Protein Synthesis in Polysomes from Houseflies and the Effect of 2,2-Bis(*p*-chlorophenyl)-1,1,1-trichloromethane*

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With the Technical Assistance of Miss P. Poblete

ABSTRACT: Polysomes from various strains of *Musca domestica* have been characterized by sucrose density gradient analysis, by their biological activity, and by the use of inhibitors of protein synthesis such as ribonuclease and puromycin. Pentamers and larger ribosome aggregates predominated in three of the strains studied, while trimers were more abundant in the fourth one. The polysomes are disaggregated by the effect of ribonuclease, indicating that messenger ribonucleic acid participates in holding together the ribosomes. Labeling experiments with L-phenylalanine-2-^{14}C indicate that aggregated ribosomes rather than monometric species are involved in protein synthesis. Puromycin releases between 19 and 25% of the newly synthesized peptides. DDT increases the *in vivo* and the *in vitro* incorporation of ^{14}C-labeled amino acids into polysomal proteins in DDT-resistant strains. Furthermore, heavier ribosome aggregates are labeled as compared with normal insects. Injected DDT-^{14}C accumulates mainly in the nuclei and polysomes, apparently through binding to proteins. In the case of polysomes, binding is essentially to nascent proteins. 2,2-Bis(*p*-chlorophenyl)-1,1,1-trichloroethane (DDT) also increases the amount of nuclear ribonucleic acid and its template activity in an *in vitro* system. It is concluded that the role of DDT as an enzyme inducer is mediated through the deoxyribonucleic acid dependent synthesis of ribonucleic acid species, presumably messenger ribonucleic acid, although a translational mechanism at the polysomal level cannot be discarded at present.

The available evidence points out to ribonucleoprotein particles, called polysomes or ergosomes (Wettstein *et al.*, 1963; Warner *et al.*, 1963), as the main site of protein synthesis in the cell. The basic unit is considered to be composed of five 73S monomers held together by one mRNA molecule. Very low concentrations of RNase convert these aggregates into single ribosomal units. On the other hand, it has been recently suggested that monomers, dimers, and trimers instead of larger aggregates would play a dominant part in protein synthesis in housefly (Tsiapalis *et al.*, 1967). An active cell-free protein synthesis system has been described in houseflies in our laboratory (Litvak *et al.*, 1967) which is similar as to requirements and activity and compared with those described for mammalian and bacterial tissues (Simpson, 1962). Since microsomes were used in our system, it was considered important to establish the role that polysomes may have in protein synthesis in houseflies, as well as to correlate these studies with previous postulated mechanisms explaining the effect of DDT.[1] This compound is known to increase protein synthesis in insects as well as mammalian tissues (Morello, 1965). It has been also shown that the effect of DDT is blocked by puromycin (Morello, 1965). Furthermore, it has been shown that actinomycin D at low concentrations blocks the incorporation of labeled precursors into fly RNA (I. Balazs and M. Agosin, unpublished data), implicating that the DNA-directed synthesis of RNA is involved in the effect of DDT.

The present paper describes the presence of typical polysome structures in houseflies, their fractionation by sucrose density gradients, their biological activity, and the effect of RNase and puromycin which are considered typical inhibitors of protein synthesis. An important part of this paper deals with the *in vivo* and the *in vitro* effect of DDT on these polysome structures. Most of the work was conducted with two housefly strains (Fc and P_2/sel) which have higher and DDT-inducible microsomal mixed-function oxidase activity (Agosin *et al.*, 1966).

Experimental Section

Reagents. L-Phenylalanine-2-^{14}C was purchased from Schwarz BioResearch, and DL-leucine-1-^{14}C from Volk Isotopes. ^{14}C-ring-labeled DDT was obtained from the Radiochemical Centre, Amersham. DDT was chromatographically purified as described previously (Agosin *et al.*, 1964). Puromycin dihydrochloride was purchased from Mann Research Biochemicals; spermine from Nutritional Biochemicals Co. The rest of the reagents were of the highest purity commercially available.

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[1] Abbreviations used: DDT, 2,2-bis(*p*-chlorophenyl)-1,1,1-trichloroethane; TCA, trichloroacetic acid; SDS, sodium dodecyl sulfate; DOC, sodium deoxycholate; PPO, 2,5-diphenyloxazole; POPOP, 1,4-bis[2-(5-phenyloxazolyl)]benzene.

