Liver Enzyme Induction by 1,1,1-Trichloro-2,2-bis-(*p*-chlorophenyl)ethane (DDT) is Accompanied by an Increase in the Specific Activity of Elongation Factor 1

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Homogenates of liver were obtained from control rats and from rats that had received DDT [1,1,1-trichloro-2,2-bis-(*p*-chlorophenyl)ethane]. The postmicrosomal supernatant fractions were used for the purification of elongation factor 1 by hydroxyapatite chromatography and phosphocellulose chromatography. The amount of binding factor present was essentially the same for both groups of animals, but the specific activity, as measured by the binding assay, was about twice as high in the DDT-treated preparations. After sucrose-gradient sedimentation, the difference in specific activity was found to reside in the low-molecular-weight (50000) form of elongation factor 1. The implications of an increased reactivity of elongation factor 1 during the induction of membrane enzymes are discussed.

DDT induces the synthesis of hepatic drug-metabolizing enzymes *de novo* in a manner that is somewhat similar to that caused by phenobarbital and certain hydrocarbons such as methylcholanthrene, since the administration of DDT to rats results in about a 20% increase in liver weight and microsomal protein content (Platt & Cockrill, 1969; Cappon & Nicholls, 1975), and an increase in the activity of a number of enzymes (Gielen & Nebert, 1971; Bunyan *et al.*, 1972). Studies in this laboratory have shown that during liver enzyme induction there are several changes in the protein-synthetic machinery, namely a decrease in a ribosomal-bound inhibitory protein and an increase in aminoacyl-tRNA-binding activity, including initiator methionyl-tRNA^{Met}-binding activity (Cappon & Nicholls, 1974a; Goodchild & Nicholls, 1976; Cappon *et al.*, 1976; Chan *et al.*, 1977). These changes appear to parallel the increased binding of mRNA specific for the induced proteins (Gelboin & Blackburn, 1964; Dehlinger & Schimke, 1972), and probably of mRNA for other proteins of the endoplasmic reticulum.

Elongation factor 1 from a number of eukaryotic species occurs both in high- and low-molecular-weight forms. The size of the heavy form of the enzyme (EF 1_H), which is an aggregated form of the light species (EF 1_L) with mol.wt. between 47000 and 60000, depends on the source and the purification

procedure (for a review see Weissbach & Ochoa, 1976). The light form is believed to be the species involved in aminoacyl-tRNA binding (Tarrago *et al.*, 1973; Grasmuk *et al.*, 1976). Changes in the relative proportion of the low-molecular-weight form have been found during the development of brine shrimp (*Artemia salina*) and nematodes (*Turbatrix aceti*) (Slobin & Möller, 1975, 1976; Bolla & Brot, 1975). In the liver of starved mice or aging rats, soluble cytosol preparations exhibited a lower specific activity of EF 1 rather than a change in the proportion of EF 1_L to EF 1_H (Liu *et al.*, 1974; Bolla *et al.*, 1975). Thus we decided to assess the amount and activity of purified EF 1 obtained from the soluble cytosol fraction of liver of rats after the induction of membrane proliferation by DDT.

Experimental

Animals

Male Wistar rats weighing 180–200 g were used and fed on fox cubes (Teklad Inc., Monmouth, IL, U.S.A.) and water *ad libitum*. DDT, dissolved in corn oil at a concentration of 50 mg/ml, was administered in two consecutive daily intraperitoneal injections. The dosage used was 200 mg of DDT/kg body wt. Control animals received an equal volume of the corn-oil vehicle. At 72 h after the second injection the animals were killed and the livers removed and placed in ice-cold containers. Usually four pairs of rats (treated and control) were used for each experiment.

Abbreviations used: DDT, 1,1,1-trichloro-2,2-bis-(*p*-chlorophenyl)ethane; EF 1, elongation factor 1; EF 2, elongation factor 2; SDS, sodium dodecyl sulphate.

