that bivalent ions may play in this toxic effect, particularly in polysome formation (Smuckler, 1966). The present paper examines the bivalent-ion requirements for amino acid incorpora-

tion with and without stimulation by synthetic messenger (polyuridylic acid).

It was observed that ribosomes and microsomes isolated from carbon tetrachloride-treated rats could be stimulated to control values of phenylalanine-incorporating activity by the addition of synthetic messenger, that these microsomes could be programmed with synthetic messenger to a degree exceeding controls, and that differences in the effect of Mg^{2+} were readily apparent in both programming and preprogramming experiments. It is concluded that early in carbon tetrachloride poisoning an alteration of ribosome-messenger interaction occurs.

MATERIALS AND METHODS

Materials. ATP, phosphoenolpyruvate and phosphoenolpyruvate kinase were purchased from Sigma Chemical Co., St Louis, Mo., U.S.A., GTP from P-L Laboratories, Milwaukee, Wis., U.S.A., polyuridylic acid and polyadenylic acid from Miles Chemical Co., Elkhart, Ind., U.S.A., and

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radioactive amino acids from New England Nuclear Corp., Boston, Mass., U.S.A., or from The Radiochemical Centre, Amersham, Bucks.

Animals. In all the following experiments male Sprague-Dawley rats weighing 225–275 g. were used. The animals were starved for 16–18 hr. before the experiments but had access to tap water *ad libitum*. Treated animals received 0.25 ml. of CCl_4 /100 g. body wt. by stomach tube without anaesthesia. The CCl_4 was dissolved in an equal volume of mineral oil; control animals received an equal volume of mineral oil. The animals were killed by a sharp blow to the head, or by ether narcosis with exsanguination, 1 and 2 hr. after administration of the CCl_4 or the mineral oil. All experiments were started in the morning to minimize diurnal variation.

The livers were perfused *in situ* with ice-cold 0.9% NaCl until grossly free of blood; they were then removed and placed in tared beakers containing the appropriate homogenizing medium. The livers were disintegrated in a glass homogenizer with eight strokes of the Teflon pestle, while the homogenizing apparatus was kept in ice. All following operations were performed at 0–4° unless otherwise specified.

Preparation of microsomes. Perfused livers were homogenized in 4 vol. of 0.15 M-sucrose containing 5 mM- MgCl_2 , 2.5 mM-KCl, 5 mM-mercaptoethanol and 0.1 M-tris hydrochloride buffer, pH 7.3 at 20° (Hoagland & Askonas, 1963). The homogenate was centrifuged in a Beckman Spinco model L preparative ultracentrifuge for 15 min. at 15000g, and the supernatant fluid was centrifuged at 105000g for 60 min. The 105000g supernatant fluid was saved as a source of soluble factors necessary for amino acid incorporation. The microsomal pellet was resuspended in the same buffer by gentle homogenization in a Dounce homogenizer.

Preparation of ribosomes. Perfused livers were homogenized in 2.5 vol. of 0.15 M-sucrose containing 75 mM-KCl, 5 mM- MgCl_2 and 35 mM-tris hydrochloride buffer, pH 7.7 at 20° (Rendi & Hultin, 1960). The homogenate was centrifuged at 15000g for 15 min. and the supernatant fluid removed. To the equivalent of 6.5 ml. of the 15000g supernatant fluid were added, in order, 2.0 ml. of 2.5 M-KCl in 10 mM- MgCl_2 , 0.5 ml. of a 10% (w/v) Lubrol W solution in 10 mM- MgCl_2 and 1.0 ml. of 10% (w/v) sodium deoxycholate solution. The additions were made to the 15000g supernatant fluid with constant agitation, and the temperature was maintained at 0°. Samples (10 ml.) of the resulting suspension were layered on 20 ml. of 0.3 M-sucrose containing 0.6 M-KCl, 5 mM- MgCl_2 and 35 mM-tris hydrochloride buffer, pH 7.7 at 20°, and the resulting gradients were centrifuged at 78410g for 75 min. After centrifugation the tubes were drained and the inner surface of the tube and the surface of the pellet washed repeatedly with the original homogenizing buffer. The ribosomal pellets were resuspended in a minimal amount of the original homogenizing medium with a Dounce homogenizer.

Preparation of polysomes. Perfused livers were homogenized in 4 vol. of 0.15 M-sucrose containing 5 mM- MgCl_2 , 2.5 mM-KCl, 5 mM-mercaptoethanol and 0.1 M-tris hydrochloride buffer, pH 7.3 at 20°. The liver brei was centrifuged at 15000g for 15 min. Polysomes were prepared from the 15000g supernatant fluid by the addition of sufficient 7% sodium deoxycholate in 0.1 M-glycylglycine buffer, pH 7.8 at 20°, to result in a 1% sodium deoxycholate solution. The resulting suspension was centrifuged at 105000g for 1 hr. and the polysomal pellet so obtained was resuspended in a

solution containing 0.5 mM- MgCl_2 , 1.0 mM-KCl and 5 mM-tris buffer, pH 7.4 at 20° (Smuckler & Benditt, 1965).

Amino acid incorporation. Amino acid incorporation was most frequently measured by using micro-scale incubation systems. Microsomal, ribosomal and polysomal preparations were adjusted to 4 mg. of protein/ml. of solution and supernatant fluid protein to 10 mg./ml. Incubation was carried on in a total of 125 μl . or 1.1 ml., containing 2 mg. of particulate protein/ml. (adjusted to the same RNA concentration), 2 mg. of 105000g supernatant fluid protein/ml., 1 mM-ATP, 0.25 mM-GTP, 20 mM-phosphoenolpyruvate, 20–50 μg . of phosphoenolpyruvate kinase/ml., 100 mM-KCl, 80–120 mM-tris buffer, pH 7.4 at 20°, and 0.05 mM-[1- ^{14}C]-phenylalanine, -[1- ^{14}C]leucine, -[1- ^{14}C]lysine or -[1- ^{14}C]-valine (specific radioactivities are indicated in the legends of the Tables and Figures). The amounts of polyuridylic acid, polyadenylic acid or MgCl_2 added are specified in the legends of the Figures and Tables. (In experiments with polysomes an amino acid mixture was added, which was 2 mM with respect to each of threonine, tyrosine, methionine, cysteine, isoleucine, glutamic acid, glutamine, aspartic acid, histidine, proline, alanine, glycine, tryptophan, arginine and valine, and leucine, lysine or phenylalanine depending on the tracer amino acid used in the procedure.) Incubation was carried out for 20 min. at 35° aerobically, after which time 100 μl . of the material was pipetted on to filter-paper disks and processed by the method of Mans & Novelli (1961). The filter-paper disks were placed in vials containing 10 ml. of a solution of 0.5% 2,5-diphenyloxazole and 0.015% 1,4-bis-(4-methyl-5-phenyloxazol-2-yl)benzene. Alternatively, 9.0 ml. of ice-cold 5% (w/v) trichloroacetic acid containing non-radioactive amino acid (0.1 M) was added and the protein isolated by the method of Siekevitz (1952). The radioactivity was determined in a Packard Tri-Carb liquid-scintillation counter with an efficiency of approx. 55% for ^{14}C .