Methods

Insect Material. Nonsexed-5-day-old flies of the following strains were used throughout: P_2/sel, selected with pyrethrins and resistant to pyrethrins and cross-resistant to DDT and naphthalene vapors, tolerant to carbamates and some organophosphorus compounds; Fc, selected with diazinon, resistant to diazinon and cross-resistant to DDT and carbamates; P_2, derived from the P_2/sel strain by removal of the selection pressure for about 70 generations, less resistant to DDT than the P_2/sel and susceptible to other insecticides; Ns, susceptible to all insecticides. The characteristics of these strains have been previously reported (Litvak *et al.*, 1967).

Polysomes Preparation. Microsomes were obtained as described previously (Litvak *et al.*, 1967), using medium I (0.35 M sucrose, 0.035 M $KHCO_3$, 0.004 M $MgCl_2$, and 0.025 M KCl). The microsomal pellet was resuspended in medium II consisting of 0.1 M sucrose, 0.005 M $MgCl_2$, and 0.025 M KCl in 0.05 M Tris-HCl buffer (pH 7.5). The microsomal suspension (4–5 ml) was carefully layered on top of a discontinuous gradient (2.3 ml of 51.3% sucrose and 3.1 ml of 17.1% sucrose containing 0.005 M $MgCl_2$ and 0.025 M KCl in Tris-HCl buffer, pH 7.5). The gradients were spun at 40,000 rpm in the no. 40 rotor of the Spinco Model L centrifuge for 10–15 hr at 0–4°. The translucent brownish polysome pellet was resuspended in medium II. Final protein concentration was adjusted to approximately 1 mg/ml. Polysomes could also be prepared in similar yields and puried from a 15,000*g*/10-min supernatant centrifuged through the above discontinuous sucrose gradient. Furthermore, the yield of polysomes was not improved by the addition of DOC at 1% final concentration. The E_{260}/E_{280} of these polysome preparations was 1.7–1.9.

Sucrose Density Gradient Analysis of Polysomes. Linear sucrose density gradients from 10 to 34 g per 100 ml were prepared essentially as described by Talal (1966). The polysomes (approximately 0.5 mg of RNA) in 1.0 ml of medium II were carefully layered on top of a 27-ml gradient. They were centrifuged at 25,000 rpm/120 min in the SW 25-1 rotor of the Spinco Model L centrifuge. The tubes were punctured at the bottom and 25–30 fractions were collected in small test tubes. Optical density of the fractions was measured in a Beckman Model DU spectrophotometer at 260 mμ.

Incorporation of Amino-^{14}C Acids into Polysomes. Groups of 40 insects were injected intrathoracically with 0.6 μl of 0.001 M neutral aqueous solutions of either L-phenylalanine-2-^{14}C or leucine-1-^{14}C (3×10^4 cpm/fly) as described previously (Agosin *et al.*, 1966). After standing for periods indicated in tables and figures at 27°, polysomes were prepared and fractionated by sucrose density gradients as described above.

To measure radioactivity, 200 μg of crystallized bovine serum albumin was added to each sucrose density gradient fraction and protein was precipitated with one volume of 10% TCA. After standing 15 min at 4° the precipitate was collected on 0.45-μ Millipore filters and washed with 10 ml of 5% TCA. The filters were dried under an infrared lamp and placed into scintillation vials containing 6 ml of scintillation mixture (0.1 mg of POPOP and 4 g of PPO per l. of toluene) and counted in a Nuclear-Chicago Mark I scintillation counter.