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Chemicals

L-[U-¹⁴C]Phenylalanyl-tRNA (specific radioactivity 385 mCi/mmol, 0.156 μ Ci/mg of tRNA) containing 19 other unlabelled aminoacyl-tRNA species from *Escherichia coli* strain B and Protosol were obtained from New England Nuclear Corp., Lachine, Que., Canada. Sodium fusidate was a gift from the Squibb Institute for Medical Research, New Brunswick, NJ, U.S.A. Hydroxyapatite was from Clarkson Chemical Co., Williamsport, PA, U.S.A., phosphocellulose (Whatman P11) was from Mandel Scientific Co., Montreal, Que., Canada, and ethoxyethanol and nitrocellulose filters (Sartorius, 0.45 μ m) were from BDH Chemicals, Toronto, Ont., Canada. Other chemicals were obtained as previously described (Girgis & Nicholls, 1972; Cappon & Nicholls, 1973).

Liver homogenization and preparation of the pH5-supernatant fraction

All work was performed on ice at 2–4°C. The livers were homogenized in a glass homogenizer with 10–12 passes of a Teflon pestle in 3 vol. of buffer 1 [0.25 M-sucrose/50 mM-Tris/HCl (pH 7.6 at 22°C)/80 mM-KCl/6 mM-MgCl₂/10 mM-2-mercaptoethanol/20% (v/v) glycerol]. The homogenate was centrifuged at 15000g for 20 min at 2°C in a Sorval type SS34 rotor (r_{av} 5.1 cm). The postmitochondrial supernatant fraction (S15) was then centrifuged at 105000g for 70 min at 2°C in a Spinco type 40 rotor (r_{av} 5.9 cm). The microsomal pellet was washed with buffer 1 and finally resuspended in this buffer. This preparation was stored at –20°C or used immediately for the preparation of preincubated salt-washed ribosomes from control rat liver as described previously (Cappon & Nicholls, 1974a).

The postmicrosomal supernatant fraction (S105), obtained from the 70 min centrifugation step above, was either stored at –20°C in buffer 1 or used for the preparation of the pH5-supernatant fraction. The pH of the postmicrosomal supernatant fraction was lowered from 7.6 to 5.2 by the addition of 1.0 M-acetic acid. This preparation was stirred on ice for 1 h, after which it was centrifuged at 15000g for 20 min. The resultant supernatant fraction (that had thus been freed of synthetases and tRNA species) was then adjusted back to a pH of 7.6 by the addition of 1 M-KOH. The resultant pH5-supernatant fraction which contained approx. 50% of the protein of the S105 fraction was stored at –20°C.

Preparation of EF 2

The chromatography was performed at 4°C in the cold-room. Enzyme-grade (NH₄)₂SO₄ was dissolved in the pH5-supernatant fraction (approx. 1.6 g of protein), with constant stirring on ice, to a final concentration of 30% (w/v) saturation. After

equilibration for 45 min, the precipitated protein was removed by centrifugation at 15000g for 20 min at 2°C. To the resulting supernatant (NH₄)₂SO₄ was added until the final concentration was 70% saturation. The precipitated protein was obtained by centrifuging at 15000g for 20 min at 2°C and then resuspended in buffer 1. The resuspended protein preparation was dialysed overnight against two changes of 1 litre of buffer 1.

The dialysed preparation was adsorbed on a column (2.5 cm $\times$ 8.5 cm) of hydroxyapatite equilibrated with 50 mM-Tris/HCl buffer (pH 7.6), containing 10% (v/v) glycerol. The column was washed with the equilibrating buffer, at a rate of 25 ml/h, until the eluted material no longer showed absorbance at 280 nm. The column was then developed with 200 mM-potassium phosphate/50 mM-Tris/HCl/10% (v/v) glycerol buffer (pH 7.2) at a flow rate of 25 ml/h. The column was eluted with this buffer until no further absorbance at 280 nm was detected. The eluted protein was concentrated by ultrafiltration in an Amicon cell fitted with a PM-10 membrane. The concentrated preparation was then dialysed overnight against two changes of 1 litre of buffer 1.