Preincubation and washing experiments. In preincubation experiments microsomes were suspended in 0.15 M-sucrose containing 5 mM- MgCl_2 , 2.5 mM-KCl, 5 mM-mercaptoethanol and 0.1 M-tris hydrochloride buffer, pH 7.4 at 20°. The particles were adjusted to 4 mg. of protein/ml. and preincubated for 10 min. at 0° with 100 μg . of polyuridylic acid or polyadenylic acid/ml. of solution or with a mixture of polyuridylic acid and polyadenylic acid. In various experiments the Mg^{2+} concentration and the polyuridylic acid concentration were varied as indicated in the legends of the Tables and Figures. After 10 min. the incubation system was diluted eight- to ten-fold with the same buffer with an MgCl_2 concentration of 5 mM. The resultant suspension was centrifuged for 1 hr. at 105000g or for 45 min. at 150000g. The resultant pellets were resuspended in the original homogenization buffer, and the capacity to incorporate amino acid was measured as described above. In other experiments the effect of preincubation with and without added synthetic messenger was measured, and in addition the effect of preincubation with and without added synthetic messenger was measured by the incorporation of the amino acids valine, lysine and leucine. Experiments were included in which preincubation was carried out with polyadenylic acid and the effect of subsequent polyuridylic acid stimulation was measured. Further, experiments were carried out in which the RNA content of the microsomes and amino acid incorporation were measured after centrifugation and resuspension had been repeated twice.

Measurement of polyuridylic acid inactivation. Polyuridylic acid (500 $\mu\text{g./ml.}$) was incubated with rat liver fractions (microsomes, ribosomes, 15000g supernatant or 105000g supernatant fluid) suspended in 0.15M-sucrose containing 5mM-MgCl₂, 75mM-KCl and 35mM-tris hydrochloride buffer, pH 7.7. Controls included samples of polyuridylic acid incubated in the absence of liver fractions, samples of liver fraction + polyuridylic acid incubated at 0° and samples of liver fraction + polyuridylic acid used immediately after mixing (Parthier, Smuckler & Hultin, 1967). During the period of incubation, samples of the fluid containing the rat liver fraction with and without added polyuridylic acid were removed and added to a test system containing ribosomes, cell sap and transfer RNA from *Escherichia coli*. The *E. coli* ribosomes were incubated in this system for 60 min., and amino acid incorporation was tested as described by Matthaei & Nirenberg (1961).

Measurement of polyuridylic acid degradation. Tritiated polyuridylic acid (prepared by Dr Tore Hultin) was suspended in 0.15M-sucrose with 5mM-MgCl₂, 5mM-mercaptoethanol and 0.1M-tris hydrochloride buffer, pH 7.3, and precipitated with 2 vol. of ethanol (Decken & Campbell, 1964). The polyuridylic acid was resuspended and reprecipitated twice. The non-sedimenting radioactivity was assayed. Resuspension and reprecipitation was repeated until the non-sedimenting activity represented less than 10 counts/min./ml. of ethanolic solution, and remained constant. Samples of this material were incubated at 37° with 4mg. of microsomes from treated or control animals/ml. Control flasks included polyuridylic acid without microsomes, microsomes without polyuridylic acid, and microsomes with polyuridylic acid precipitated immediately on mixing. Microsomes and polyuridylic acid were precipitated with 25% (w/v) HClO₄ and centrifuged at 1000g for 15 min. The radioactivity in the supernatant fluid was measured in a Packard Tri-Carb liquid-scintillation counter with an efficiency for ³H of 8%.

Deprogramming experiments. Microsomes were suspended in 0.15M-sucrose, 5mM-MgCl₂, 2.5mM-KCl, 5mM-mercaptoethanol and 0.1M-tris hydrochloride buffer, pH 7.3 at 20°, and adjusted to 4mg./ml. To this suspension were added (final concentrations) 105000g supernatant fluid (2mg./ml.), ATP (1mM), GTP (0.25mM), phosphoenolpyruvate (20mM), phosphoenolpyruvate kinase (20 $\mu\text{g./ml.}$), KCl (100, 450 or 750mM), MgCl₂ (10mM) and tris hydrochloride buffer, pH 7.4 at 20° (80mM). These suspensions were incubated at 0° and 37° for 0, 15, 30, 45, 60 or 90 min., with or without the various cofactors. At the indicated times the suspensions were diluted ninefold with an ice-cold solution containing 0.15M-sucrose, 1mM-MgCl₂, 5mM-KCl and 35mM-tris hydrochloride buffer, pH 7.3 at 20°, and resedimented at 150000g for 45 min. The resulting pellet was resuspended in the same buffer and utilized for amino acid incorporation as indicated above.

Protein and RNA determinations. Protein was determined by the Lowry method with crystalline bovine albumin as a standard (Lowry, Rosebrough, Farr & Randall, 1951) and RNA was measured by the orcinol technique (Mejbaum, 1939) with yeast RNA as a standard.

RESULTS

Ribosomes isolated from carbon tetrachloride-treated animals by the method of Rendi & Hultin

Fig. 1. Ribosomes were prepared from rat liver 1 hr. after the animal had received mineral oil or 0.25 ml. of CCl₄/100g. body wt. The particles were isolated by the use of detergent at high ionic strength by the method of Rendi & Hultin (1960). The particles were resuspended in a solution containing 1mM-MgCl₂, 5mM-KCl and 35mM-tris buffer, pH 7.4, and incubated with 105000g supernatant fluid. The incubation was carried out in a total volume of 125 $\mu\text{l.}$ at 35° in air. The medium contained 0.08mg. of ribosomal RNA, 0.2mg. of 105000g-supernatant protein, 1.0mM-ATP, 0.25mM-GTP, 20mM-phosphoenolpyruvate, 20 $\mu\text{g.}$ of phosphoenolpyruvate kinase/ml., 110mM-KCl, 16mM-tris, pH 7.4, 100 $\mu\text{g.}$ of polyuridylic acid/ml., 0.05mM-[1-¹⁴C]-phenylalanine (6mc/m-mole) and the amounts of MgCl₂ indicated in the graph. After 20 min. incubation, 100 $\mu\text{l.}$ samples were placed on filter-paper disks and processed by the method of Mans & Novelli (1961). The results are expressed as counts/min./mg. of protein. In all instances three or more experiments were carried out. ○, Control ribosomes; ●, ribosomes from CCl₄-treated livers. Symbols represent the mean, and vertical bars 1 s.d. from the mean.