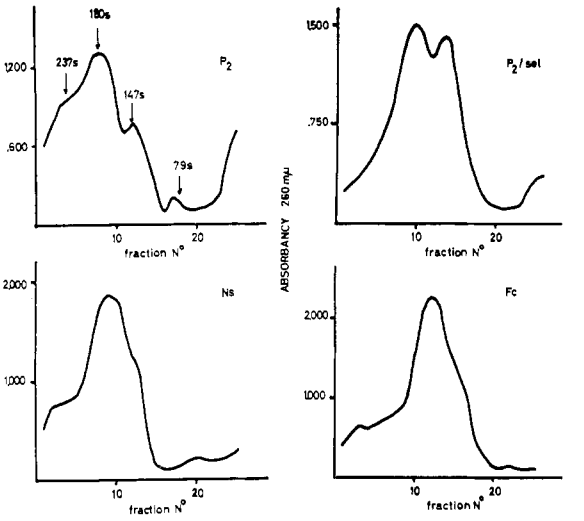

FIGURE 1: Sucrose gradient centrifugation of housefly polysomes. Medium II (1 ml) containing 0.5 mg of RNA polysomes from the indicated strains was layered on top of a 10–34% sucrose gradient in medium II. The tubes were centrifuged in the SW 25.1 rotor of the Spinco Model L centrifuge at 25,000 rpm for 2 hr. The tubes were punctured at the bottom and 25–30 fractions were collected. For further details, see under Experimental Section.

Intracellular Distribution of DDT-^{14}C. Groups of 40 insects each were intrathoracically injected with 1 μg in 1 μl of acetone of ring-labeled DDT-^{14}C (specific activity 24,000 cpm/μg). After standing 20 min at 27° the insects were homogenized in 9 volumes/g fresh wt of medium I in an all-glass Potter-Elvejhem homogenizer inmersed onto crushed ice. All the following operations were carried out at 0–4°. The homogenate was filtered through a double layer of cheesecloth and then centrifuged at 600*g* (average) for 10 min in a Sorvall SS-2 centrifuge. The pellet was washed three times with the same medium and finally suspended in 2 ml of medium II. This pellet was considered to be the nuclear fraction. The 600*g* supernatant fluid was centrifuged at 10,000*g* for 10 min; this pellet was washed twice with the same medium and finally resuspended in 2 ml of medium II. This fraction was assumed to correspond to sarcosomes. The 10,000*g* supernatant fluid was centrifuged at 105,000*g* for 90 min in the no. 40 rotor of the Spinco Model L centrifuge; this pellet corresponding to microsomes was suspended in 2 ml of medium II. The supernatant fluid after the 105,000*g* for 90-min centrifugation corresponds to the soluble fraction. Protein in each fraction was purified as described previously (Agosin and Repetto, 1963). The protein was finally dissolved in 0.2 ml of 98% formic acid, plated onto aluminium planchets, dried, and counted for radioactivity in a thin, end-window Nuclear-Chicago gas-flow counter.

When the distribution of radioactivity from DDT-^{14}C was followed in polysomes fractionated in sucrose density gradients as above, carrier crystallized serum albumin (200 μg) was added to each fraction, the protein was precipitated and purified as in the case of the sub-

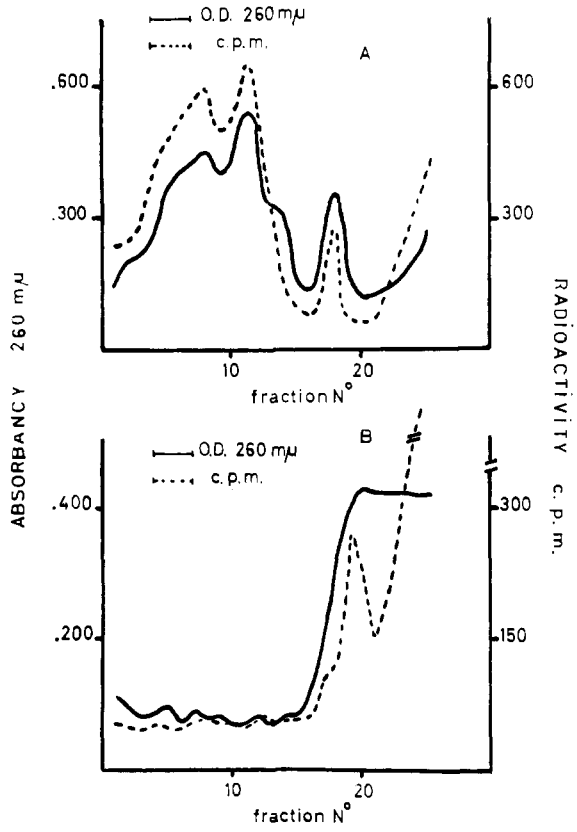

FIGURE 2: *In vivo* incorporation of L-phenylalanine-2-^{14}C into polysomes and the effect of RNase. Insects of the P_2/sel strain were injected with L-phenylalanine-2-^{14}C and after 20 min polysomes were prepared and fractionated by sucrose density gradients as in Figure 1. (A) Approximately 0.2 mg of RNA polysomes in 1.0 ml of medium II was fractionated. (B) The same amount of polysomes was incubated with 5 μg of RNase at 37° for 5 min and then fractionated.