The sample obtained above from hydroxyapatite chromatography was placed on a column (2.5 cm $\times$ 45.0 cm) of Sephadex G-100 and washed with 50 mM-Tris/HCl (pH 7.6)/80 mM-KCl/6 mM-MgCl₂/10 mM-2-mercaptoethanol/10% glycerol buffer at a flow rate of 30 ml/h. On the basis of molecular-weight determinations of EF 2 by Twardowski & Legocki (1973) and Raeburn *et al.* (1971), fractions corresponding to mol. wts. between 50000 and 100000 were collected. These were pooled and concentrated by ultrafiltration on a PM-10 membrane. This EF 2 preparation was highly active in the polymerization assay but it showed no EF 1 activity in either the binding or polymerization assay.

Preparation of EF 1

(NH₄)₂SO₄ (0–30% satd.) precipitated approx. 50% of the total protein in the pH5-supernatant and most (49–50%) of the remaining protein was precipitated by 30–70% satd. (NH₄)₂SO₄. The samples were resuspended in buffer 1, then dialysed overnight at 2°C against two changes of 1 litre of buffer 1. The fractions obtained by the above procedure were assayed for EF 1 activity. Both polymerization and binding assays were performed. The resuspended precipitate from the 0–30%-satd.-(NH₄)₂SO₄ step and the supernatant from the 70%-saturation step showed no EF 1 activity. All the EF 1 activity was found in the resuspended precipitate from the 30–70%-satd.-(NH₄)₂SO₄ step.

To detect changes in EF 1_L, as well as changes in EF 1_H, the gel-filtration step used by other workers

was omitted (Gasior & Moldave, 1965; Collins *et al.*, 1972; Drews *et al.*, 1974). Chromatography on hydroxyapatite and phosphocellulose was carried out before sedimentation of EF 1.

EF 1 was retained on the hydroxyapatite column used above after washing with buffer [200mM-potassium phosphate/50mM-Tris/HCl/10% (v/v) glycerol (pH 7.2)]. The column was washed with this buffer until the eluted material showed no absorbance at 280nm. It was then developed with 350mM-potassium phosphate/50mM-Tris/HCl/10% glycerol (pH 7.2) at a flow rate of 25 ml/h. This concentration of buffer was determined from prior experiments with buffer containing a linear gradient of 0–500mM-potassium phosphate. The eluted protein was concentrated by ultrafiltration using a PM-10 membrane. The concentrated sample was then dialysed overnight against two changes of 1 litre of buffer 1.

The dialysed sample was assayed for EF 1 activity and then adsorbed on a column (1.8cm × 12.0cm) of phosphocellulose equilibrated with 50mM-Tris/HCl/6mM-MgCl₂/10mM-2-mercaptoethanol/10% glycerol (pH 7.6). The column was washed with this equilibrating buffer until there was no absorbance at 280nm. The column was then washed with equilibrating buffer adjusted to a KCl concentration of 100mM until no more protein was eluted. The column was developed with the equilibrating buffer containing 350mM-KCl. This concentration of buffer was determined from previous experiments using equilibrating buffer containing a linear gradient of 100–350mM-KCl. The eluted protein was collected and concentrated by ultrafiltration through a PM-10 membrane. The KCl concentration was decreased to 80mM during the ultrafiltration procedure. This preparation was assayed for EF 1 activity and stored at –20°C.

The phosphocellulose preparation described above was placed on a 5ml linear (10–30%, w/v) sucrose gradient in 50mM-Tris/HCl (pH 7.6)/80mM-KCl/6mM-MgCl₂/10mM-2-mercaptoethanol. It was centrifuged at 105000g for 16h in a Spinco type SW 65 rotor (r_{av} , 6.4cm). The gradients were fractionated into 0.3ml samples using an ISCO fractionator and assayed for EF 1 activity and protein concentration.