(1960) contained 40% less ribosomal material than ribosomes from controls of the same weight. However, these particles had the same E_{260}/E_{280} ratios [controls 1.87 ± 0.038 , treated 1.86 ± 0.037 (means $\pm$ s.d. of eight experiments)]. Equivalent amounts of ribosomal material (measured as RNA) from carbon tetrachloride-treated animals did not support incorporation of phenylalanine and leucine to the same degree as control particles. The Mg²⁺ requirements were identical for both types of particle. Optimum incorporation was achieved at about 8mM-magnesium chloride. Addition of polyuridylic acid resulted in a three- to four-fold increase in phenylalanine incorporation by control particles, with an optimum Mg²⁺ concentration identical with that for unstimulated particles.

Table 1. *Effect of polyuridylic acid on phenylalanine incorporation in carbon tetrachloride poisoning*

Polysomes were prepared from control and CCl₄-treated (0.25 ml./100 g. body wt.) rats 2 hr. after intubation. The polysomes were incubated in a volume of 1.1 ml. containing 2 mg. of polysomal protein, 2 mg. of pH 5 enzyme preparation, 1 μ mole of ATP, 0.25 μ mole of GTP, 20 μ moles of phosphoenolpyruvate, 50 μ g. of phosphoenolpyruvate kinase, 100 μ moles of KCl, 0.2 μ mole of an amino acid mixture, 120 μ moles of tris buffer, pH 7.6, and 0.01 μ mole of [1-¹⁴C]phenylalanine. Amounts of MgCl₂ and polyuridylic acid were added as indicated. Incubation was carried out aerobically for 30 min. at 37°, after which the proteins were precipitated with 5% (w/v) trichloroacetic acid containing non-radioactive phenylalanine (0.1 M), and were isolated as described by Smuckler & Benditt (1965).

	Radioactivity		
	Control (disintegrations/min./mg.)	CCl ₄ -treated (disintegrations/min./mg.) (% of control)	
Polysomes without polyuridylic acid in 6 mM-MgCl ₂	2383	1181	50
Polysomes with 100 μ g. of polyuridylic acid in 6 mM-MgCl ₂	4660	2859	61
Polysomes with 100 μ g. of polyuridylic acid in 16 mM-MgCl ₂	6524	5925	91
Polysomes with 100 μ g. of polyuridylic acid in 21 mM-MgCl ₂	5337	2862	54

When polyuridylic acid was added to particles obtained from carbon tetrachloride-treated animals a greater stimulation of phenylalanine incorporation was obtained yielding specific activities equivalent to those of the control structures. One readily apparent difference between particles from control and carbon tetrachloride-treated animals was the shift in optimum Mg²⁺ concentration to a higher value (from 8 mM- to 12 mM-magnesium chloride) (Fig. 1).

Microsomes and material isolated as polysomes from carbon tetrachloride-treated animals incorporated less leucine or phenylalanine (in the absence of polyuridylic acid) than did controls. The optimum Mg²⁺ concentration for incorporation was identical for material from control or carbon tetrachloride-treated rats. Polysomal preparations from treated rats showed significant stimulation with addition of polyuridylic acid (Table 1), but with a response more critically dependent on Mg²⁺ concentration. The stimulation of phenylalanine incorporation by polysomes prepared from treated materials did not reach control values; it is not apparent whether the small difference (less than 10%) is significant; in each experiment the treated particles had a lower specific activity than the controls, but the specific activities varied sufficiently from experiment to experiment to make a statistical test impracticable.

Microsome yields from control and treated animals were identical [10.92 $\pm$ 1.36 mg. of microsomal protein/g. wet wt. of liver from controls (mean $\pm$ s.d. of ten experiments) and 11.19 $\pm$ 1.42 mg. of microsomal protein/g. wet wt. of liver (six experiments) from carbon tetrachloride-treated

animals], but the RNA content of the microsomes from treated animals exceeded controls [177 $\pm$ 17.6 μ g. of RNA/mg. of microsomal protein for control (ten experiments) and 219 $\pm$ 13.6 μ g. of RNA/mg. of microsomal protein for carbon tetrachloride-treated animals (six animals) ($P < 0.05$)]. Addition of polyuridylic acid resulted in a sixfold increase in control activity, and stimulation of treated material resulted in even greater increases (15–20-fold), with a narrowed optimum concentration of Mg²⁺ (Fig. 2).

In an attempt to measure differences in messenger attachment and translation, control and carbon tetrachloride-treated particles were preincubated with polyuridylic acid. Preincubation of rat liver microsomes and ribosomes with synthetic messenger (polyuridylic acid) resulted in an interaction between the messenger and the particles. This interaction was associated with enhanced phenylalanine incorporation, suggesting that the ribosomes now read a specific coding message, that is they had been programmed before incorporation for such a response. For convenience in this paper we term this 'preprogramming'. Preincubation of microsomes or ribosomes with cell sap and an energy source resulted in decreased endogenous amino acid incorporation and was related to decreased messenger-ribosome interaction (Parthier *et al.* 1967). The removal of a coding messenger in this instance is termed 'deprogramming'. Preliminary experiments with ribosomes indicated that the effects of preprogramming were similar in both control and treated groups. Preliminary experiments with microsomes showed that carbon tetrachloride-treated microsomes could be pre-

Fig. 2. Microsomes and 105000g supernatant fluid were prepared from control and CCl_4 -treated (0.25 ml./100 g. body wt., 1 hr. previously) rats. The perfused livers were homogenized in a solution containing 0.15M-sucrose, 5mM- $MgCl_2$, 2.5mM-KCl, 5mM-mercaptoethanol and 0.1M-tris buffer, pH 7.3. Microsomes were isolated from the 15000g supernatant by centrifugation at 105000g for 1 hr. The 105000g supernatant was used as the source of the soluble factors. Incubation was carried out as described in Fig. 1 with the exceptions that microsomes were used at a concentration of 2mg./ml. in place of the ribosomes and the concentration of tris was increased to 80mM. ○—○, Control systems, polyuridylic acid-stimulated activity; ●—●, materials derived from treated animals, polyuridylic acid-stimulated activity; □—□, control systems, endogenous activity; ■—■, materials derived from treated animals, endogenous activity. The results are expressed as counts/min./mg. of protein. The vertical lines represent 1 s.d. from the mean. The significance between control and treated points was tested with Student's *t* test; significant differences are indicated by $P < 0.05$, for the upper curves. In the unstimulated materials all control and treated points were found to differ significantly from one another.

programmed to a considerably greater extent. The design of the preprogramming experiments included preincubation of particles with synthetic messenger in the presence of various Mg^{2+} concentrations, followed by resedimentation and

resuspension of these particles. A series of experiments were undertaken to measure the effects of preincubation on amino acid incorporation by microsomes.