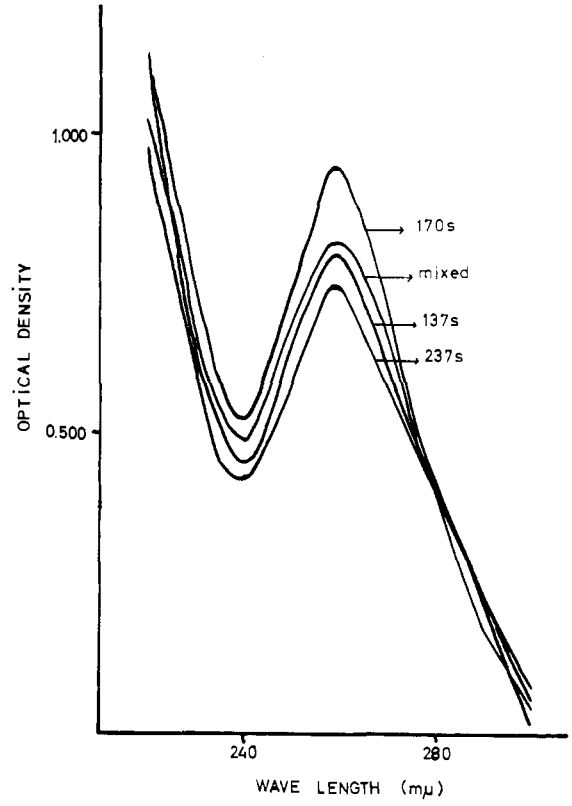

FIGURE 3: Ultraviolet spectra of ribosomal aggregates of different sizes. The spectra were obtained from the peaks of Figure 2A.

cellular fractions, and the radioactivity was measured by the filter disk procedure in a Mark I Nuclear-Chicago scintillation counter.

Amino Acid Incorporation in a Cell-Free System. Amino acid incorporation into protein in a cell-free system was followed as described previously (Litvak *et al.*, 1967) with the exception that polysomes (0.2 mg of protein) were used instead of microsomes.

Preparation of Nuclear RNA. RNA was extracted from nuclei obtained from groups of 50 flies each (1 g fresh wt) by the phenol method. The nuclei were homogenized with ten volumes of a medium consisting of 5.0% SDS, 0.01 M sodium acetate buffer (pH 5.2), and 0.001 M $MgCl_2$ in a "Virtis" homogenizer. RNA was extracted from the homogenate with 90% phenol at 4° for 45 min; the aqueous phase was reextracted once with 90% phenol at 4° for 30 min. Residual phenol was eliminated from the aqueous layer by three extractions with two volumes each of ethyl ether. RNA was precipitated from the aqueous layer by the addition of two volumes of 95% ethanol containing 1.5 M potassium acetate and 0.1 M NaCl. The E_{260}/E_{280} of this RNA was approximately 2.1 and contained 20% DNA as determined by the diphenylamine method (Dische, 1930). Total nuclear nucleic acids were isolated by the procedure of Schneider (1957). The amount of RNA and DNA in the extracts was determined as indicated under Analytical Procedures.

DDT Treatment. Acetone solutions of DDT were topically applied at the dose of 1 μg/fly as described previously (Agosin *et al.*, 1966).

Analytical Procedures. Protein was determined by the Folin method (Lowry *et al.*, 1951). RNA was determined by the ultraviolet absorbancy and by the orcinol method (Mejbaum, 1939). DNA was estimated by the diphenylamine procedure (Dische, 1930).