Gel electrophoresis

Purification steps were monitored by electrophoresis in polyacrylamide gels containing SDS as first described by Laemmli (1970). The slab-gel apparatus was a modification of the design described by Studier (1973). Preparation of the SDS/polyacrylamide gels has been described by O'Farrell (1975). The discontinuous system consisted of a 10% (w/v) acrylamide gel, pH 8.8, on which 4.75% polyacrylamide gel, pH 6.8, was layered. The samples were electrophoresed for 16h at 22°C, at a constant current of 20mA. The slab gel was stained

with a solution of 0.1% (w/v) Coomassie Blue and 50% (w/v) trichloroacetic acid for 30min. It was destained by several changes of 7.5% (v/v) acetic acid.

Measurement of radioactivity

The incorporation of labelled amino acids into peptide in the polymerization assay was terminated by the addition of cold 5% (w/v) trichloroacetic acid containing 0.02% (w/v) phenylalanine as a carrier. The precipitated protein was heated at 90°C with 5% (w/v) trichloroacetic acid, and washed as described by Girgis & Nicholls (1971). The protein was solubilized in 1.0ml of Protosol, and transferred to scintillation vials for counting. Quenching efficiency was about 75% by the internal-standardization method. Activity was expressed as d.p.m./mg of ribosomal RNA.

The binding assay *in vitro* was terminated by the addition of 7 vol. of cold buffer 1. The ribosomal-aminoacyl-tRNA complex was collected on nitrocellulose filters and washed twice with cold buffer 1. The filters were placed in 3ml of ethoxyethanol and the samples were left to equilibrate for 1h. Scintillation solution (3vol.) was added and counting was carried out with an efficiency of about 55%. Activity was expressed in d.p.m./mg of ribosomal RNA.

Determination of protein and RNA concentrations

RNA concentration was measured by the method of Schneider (1945), or by measuring its A_{260} . Protein concentration was measured by the method of Lowry *et al.* (1951), with bovine serum albumin as a standard. For purified samples free of nucleic acids, protein concentration was determined at A_{210} as outlined by Tombs *et al.* (1959).

Results

Binding activity after DDT treatment

In the experiments shown in Table 1, EF 1 was assayed directly by means of the binding assay as described by Malkin & Lipmann (1969), which uses 4.0mM-sodium fusidate to inhibit translocation, and then traps the ribosomal-aminoacyl-tRNA complex on nitrocellulose filters. There was a statistically significant increase in binding in the pH 5 supernatant fraction obtained from DDT-treated animals. This increase was present for limiting concentrations of the pH 5-supernatant fraction (Fig. 1a). These results for binding confirm those obtained when bond synthesis was measured after DDT treatment and when it was observed that EF 1 activity was limiting relative to EF 2 activity in supernatant preparations from both control and DDT-treated animals (Cappon & Nicholls, 1974a).

Table 1. Binding of [14 C]phenylalanyl-tRNA to ribosomes in pH5-supernatant fractions obtained from control and DDT-treated animals

The pH5-supernatant fractions were obtained as described in the Experimental section. Excess of salt-washed pre-incubated ribosomes (96 μ g of RNA) from control rat liver were incubated for 7 min at 37°C with 100 μ g of pH5-supernatant protein obtained from livers from control and DDT-treated rats. The incubation mixture had a final volume of 0.4 ml and contained 0.25 M-sucrose, 50 mM-Tris/HCl buffer (pH 7.6), 80 mM-KCl, 0.5 mM-GTP, 4 mM-fusidic acid, 6 mM-MgCl₂, 15% glycerol and [14 C]phenylalanyl-tRNA (13 600 d.p.m.). Values are means $\pm$ S.E. for the numbers of pairs of animals given in parentheses. *P* value was obtained by Student's *t* test.