The effect of resedimentation and resuspension on microsomal RNA content and amino acid incorporation was measured. The yield of microsomes was not different in the two groups, and the RNA content did not change [the RNA contents of microsomes (μ g. of RNA/mg. of protein) from controls and carbon tetrachloride-treated animals, initially and after one and two washes respectively were, in controls, 166 ± 25 , 185 ± 36 , 165 ± 43 , and in treated samples, 168 ± 32 , 205 ± 43 , and 201 ± 32]. The capacity of microsomes to incorporate amino acids (leucine and lysine) in the absence of polyuridylic acid was decreased after washing (endogenous activity) (Table 2), but their capacity to incorporate phenylalanine after addition of polyuridylic acid was not affected (Table 3). The effect of washing of the microsome was the same for both control and carbon tetrachloride-treated animals.

The difference in polyuridylic acid-stimulated amino acid incorporation in these and subsequent experiments could have been due to different rates of inactivation of the added synthetic messenger. To test whether there were differences in the destruction of this messenger by the liver fraction, the residual capacity of the polyuridylic acid was analysed with *E. coli* ribosomes. No differences in the rate of polyuridylic acid degradation were apparent in the various cell fractions from the control and treated material (Fig. 3). Similarly no differences in the release of acid-soluble radioactivity from tritiated polyuridylic acid were noted.

An indirect assay of polyuridylic acid binding by microsomes was carried out by measuring the capacity of these structures to be programmed for phenylalanine incorporation. Preincubation of microsomes from control animals with polyuridylic acid resulted in a doubling of the incorporation, but required a Mg^{2+} concentration of 10mM or more (Fig. 4). Microsomes from treated animals exhibited a progressive effect of preprogramming with increasing Mg^{2+} concentration. This increase in amino acid incorporation was not the result of washing the microsomes at high Mg^{2+} concentration, since the endogenous activity as measured by the incorporation of leucine remained the same (Fig. 5). It is unlikely that the apparent increase in the effect of preprogramming at high Mg^{2+} concentration was due to simple non-specific binding, since after preincubation the particles were diluted eight- to ten-fold with buffer containing 5mM-magnesium chloride and resedimented before the incorporation experiment. Thus the effects of high concentrations of Mg^{2+} during sedimentation were minimized, and

Table 2. *Effect of preincubation and resedimentation on leucine and lysine incorporation by microsomes*

Microsomes were prepared from rat liver 1 hr. after the animal had received mineral oil (control) or 0.25 ml. of $\text{CCl}_4/100\text{g. body wt.}$ (treated) as described in the legend of Fig. 2. The microsomes were resuspended in the homogenization medium, and saved for amino acid incorporation (unwashed microsomes) or preincubated for 10 min. at 0° without polyuridylic acid (washed microsomes) or with $100\mu\text{g.}$ of polyuridylic acid/4 mg. of microsomal protein. The two last-named groups were diluted ninefold with buffer and resedimented at $150000g$ for 45 min. The resuspended particles were used to measure leucine or lysine incorporation. The results are expressed as counts/min./mg. of protein and represent the means $\pm$ S.D. of three or more experiments.

	Radioactivity (counts/min./mg. of protein)	
	Control	CCl_4 -treated
Leucine incorporation by unwashed microsomes	775 ± 155	350 ± 45
Leucine incorporation by washed microsomes	244 ± 30	265 ± 40
Leucine incorporation by microsomes preincubated with $100\mu\text{g.}$ of polyuridylic acid/4 mg. of microsomal protein	230 ± 65	260 ± 15
Lysine incorporation by unwashed microsomes	525 ± 20	220 ± 75
Lysine incorporation by microsomes preincubated with $100\mu\text{g.}$ of polyuridylic acid/4 mg. of microsomal protein	280 ± 30	180 ± 30

Table 3. *Effect of washing on polyuridylic acid-stimulated and unstimulated phenylalanine incorporation in microsomes from control and carbon tetrachloride-treated rats*

Microsomes were prepared from the livers of control and CCl_4 -treated (0.25 ml./100 g. body wt., given 1 hr. before killing) rats by homogenization in 0.15 M-sucrose containing 5 mM-MgCl₂, 2.5 mM-KCl, 5 mM-mercaptoethanol and 0.1 M-tris buffer, pH 7.3. The brei was centrifuged at $15000g$ for 15 min., and the supernatant from this step was centrifuged for 1 hr. at $105000g$. This first pellet was resuspended in the same buffer and is referred to as the unwashed microsomes; the material was diluted tenfold and centrifuged for a second hour. The pellet is referred to as the material washed once. It was resuspended and centrifuged again for 1 hr. and resuspended; this is referred to as the material washed twice. The protein concentration of the microsomal suspensions was adjusted to 4 mg./ml. and the incubation mixture (total vol. $125\mu\text{l.}$) was as follows: 1 mM-ATP, 0.25 mM-GTP, 20 mM-phosphoenolpyruvate, $20\mu\text{g.}$ of phosphoenolpyruvate kinase, 100 mM-KCl, 10 mM-MgCl₂, 80 mM-tris buffer, pH 7.4, 0.05 mM-[1- ^{14}C]phenylalanine, 0.2 mg. of microsomal protein and 0.2 mg. of $105000g$ supernatant protein. Incubation was carried on for 20 min. at 35° aerobically. Of the material, $100\mu\text{l.}$ was applied to a filter-paper disk and processed by the method of Mans & Novelli (1961). Statistical differences were measured by Student's *t* test. The figures represent the means $\pm$ S.D.

Microsome source	Polyuridylic acid ($\mu\text{g.}$)	Radioactivity of microsomes (counts/min./mg. of protein)			
		Unwashed		Washed once	Washed twice
Control rats	—	1355	$\longleftrightarrow P < 0.05 \longleftrightarrow$	430 ± 120	$\longleftrightarrow P < 0.05 \longleftrightarrow$ 265 ± 70
Control rats	100	4795 ± 1220	$\longleftrightarrow P = 0.15 \longleftrightarrow$	4825 ± 965	$\longleftrightarrow P = 0.4 \longleftrightarrow$ 3545 ± 1025
CCl_4 -treated rats	—	460 ± 100	$\longleftrightarrow P < 0.05 \longleftrightarrow$	280 ± 30	$\longleftrightarrow P < 0.05 \longleftrightarrow$ 90 ± 10
CCl_4 -treated rats	100	9680 ± 1745	$\longleftrightarrow P = 0.04 \longleftrightarrow$	8580 ± 2250	$\longleftrightarrow P = 0.4 \longleftrightarrow$ 7855 ± 980

at the same time the concentration of polyuridylic acid was significantly lowered. Preprogramming by polyuridylic acid could be blocked by preincubation with polyadenylic acid which showed a specific Mg^{2+} requirement of 10 mM or greater (Fig. 6).