Results

The yield of mixed ribosomes from houseflies was approximately 1 mg of RNA/g of tissue. The ratio of RNA to RNA plus protein was 0.63, which is similar to what has been observed for other tissues (Hanzon and Toschi, 1960). Figure 1 shows the sucrose density gradient patterns of housefly polysomes. The small peak observed in tube 17 coincides exactly with the 79S monomer described for rat spleen by Talal (1966). By the use of this 79S monomer as a reference it can be calculated from Martin and Ames's (1961) equations that components larger than monomers predominate in these preparations. Pentamers (180 S) are definitely in larger proportions in the Ns, P_2, and P_2/sel strains, while trimers seem to be more abundant in the Fc strain. However it should be pointed out that in the latter strain the polysome profile varied from one preparation to another, pen-

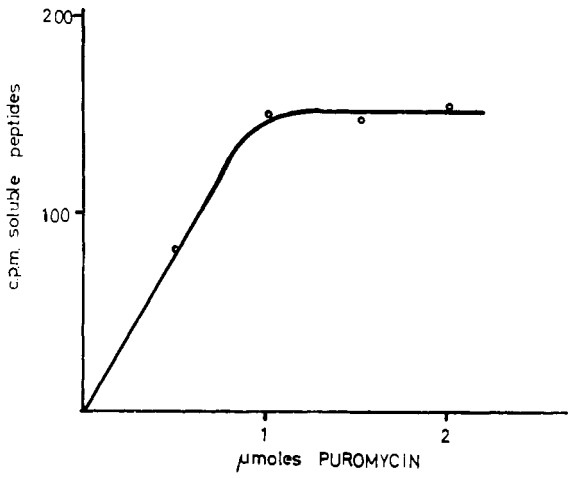

FIGURE 4: Effect of puromycin concentration on the puromycin-dependent release of newly synthesized peptides. Insects of the Fc strain were injected with L-phenylalanine-2-^{14}C and after 20 min polysomes were prepared as indicated in the text. The ^{14}C-labeled polysomes (0.2 mg of RNA) were incubated in a medium containing spermine, 0.5 μmole; $MgCl_2$, 3.5 μmoles; Tris-HCl buffer (pH 9), 20 μmoles; KCl, 140 μmoles; pH 5 fraction; 0.15 mg of protein; and puromycin as indicated. Final volume was 0.5 ml. Incubation was done for 4 min at 4°. The reaction mixtures were then centrifuged at 105,000*g* (120 min) and the radioactivity remaining in the supernatant fluid was measured. Every point corresponds to the average of three closely agreeing experiments.

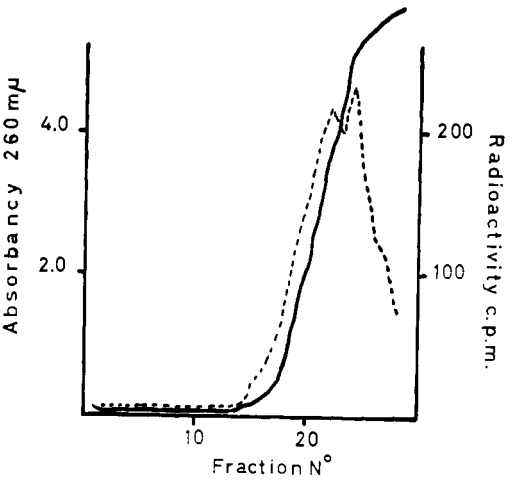

FIGURE 5: Sucrose density centrifugation of newly synthesized peptides released by the effect of puromycin. Polysomes obtained from the Fc strain injected with L-phenylalanine-2-^{14}C for 20 min were incubated with puromycin as indicated in Table I. The polysomes were then centrifuged at 105,000*g* (90 min) in the no. 40 rotor of the Spinco Model L centrifuge. The supernatant fluid was then layered on top of a 10–30% sucrose density gradient prepared in medium II, and centrifuged at 24,000 rpm for 16 hr in the SW 25.1 rotor of the Spinco Model L centrifuge. Fractions were obtained as in Figure 1. (——) Absorbancy and (- - -) radioactivity.

tamers predominating in some cases. These results are in disagreement with those reported by Tsiapalis *et al.* (1967) where monomers, dimers, and trimers were predominant.

When polysomes were prepared from insects which had been injected with L-phenylalanine-2-^{14}C for 20 min, the radioactivity followed closely the absorbancy profile as shown in Figure 2A. These results suggested that the aggregated ribosomes rather that monomeric species are involved in protein synthesis.