Fraction	Percentage increase = $\left(\frac{\text{DDT-control}}{\text{control}} \right) \times 100$.		Percentage increase	<i>P</i>
	Control	DDT-treated		
pH5 supernatant	3.88 $\pm$ 0.73	5.12 $\pm$ 0.64	35.0 $\pm$ 4.78 (33)	<0.001

Fig. 1. Binding of [14 C]phenylalanyl-tRNA to control liver ribosomes in paired samples from control and DDT-treated animals. Incubation as for Table 1. (a) pH5-supernatant fraction, (b) hydroxyapatite preparation, (c) phosphocellulose preparation. O, Control; ●, DDT-treated.

Table 2. Binding of [14 C]phenylalanyl-tRNA to ribosomes in samples from control and DDT-treated animals obtained during the purification steps

Fractions were obtained as described in the Experimental section. Incubation was performed as described in Table 1. Symbols are as for Table 1.

Step and treatment	Percentage increase	<i>P</i>
1. pH 5-supernatant	42 $\pm$ 15 (18)	<0.05
3. Hydroxyapatite chromatography	75 $\pm$ 14 (18)	<0.01
4. Phosphocellulose chromatography	81 $\pm$ 12 (16)	<0.01

Chromatography

Between 600 and 700 mg of the precipitated protein from the 30–70% satd.-(NH₄)₂SO₄ step from both control and DDT-treated preparations was adsorbed on separate hydroxyapatite columns. The 350 mM-potassium phosphate buffer eluted approx. 10% of

the total protein and contained all the EF 1 activity. Fig. 1(b) shows the binding assay for samples from control and DDT-treated preparations. As was observed in the crude pH5-supernatant fractions, the DDT-treated liver preparation had a greater capacity (75%) to bind [14 C]phenylalanyl-tRNA to ribosomes (Table 2).

Approx. 50 mg of protein obtained as above from the hydroxyapatite chromatography step was adsorbed on phosphocellulose. The buffer containing 350 mM-KCl contained approx. 5% of the protein and all of the EF 1 activity. Fig. 1(c) shows the binding assay for the control and DDT-treated preparations. Treatment with DDT resulted in an increase (81%) in the binding of [14 C]phenylalanyl-tRNA to the ribosomes (Table 2).

The average specific activity of the phosphocellulose preparations from four experiments was 39.9 pmol/mg of protein for control liver and 69.9 pmol/mg of protein for liver from DDT-treated animals under the conditions of the binding assay. The average yield

of protein from these experiments was 2.44 mg for control and 2.62 mg for DDT-treated preparations.

Fractions obtained from the above steps were subjected to electrophoresis on SDS/polyacrylamide gels to assess the degree of purification. The pH 5 supernatant fractions showed a large number (over 100) of protein bands (not shown). Close examination did not reveal any major differences between control and treated samples. After hydroxyapatite chromatography many bands were still present in both preparations. Phosphocellulose chromatography, however, removed much of the contaminating protein so that only one major band was detected visually. When placed in a sensitive gel scanner, six minor protein bands were also detected but the absence of other major bands strongly suggested that the EF 1 was composed of aggregates of a single low-molecular-weight protein. The molecular weight of this major band both from the control and the DDT-treated preparations was estimated by the method of Weber & Osborn (1969) and by comparison with a number of protein standards to be approx. 50 000, which agreed with the weight of EF 1_L reported by Liu *et al.* (1974) for calf liver.

The activity of the phosphocellulose preparations

was lost after Sephadex gel filtration or after gel electrophoresis (in the absence of SDS), presumably because of the relatively small amount of protein available from each experiment. However, sedimentation of the EF 1 on sucrose gradients yielded an active fraction (fraction 5) of about 50 000 mol.wt. and relatively inactive fractions (fractions 13–20) of greater molecular weight (Fig. 2). A similar distribution of activity between low- and high-molecular-weight forms was noted by Nagata *et al.* (1976) for rat liver and was believed to depend on the presence of glycerol throughout chromatography. The specific activity of EF 1_L (fractions 4–6) from the DDT-treated preparations was markedly increased compared with controls. The concentration of the low-molecular-weight protein (fractions 4–6) was about 20% of that of the total protein as estimated by the relative area under the curves (Fig. 3) and was essentially the same for control as for DDT-treated preparations. No significant changes could be detected in the activity or in the concentration of the high-molecular-weight protein after DDT treatment (Figs. 2 and 3). No difference between control and DDT-treated preparations could be detected when the low-molecular-weight form (fractions 4–6) of