Preprogramming of control particles did not change the general shape of the Mg^{2+} response curve, and the optimum Mg^{2+} concentration for incorporation remained about 8 mM. Preprogramming of carbon tetrachloride-treated particles showed an increase in the steepness of the Mg^{2+} response curve, but did not show the sharp peak seen when polyuridylic acid was added directly to the incubation mixture (Fig. 7).

The concentration of polyuridylic acid used in these experiments was at or beyond the saturation limit both during incubation (Fig. 8) and preincubation (Fig. 9). Addition of more polyuridylic acid during amino acid incorporation did not enhance phenylalanine incorporation further (Fig. 8); addition of more polyuridylic acid during preincubation experiments indicated an enhanced activity with microsomes from carbon tetrachloride-treated liver (Fig. 9).

Attempts were made to disrupt messenger-ribosome interaction by deprogramming. Microsomes resuspended in cell sap and supplied with an energy source were less able to incorporate amino

Fig. 3. Microsomes were prepared from control and CCl₄-treated (0.25 ml. of CCl₄/100 g. body wt., 1 hr. previously) animals, as indicated in Fig. 2. They were suspended in the initial homogenization medium and diluted to 1 mg. of protein/ml. These suspensions were mixed with polyuridylic acid (200 μ g./ml.), and after 0, 3, 6, 12, 24 and 48 min. duplicate samples were removed and added to a suspension of *E. coli* ribosomes and cell sap, and the polyuridylic acid-directed phenylalanine incorporation was measured. ○, Control microsomes; ●, microsomes from CCl₄-treated animals.

acid after incubation at 37° compared with identical microsome-cell sap mixtures maintained at 0°. The decrease in endogenous amino acid incorporation was more marked with microsomes from control than from carbon tetrachloride-treated animals (Table 4). Omission of cell sap had no effect, but the decrease in amino acid incorporation was more pronounced in the presence of ATP and GTP. After incubation at 37° in the presence of cell sap and an energy source, control microsomes showed increased activity to polyuridylic acid, exceeding that of microsomes incubated at 0°. Microsomes from carbon tetrachloride-treated animals failed to show such an enhanced response to the synthetic messenger.

Preincubation of microsomes in the presence or absence of an energy source did not change the RNA content (Table 5), and as indicated above carbon tetrachloride-treated microsomes had an increased RNA content that did not appear to be altered by washing or preincubation.

Incubation of microsomes at 37° without cell sap or an energy source resulted in decreased capacity for incorporation of leucine and lysine compared with microsomes incubated at 0° (Table 6). Preincubation of microsomes and subsequent stimulation of phenylalanine incorporation by polyuridylic acid resulted in enhanced incorporation in particles from control and carbon tetrachloride-treated animals, and this enhancement was greater in the

Fig. 4. Microsomes were prepared from perfused livers of control rats and of rats that had received CCl₄ (0.25 ml./100 g. body wt.) 1 hr. previously. The microsomes were suspended in a solution containing 0.15 M-sucrose, 2.5 mM-KCl, 5 mM-mercaptoethanol and 0.1 M-tris buffer, pH 7.4. Sufficient MgCl₂ was added to give the concentrations indicated. The microsomes were incubated with polyuridylic acid in a ratio 4 mg. of microsomal protein/100 μ g. of polyuridylic acid for 10 min. at 0°. Then 9 ml. of the buffer with 5 mM-MgCl₂ was added (a ninefold dilution) and the microsomes were resedimented at 150 000g for 45 min. The washed microsomal pellet was resuspended and incubated as described in Fig. 2. The vertical lines indicate 1 s.d. from the mean of four or more experiments. ○, Control microsomes; ●, microsomes from treated animals. The means on the ordinate line ('No polyU') represent incubations with 5 mM-MgCl₂ without added polyuridylic acid. The first scale division represents incubation at 5 mM-MgCl₂ with polyuridylic acid.

control samples (Table 7). Preincubation at high K⁺ concentration decreased the activity of both treated and control microsomes (Table 7).

DISCUSSION

Administration of carbon tetrachloride to a variety of animal species or preincubation of liver slices with carbon tetrachloride results in decreased capacity for hepatic protein synthesis (Smuckler *et al.* 1961, 1962; Smuckler & Benditt, 1963, 1965; Smuckler, 1966; Seakins & Robinson, 1963). Investigation of the mechanism responsible for the

Fig. 5. Microsomes were prepared from control and CCl_4 -treated animals as previously described, and preincubated with polyuridylic acid (a) or without polyuridylic acid (b) as described in Fig. 4. In (a), after resedimentation, the capacity to incorporate leucine after preincubation in the presence of polyuridylic acid was measured. In (b) the effect on phenylalanine incorporation of preincubation without polyuridylic acid was measured. These graphs represent experiments with control preparations. Similar results, but with lower specific activities, were obtained from CCl_4 -treated animals. Concentrations of Mg^{2+} (mM) during preincubation: ●, 5; ○, 10; △, 24; ▲, 48; ■, 75.

Fig. 6. Microsomes were prepared from control animals, preincubated with or without polyadenylic acid and resedimented. The particles were resuspended and used in amino acid-incorporation systems with or without polyuridylic acid, as described in Fig. 2. It is apparent that preincubation without polyadenylic acid with 5 and 72 mM- $MgCl_2$ does not alter amino acid incorporation and that preincubation with polyadenylic acid does not effect subsequent polyuridylic acid-directed incorporation unless a higher concentration (10 mM) of $MgCl_2$ is used. ○, Preincubated with polyadenylic acid at 5 mM- $MgCl_2$; ●, preincubated without polyadenylic acid at 5 mM- $MgCl_2$; ▼, △, ▲, □, preincubated with polyadenylic acid at 10, 24, 48 or 72 mM- $MgCl_2$ respectively; ●, ■, preincubated (without polyadenylic acid or polyuridylic acid) at 5 or 72 mM- $MgCl_2$ respectively.

decreased activity has indicated that polysomes are found in decreased numbers in poisoned livers and liver preparations (Smuckler *et al.* 1962; Smuckler & Benditt, 1965; Webb, Blobel & Potter, 1966), ribosomes and microsomes have decreased capacity to incorporate amino acid in cell-free preparations and ribosomes have altered sedimenting activity (Smuckler & Benditt, 1963, 1965). It was also shown the carbon tetrachloride itself was not directly responsible for these changes (Smuckler, 1966).