Further evidence that the 180S and 147S peaks corresponded to polysomes was obtained through the use of RNase. When polysomes obtained as in Figure 2A were treated with 5 μg of RNase/ml for 5 min at 37°, the pattern in Figure 2B was obtained. The polysomes were disaggregated into monomers with a sedimentation coefficient of 79 S. A similar situation occurs with the radioactivity profile; part of it coincided with the 79S absorbancy peak and the rest appeared at the top of the gradient. This latter radioactivity may correspond to newly released peptides. The profile of polysomes incubated at 37° in the absence of RNase was unchanged, which indicates that a temperature effect was not involved in this phenomenon, and that mRNA as expected participates in holding together the ribosomes. It should be pointed out that RNase at the concentration of 2 μg/ml at 0° has no effect on the polysomes pattern, which may explain the lack of effect of RNase on housefly polysomes reported by Tsiapalis *et al.* (1967), under the latter conditions.

That all ribosomal aggregates evidenced by the data of Figures 1 and 2 really correspond to ribonucleoprotein particles is shown by the spectra of the various absorbancy peaks (Figure 3) which indicate that the 237, 180, and 147 S have all typical spectrum with a 260 mμ/280 mμ ratio of approximately 2.0.

To show that the *in vivo* incorporation of ^{14}C-labeled amino acids into polysomes corresponds to true protein synthesis, polysomes obtained under the conditions of Figure 2A were incubated in the presence of puromycin as described by Hultin (1966). It is known that puromycin is capable of releasing polypeptide chains from rat liver ribosomes in amounts approaching 30–35% (Hultin, 1962) and it was assumed that a similar effect could be expected with our material. The optimal concentration of puromycin for the release of labeled polypeptides, as calculated from the data of Figure 4, was 5 μmoles/mg of polysomal RNA. The amount of released peptides was essentially the same in all four strains tested (Table I). Figure 5 shows the absorbancy and radioactivity patterns of the released peptides in puromycin-treated polysomes as analyzed by sucrose density gradients. The heavier components and subunits are absent and the radioactivity appears only in the region corresponding to soluble peptides. The latter were essentially not precipitable at pH 5.

DDT has been shown to increase the incorporation of ^{14}C-labeled amino acids into total proteins of *Musca domestica* (Agosin *et al.*, 1966). It was then considered of interest to establish the pattern of *in vivo* incorporation of ^{14}C-labeled amino acids into polysomes in insects pretreated with DDT. As shown in Figure 6, the rate of incorporation of L-phenylalanine-^{14}C into polysomes was linear during the entire experimental period in control as well as in DDT-treated insects. However, DDT markedly increases the amount of isotope incor-

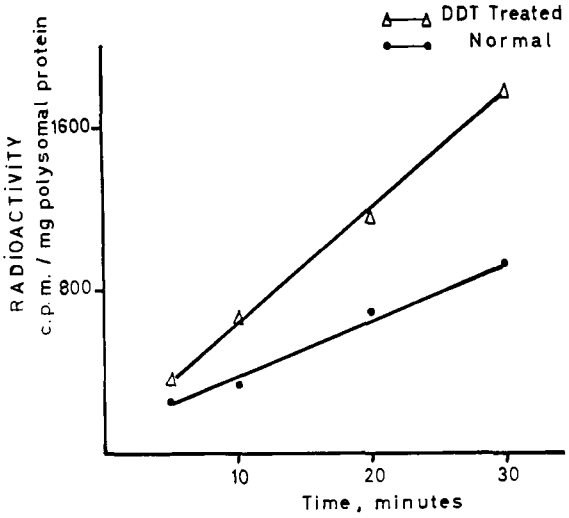

FIGURE 6: Effect of the *in vivo* DDT treatment on the incorporation of L-phenylalanine-2-^{14}C into polysomal protein. The insects (Fc strain) were topically exposed to DDT (1 μg/fly) for 60 min. Then they were injected with L-phenylalanine-2-^{14}C for periods of 5–30 min. At the end of the corresponding periods, polysomes were prepared, and the radioactivity incorporated into protein was determined as described under Experimental Section. Each point represents the average of five separate experiments.

poration into polysomal protein. These effects were observed only with DDT-resistant strains, but not with susceptible ones, such as the P_2 and Ns. Similar results were obtained with DL-leucine-1-^{14}C. When polysomes labeled with L-phenylalanine-2-^{14}C, obtained from control acid DDT-treated insects in the experiment described in Figure 7, were fractionated in a sucrose density gradient, the control insects showed a co-