Fig. 2. Incorporation of [¹⁴C]phenylalanyl-tRNA into peptide in the phosphocellulose preparations of EF 1 from rat liver of control and DDT-treated animals that were subjected to centrifugation in a linear sucrose gradient

Purified preparations were placed on 10–30% (w/v) linear sucrose gradient and centrifuged as described in the Experimental section. Gradients were fractionated into 300 μ l samples (top of tube at left). For the polymerization assay, 100 μ l of each fraction was incubated with an excess of salt-washed preincubated ribosomes (100 μ g of RNA) from control rat liver for 10 min at 37°C in the presence of excess of purified EF 2 (100 μ g). The incubation mixture had a final volume of 0.4 ml and contained 0.25 M-sucrose, 50 mM-Tris/HCl (pH 7.6), 80 mM-KCl, 6 mM-MgCl₂, 0.5 mM-GTP, 15% glycerol and [¹⁴C]phenylalanyl-tRNA (13 600 d.p.m.). Molecular-weight standards were (A) ovalbumin (43 000), (B) bovine serum albumin (68 000) and (C) γ -globulin (155 000). $\circ$, Control; $\bullet$, DDT-treated.

Fig. 3. Profile of protein in the phosphocellulose preparations of EF 1 from liver of control and DDT-treated animals that were subjected to sedimentation in a linear sucrose gradient. Gradients and centrifugation as described in the Experimental section. Fractions (0.100 ml) were diluted to 1.1 ml and the A_{280} was measured. ○, Control; ●, DDT-treated.

EF 1 was subjected to SDS/polyacrylamide electrophoresis (Fig. 4). The molecular weight (45 000) of the major band observed in Fig. 4 was similar to that of the major band of the phosphocellulose preparation. This suggests that the major band of the phosphocellulose preparation was EF 1.

Discussion

Previous results from this laboratory showed that there was an increased capacity for the incorporation of [^{14}C]phenylalanyl-tRNA into peptide in the S105 and pH5-supernatant fractions of the cytosol derived from the liver of rats undergoing proliferation of the smooth microsomal membranes after DDT treatment (Cappon & Nicholls, 1974a). This increased activity was attributed chiefly to an increase in EF 1 activity, as an increased bond synthesis could be induced in control preparations that were supplemented with exogenous EF 1, and activity was increased in fractions after Sephadex G-200 chromatography, which resolved EF 1 from EF 2. Such results could not distinguish between an increase in amount and/or an increase in reactivity of EF 1, nor did they eliminate the possibility of tightly bound activators or inhibitors that remain associated during gel filtration.

The present results on paired samples of the liver pH5-supernatant fraction from control and DDT-treated rats show that the binding activity is significantly elevated by DDT treatment when EF 1 is purified by hydroxyapatite and phosphocellulose chromatography. From the postmicrosomal fraction, a decrease in protein from 3200 mg to about 2.5 mg (i.e. 1600-fold) was obtained for both control and DDT-treated samples in more than six separate experiments. The relatively high salt concentration necessary to remove the activity adsorbed on hydroxyapatite and phosphocellulose would be expected to remove any activators tightly bound to EF 1. A disadvantage of the relatively high salt concentrations and small tissue samples used is the severe loss of EF 1 activity, as noted by Schneir & Moldave (1968), and as seen in the low reactivity of the phosphocellulose preparation and of the preparation subjected to sucrose-gradient sedimentation.