Protein synthesis is carried on by ribosomes acting in concert with messenger RNA and certain cellular enzymes (Warner, Rich & Hall, 1962; Wettstein, Staehelin & Noll, 1963). Assay of the cellular enzymes (e.g. the amino acid-activating system, the transferring and binding factors) did not reveal differences from control preparations (Smuckler & Benditt, 1963, 1965; Smuckler & Barker, 1966), suggesting the defect might be in the messenger RNA-ribosome complex.

The number of polysomes could be affected by the

rate of synthesis and destruction of messenger RNA or by an alteration in messenger-ribosome interaction. The rate of messenger RNA synthesis does not appear to be a major factor since the onset of polysome loss and concomitant decreased microsome function are so rapid; direct assays of orotate uptake *in vivo* and of RNA polymerase activity *in vitro* reveal no difference in the first 6 hr. of carbon tetrachloride poisoning (E. A. Smuckler, unpublished work). Indirect evidence that an increase in messenger destruction is not a factor includes the findings that: (a) inactivation of a synthetic messenger (polyuridylic acid) by liver fractions from control or carbon tetrachloride-treated animals was not different (this paper); (b) release of acid-soluble fragments from polyuridylic acid was not different in controls and carbon tetrachloride-

Fig. 7. Microsomes were prepared from rat liver 1 hr. after intragastric deposition of 0.25 ml. of CCl_4 /100 g. body wt., as described in Fig. 2. The microsomes were diluted to 4 mg. of protein/ml. and incubated in a solution containing 0.15 M-sucrose, 25 mM- MgCl_2 , 2.5 mM-KCl, 5 mM-mercaptoethanol and 0.1 M-tris buffer, pH 7.3 at 20° , with or without 100 μg . of polyuridylic acid/ml., at 0° for 10 min. They were then diluted ninefold and centrifuged at 150000g for 45 min. The pellets were resuspended by gentle homogenization and incubated as described in Fig. 2. Δ , Microsomes preincubated, and incubated without polyuridylic acid; $\circ$, microsomes not preincubated, but incubated with polyuridylic acid; $\bullet$, microsomes preincubated with polyuridylic acid and incubated without polyuridylic acid. —, Left ordinate scale used; ----, right ordinate scale used.

treated samples (this paper); (c) cell sap from the livers of carbon tetrachloride-poisoned animals supported amino acid incorporation in cell-free systems as well as did control cell sap (Smuckler & Benditt, 1963, 1965); (d) increased nuclease activity was found only later in the course of the poisoning, after the change in protein synthesis had occurred (Ugazio, Artizzu, Pani & Dianzani, 1964; Slater & Greenbaum, 1965).

One means available for studying the interaction of messenger with ribosomes is the analysis of polyuridylic acid stimulation of amino acid incorporation. It is recognized that mammalian cell-free systems can be stimulated to incorporate phenylalanine by the addition of polyuridylic acid, which acts as a synthetic messenger (Maxwell, 1962). This stimulation involves the attachment of the polyribonucleotide to ribosomes and the

Fig. 8. Microsomes were prepared from control (○) and CCl₄-treated (●) animals as described in Fig. 2. These particles were used in the amino acid-incorporating system previously described, and the effect of increasing amounts of polyuridylic acid was measured. Each point represents the mean of three or more experiments.

Fig. 9. Microsomes were prepared from control (○) and CCl₄-treated (●) animals and preincubated as described in Fig. 4. In these experiments increasing amounts of polyuridylic acid were employed during preincubation with 10mM-MgCl₂. After sedimentation the retained (programmed) capacity for polyuridylic acid-directed phenylalanine incorporation was measured. The vertical lines indicate 1 S.D. from the mean of four or more experiments.

translation of the messenger by ribosomes, and can be used to test both messenger-ribosome interactions and messenger translation.

The response of cell-free systems from carbon tetrachloride-poisoned animal livers to synthetic-messenger stimulation has produced conflicting results (Smuckler & Barker, 1964; Mager, Bornstein & Halbreich, 1965; Richter & Michel, 1966). Polysomes could be restored to (or to near) control values of specific activity of incorporation when appropriate attention was paid to bivalent ion concentrations. Similarly, ribosomes isolated by the

use of a detergent at high ionic strength also could be restored to activities identical with those of control particles, although a higher Mg²⁺ concentration (12–13mM for carbon tetrachloride-treated particles and 8–10mM for control particles) was required. Stimulation of amino acid incorporation by microsomes through the addition of polyuridylic acid resulted in specific activities as great as or greater than controls (based on the RNA content of the microsomes). The Mg²⁺ requirements were similar to those of polysomal preparations, i.e. the response curves of the carbon tetrachloride-treated materials showed a much sharper optimum than those of the controls. It appears that bivalent ion concentration is critical in the utilization of messenger, since in all these systems the differences between control and treated particles were only evident in the presence of polyuridylic acid. A role for bivalent cations in carbon tetrachloride poisoning has previously been suggested by the difference in metal-ion content of polysomal materials after carbon tetrachloride poisoning (Smuckler, 1966) and would appear to be substantiated here.

In our experiments, incorporation of phenylalanine requires the attachment of messenger RNA to the ribosome and its translation. Since differences between control and treated materials might be manifest in either or both steps, and since bivalent ions might be important in one or the other, an attempt was made to separate these two processes experimentally. It is possible to measure messenger attachment by incubating particles with synthetic messenger, washing the particles free of unattached polynucleotide and measuring the retention of the message. It was observed that microsomes from control or treated animals were able to bind polyuridylic acid at 0° in the absence of an energy source. Incubation at higher temperatures led to inactivation of the particles, and prolonged incubation failed to increase preprogramming. It was found that preprogramming of microsomes from control livers required Mg²⁺ concentrations higher than those used in incorporation studies, and this is in agreement with the work of Revel & Hiatt (1965). Microsomes from carbon tetrachloride-treated animals showed a significantly greater capacity for preprogramming, which was enhanced at increased Mg²⁺ concentrations. The evidence for the utilization of polyuridylic acid as a messenger is that preincubation with a second synthetic messenger, not coding for phenylalanine, blocked the polyuridylic acid effect, although only at increased Mg²⁺ concentrations.