Protein factors that stimulate EF 1 activity have been reported in EF 1_H preparations from pig liver (EF 1 β) (Iwasaki *et al.*, 1973) and in rabbit reticulocytes (Prather *et al.*, 1974). Other studies with calf brain and liver (Moon *et al.*, 1973; Liu *et al.*, 1974), rat liver (Collins *et al.*, 1972) and Krebs ascites cells (Drews *et al.*, 1974) provide evidence that EF 1_H is an aggregate of EF 1_L so that if a recycling factor

Fig. 4. Electrophoresis of sucrose-gradient preparations of EF 1 from liver of control and DDT-treated animals. Fractions 4-7 were pooled, co-precipitated with 40 μ g of poly (A) by the addition of 10 vol. of cold ethanol, sedimented at 15000g for 15min and then resuspended in buffer for electrophoresis in polyacrylamide gels containing SDS as described in the Experimental section. The left gel pattern, slot 1, represents a control preparation and the right gel pattern, slot 2, is a DDT-treated preparation. Migration from top (—) to bottom (+).

(analogous to prokaryotic EF-Ts) does exist, it is not present in the purified EF 1 preparations of the latter workers. A factor such as EF 1 β in the EF 1_H fractions, if present, could not account for the effect of DDT, since the increased specific activity occurred in the EF 1_L fraction and not in the EF 1_H fractions.

In certain eukaryotes, changes in the ratio of EF 1_H/EF 1_L have been observed during development (Slobin & Möller, 1975, 1976; Bolla & Brot, 1975) and subsequent to the addition of the cyclic GMP (Lanzani *et al.*, 1974), guanosine nucleotides (Nombella *et al.*, 1976) and proteolytic enzymes (Nombella *et al.*, 1976; Twardowski *et al.*, 1977). However, in the present experiments with rat liver, the marked increase in binding activity during liver microsomal enzyme induction was solely due to a change in reactivity of the low-molecular-weight form of EF 1. During the present investigation a preliminary report appeared describing similar changes in the liver of toadfish (*Opsanus tau*) after cold acclimation or injections of tri-iodothyronine (Nielsen & Haschemeyer, 1977). These changes contrast with those in liver of starved mice or aging rats where decreases in the specific activity of EF 1, with no concomitant changes in the size distribution of EF 1, were noted (Liu *et al.*, 1974; Bolla *et al.*, 1975). In these latter studies the liver supernatant fractions were layered directly on sucrose gradients and were not previously purified as in the studies reported here.

Our results indicate that the reactivity of the soluble EF 1 is increased in cells stimulated to produce membranes and certain membrane-bound enzymes. After DDT administration, the total soluble proteins are labelled *in vivo* to a greater degree (Cappon & Nicholls, 1973), but the proteins affected are unknown. It seems likely that factors induced by DDT treatment which lead to an increased reactivity and/or stability of EF 1, are located in the soluble fraction of the cells. Either their association with EF 1_L is so close that they are not removed by hydroxyapatite and phosphocellulose chromatography or else their effects remain after they have been dissociated from the EF 1_L. For example, it has been reported that highly purified EF 1_L from rat liver is a zinc metallo-enzyme and that removal of Zn²⁺ removes activity (Kotsiopoulos & Mohr, 1975). Another possibility might be that after DDT treatment there is an increased turnover (i.e. synthesis and degradation) of EF 1 which results in a more reactive species of EF 1_L perhaps owing to the disappearance of an inactive or faulty enzyme. A differential effect of cyclic hydrocarbons on the turnover of certain membrane-bound proteins of rat liver has been documented (Arias *et al.*, 1969; Omura, 1975). An increase in aminoacyl-tRNA-binding activity was found during hyperplasia of rat liver (Cappon & Nicholls, 1974a; Nicholls *et al.*, 1974, 1977) and

kidney (Girgis & Nicholls, 1971; Cappon & Nicholls, 1974b; Nicholls *et al.*, 1975; Nicholls & Ng, 1976) and during cardiac hypertrophy (Petryshyn *et al.*, 1977), but further experiments are required to see whether these increases involve the same change as measured here during membrane proliferation.

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