The process of preincubation and washing was tested by measuring RNA content and leucine and lysine incorporation; no difference was observed between particles from control and carbon tetra-

Table 4. *Effect of cell sap and an energy source on microsomal deprogramming*

Microsomes were prepared as described in the legend of Fig. 2, and suspended in a solution containing 0.15 M-sucrose, 5 mM-MgCl₂, 2.5 mM-KCl, 5 mM-mercaptoethanol and 0.1 M-tris buffer, pH 7.3. A suspension containing 2 mg. of microsomal protein, 2 mg. of cell-sap protein, 1 mM-ATP, 0.25 mM-GTP, 20 mM-phosphoenolpyruvate, 20 μg. of phosphoenolpyruvate kinase/ml., 110 mM-KCl, 6 mM-MgCl₂ and 80 mM-tris buffer, pH 7.3, in a total volume of 1 ml. was made and incubated at 0° or 37° for 30 min. The preparations were diluted ninefold with a solution containing 1 mM-MgCl₂, 5 mM-KCl and 35 mM-tris buffer, pH 7.4, and resedimented at 150 000g for 45 min. The microsomes were resuspended and used for phenylalanine incorporation with and without polyuridylic acid. The results are expressed as counts/min./μg. of RNA (measured at 260 mμ). The results are the means of four or more experiments.

Polyuridylic acid...	Radioactivity (counts/min./μg. of RNA)			
	Control		CCl ₄ -treated	
	Absent	Present	Absent	Present
Complete system at 0°	18 224	209 094	4342	158 602
Complete system at 37°	5735	263 498	3323	150 684
No supernatant fluid	5896	237 180	3430	127 836
No ATP or GTP added	14 204	237 448	4717	167 124

Table 5. *Effect of preincubation on microsomal RNA content*

Microsomes were prepared from control and CCl₄-treated rat liver (0.25 ml./100 g. body wt., 1 hr. previously) as described in the legend of Fig. 2. The microsomes were suspended in a solution containing 0.15 M-sucrose, 5 mM-MgCl₂, 2.5 mM-KCl, 5 mM-mercaptoethanol, 0.1 M-tris buffer, pH 7.3, and adjusted to 4 mg. of microsomal protein/ml. They were incubated at 0° or 37° for 60 min., after which time they were diluted ninefold with the suspending medium and resedimented at 105 000g for 60 min. The protein of the resulting pellet was measured by the Lowry technique (Lowry *et al.* 1951) and the RNA by the orcinol method (Mejbaum, 1939). The results are expressed as μg. of RNA/mg. of protein and given as means of four or more determinations ± S.D.

Temperature	RNA content (μg./mg. of protein)	
	Control microsomes	CCl ₄ -treated microsomes
0°	184 ± 36	205 ± 43
37°	175 ± 23	204 ± 46

chloride-treated animals after these treatments, which also had no effect on phenylalanine incorporation in the absence of polyuridylic acid. No differences in the inactivation of polyuridylic acid by microsomes from control or treated animals was observed, and identical release of soluble radioactivity from polyuridylic acid was found on incubation with microsomes from control and treated animals. It is concluded that microsomes from carbon tetrachloride-poisoned animals can be preprogrammed more readily.

The enhanced capacity of microsomes from carbon tetrachloride-treated animals to accept polyuridylic acid direction suggests that they have

Table 6. *Effect of preincubation at 0° and 37° on microsomal leucine and lysine incorporation*

Microsomes were prepared as described in the legend of Fig. 2 and preincubated for 60 min. at 0° or 37°. Leucine and lysine incorporation was measured as indicated in Fig. 2. The amino acids used had specific radioactivities nearly twice those of the amino acids used in the experiments in Table 2. The results of three or more experiments are expressed as means ± S.D.; correction for RNA content is not included.

Temperature	Radioactivity (counts/min./mg. of protein)	
	Control microsomes	CCl ₄ -treated microsomes
Leucine incorporation		
0°	745 ± 135	320 ± 40
37°	345	270 ± 41
Lysine incorporation		
0°	425 ± 20	220 ± 75
37°	255	195

more open binding sites with which to accept the messenger strand. The enhanced capacity of these structures to utilize the polyuridylic acid at increased Mg²⁺ concentrations seems to show that this bivalent ion is a necessary factor in messenger attachment. Since preprogrammed microsomes do not show the narrow optimum for Mg²⁺ concentration seen with non-preprogrammed microsomes it may be that the change in Mg²⁺ requirements are the result of the attachment phenomena, and represent more specific environmental ion concentrations than seen with normal microsomes.

Our data suggest that preparations from carbon tetrachloride-treated animals do not contain as much endogenous messenger as controls. If this

Table 7. *Effect of preincubation at 0° and 37° on unstimulated and polyuridylic acid-stimulated phenylalanine incorporation by control and carbon tetrachloride-treated microsomes*

Microsomes were prepared as described in the legend of Fig. 2 and preincubated with the amino acid-incorporating mixture described in Table 5. The concentration of KCl was 0.1 M and the time of incubation was 45 min. At the end of preincubation 9 vol. of 1 mM-MgCl₂-5 mM-KCl-35 mM-tris, pH 7.4, was added, and the microsomes were isolated by centrifugation at 150000g for 45 min. The pellet was resuspended and used for phenylalanine incorporation as previously described. The results are expressed as counts/min./μg. of RNA and represent the means ± s.d.

	Radioactivity (counts/min./μg. of RNA)			
	0°		37°	
Control microsomes	25996 ± 7879	177630 ± 24616	13507 ± 5628	290780 ± 55744
CCl ₄ -treated microsomes	8683 ± 3644	161818 ± 19350	6754 ± 2305	200625 ± 56608

Table 8. *Effect of preincubation at 0° and 37° on unstimulated and polyuridylic acid-stimulated phenylalanine incorporation by control and carbon tetrachloride-treated microsomes*

Microsomes were prepared and preincubated as described in Table 7 except that the time of preincubation was 30 min., and the KCl concentration was 0.4 M or 0.75 M as indicated. The results are expressed as counts/min./μg. of RNA of microsomes, and represent the means ± s.d.

	Radioactivity (counts/min./μg. of RNA of microsomes)			
	0°		37°	
	0.4 M-KCl			
Control microsomes	24227 ± 1822	154154 ± 19457	21386 ± 3377	143380 ± 26800
CCl ₄ -treated microsomes	5520 ± 1500	131374 ± 35644	4937 ± 1608	95783 ± 19778
	0.75 M-KCl			
Control microsomes	18974 ± 3377	136610 ± 28944	16777 ± 2305	127300 ± 25192
CCl ₄ -treated microsomes	6432 ± 1938	107950 ± 17045	5199 ± 965	95301 ± 16080

were the only defect, it should then be possible to deprogramme control particles to the same endogenous activity as carbon tetrachloride-treated ones and to stimulate them to similar specific activities (Parthier *et al.* 1967; Fessenden & Moldave, 1963; Revel & Hiatt, 1965; Gelboin, 1964; Weksler & Gelboin, 1967; Fessenden, Cairncross & Moldave, 1963; Hishizawa & Terayama, 1966). The process of washing and resuspension of microsomes was associated with decreased endogenous activity of both control and carbon tetrachloride-treated materials; in fact, activities decreased in a nearly identical manner; and polyuridylic acid stimulation resulted in identical activities for both structures. These changes are not explicable on the basis of loss of RNA. Endogenous messenger, not fixed to ribosomes or membrane, might have been removed. In the next series of experiments, attempts were made to free messenger by preincubation. Incubation at 37° without the addition of cell sap or an energy source was also associated with endogenous activity loss, and after polyuridylic acid stimulation identical specific activities were attained. In our system, unlike previously reported deprogramming

systems with ribosomes and microsomes, omission of cell sap and an energy source did not prevent loss of endogenous activity to the degree noted (Parthier *et al.* 1967; Fessenden & Moldave, 1963; Revel & Hiatt, 1965; Gelboin, 1964; Weksler & Gelboin, 1967; Fessenden *et al.* 1963; Hishizawa & Terayama, 1966). In all instances there was a parallel decrease in activity in particles from control and treated animals. It seems possible that this represents a loss of the endogenous activity remaining on the carbon tetrachloride-treated particles, or that there is a greater sensitivity to the deprogramming in the treated materials. Attempts were made to deprogramme at high K⁺ concentration, known to enhance protein release (Hultin & Pedersen, 1963). Augmentation of polysome disappearance was noted with controls (Parthier *et al.* 1967), but only a peculiar aggregation and decreased activity was evident with materials from carbon tetrachloride-treated animals (E. A. Smuckler, unpublished work). The loss of endogenous activity after preincubation and incubation in high K⁺ concentration and the failure of the carbon tetrachloride-treated particles to respond to exogenous

messenger to the same extent as the controls suggest an enhanced sensitivity of the particles.

These results suggest that there may be two processes that occur in carbon tetrachloride poisoning, a loss of attachment of messenger RNA to ribosomes and an alteration in the ribosome itself. Consistent with this hypothesis is the fact that carbon tetrachloride-poisoned materials can and do respond to exogenous messenger RNA, and reach control activities. The polyuridylic acid stimulation does measure ribosome-messenger interaction since there is excess of added synthetic messenger, and the short period necessary for chain completion indicates the predominant effect of polyuridylic acid (over endogenous messenger) in these systems. The similarity of activities in control and treated groups suggests that the particles are able to respond to the messenger. That the poisoned materials are not only deprogrammed is indicated by their decreased ability to withstand the experimental manipulation, and by the altered sedimentation behaviour reported above. The role of the bivalent ions and the part they play in the messenger attachment and maintenance of ribosome integrity are not clear.

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REFERENCES

- Decken, A. von der & Campbell, P. N. (1964). *Biochem. J.* **91**, 195.
- Fessenden, J. M., Cairncross, J. & Moldave, K. (1963). *Proc. nat. Acad. Sci., Wash.*, **49**, 82.
- Fessenden, J. M. & Moldave, K. (1963). *Nature, Lond.*, **199**, 1172.
- Gelboin, H. V. (1964). *Biochim. biophys. Acta*, **91**, 130.
- Hishizawa, T. & Terayama, H. (1966). *J. Biochem., Tokyo*, **59**, 272.
- Hoagland, M. B. & Askonas, B. A. (1963). *Proc. nat. Acad. Sci., Wash.*, **49**, 430.
- Hultin, T. & Pedersen, S. (1963). *Biochim. biophys. Acta*, **72**, 421.
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). *J. biol. Chem.* **193**, 265.
- Mager, J., Bornstein, S. & Halbreich, A. (1965). *Biochim. biophys. Acta*, **95**, 682.
- Mans, R. J. & Novelli, G. D. (1961). *Arch. Biochem. Biophys.* **94**, 48.
- Matthaei, J. H. & Nirenberg, M. W. (1961). *Proc. nat. Acad. Sci., Wash.*, **47**, 1580.
- Maxwell, E. S. (1962). *Proc. nat. Acad. Sci., Wash.*, **48**, 1639.
- Mejbaum, W. (1939). *Hoppe-Seyl. Z.* **258**, 117.
- Parthier, B., Smuckler, E. A. & Hultin, T. (1967). *Biochim. biophys. Acta*, **145**, 446.
- Rendi, R. & Hultin, T. (1960). *Exp. Cell Res.* **18**, 187.
- Revel, M. & Hiatt, H. (1965). *J. molec. Biol.* **11**, 467.
- Richter, G. & Michel, S. (1966). *Proc. 3rd Meet. Fed. Europ. biochem. Soc., Warsaw*, p. 302.
- Seakins, A. & Robinson, D. S. (1963). *Biochem. J.* **86**, 401.
- Siekevitz, P. (1952). *J. biol. Chem.* **195**, 549.
- Slater, T. F. & Greenbaum, A. L. (1965). *Biochem. J.* **96**, 484.
- Smuckler, E. A. (1966). *Lab. Invest.* **15**, 157.
- Smuckler, E. A. & Barker, E. A. (1964). *Fed. Proc.* **23**, 335.
- Smuckler, E. A. & Barker, E. A. (1966). *Excerpta med. int. Congr. Ser.* no. 115, p. 83.
- Smuckler, E. A. & Benditt, E. P. (1963). *Science*, **140**, 308.
- Smuckler, E. A. & Benditt, E. P. (1965). *Biochemistry*, **4**, 671.
- Smuckler, E. A., Iseri, O. A. & Benditt, E. P. (1961). *Biochem. biophys. Res. Commun.* **5**, 270.
- Smuckler, E. A., Iseri, O. A. & Benditt, E. P. (1962). *J. exp. Med.* **116**, 55.
- Ugazio, G., Artizzu, M., Pani, P. & Dianzani, M. U. (1964). *Biochem. J.* **90**, 109.
- Warner, J. R., Rich, A. & Hall, C. E. (1962). *Science*, **138**, 1399.
- Webb, T. E., Blobel, G. & Potter, V. R. (1966). *Cancer Res.* **26**, 1022.
- Wekslar, M. E. & Gelboin, H. V. (1967). *J. biol. Chem.* **242**, 727.
- Wettstein, F. O., Staehelin, T. & Noll, H. (1963). *Nature, Lond.*, **197**, 430.