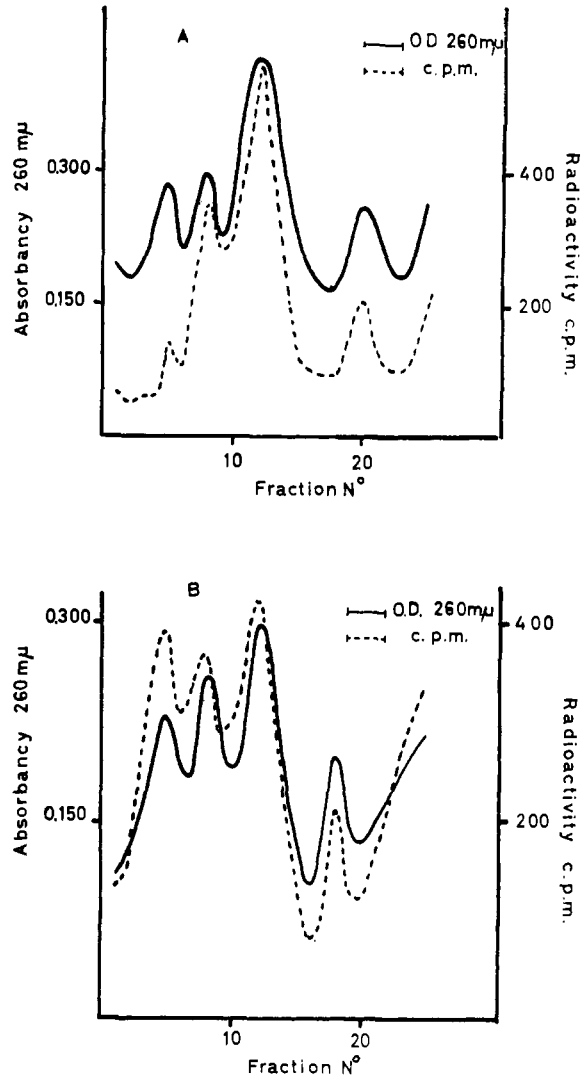

FIGURE 7: Effect of the *in vivo* treatment of DDT on the distribution of radioactivity from L-phenylalanine-2-^{14}C into polysomes. Polysomes obtained from insects of the Fc strain injected with L-phenylalanine-2-^{14}C for 20 min as in Figure 6, from control, and DDT-treated insects were fractionated by sucrose density gradients under the conditions of Figure 1.

TABLE I: Puromycin-Dependent Release of Peptides from ^{14}C-Labeled Polysomes.[a]

Strain	Released Peptides (%)
Fc	21
P_2	19
P_2/sel	21
Ns	25

[a] Insects of the indicated strains were injected with L-phenylalanine-2-^{14}C and after 20 min polysomes were prepared as described in the text. The polysomes were incubated in the following medium: spermine, 0.5 μmole; puromycin, 0.5 μmole; Tris-HCl buffer (pH 9), 20 μmoles; KCl, 150 μmoles; $MgCl_2$, 3.5 μmoles; pH 5 fraction prepared by Litvak *et al.* (1967), 0.075 mg of protein; ^{14}C-labeled polysomes; and 0.1 mg of RNA. The final volume was 0.5 ml. Incubation was done for 4 min at 4°. The reaction mixtures were then centrifuged at 105,000*g* for 120 min and the radioactivity remaining in the supernatant fluid was measured. The figures represent the average of two closely agreeing experiments.

inciding pattern of absorbancy and radioactivity (Figure 7A) although the heavier component was little labeled. On the other hand, labeling of polysomes obtained from DDT-treated insects was heavier with the most dramatic increase at the 180S and 240S particles (Figure 7B).

The above results suggested that DDT in some manner could be affecting translational mechanisms at the ribosomal level. This possibility is supported by the data presented in Table II. About 69% of the total radioactivity found in the various subcellular fractions corresponded to the nuclei, while 22% was associated with the microsomal fraction. It could be argued that the latter may be a consequence of metabolic inactivation of DDT (Agosin *et al.*, 1961). However this could be only a partial explanation in view of the data of Figure 8. Polysomes derived from the microsomal fraction obtained under the conditions of Table II showed by

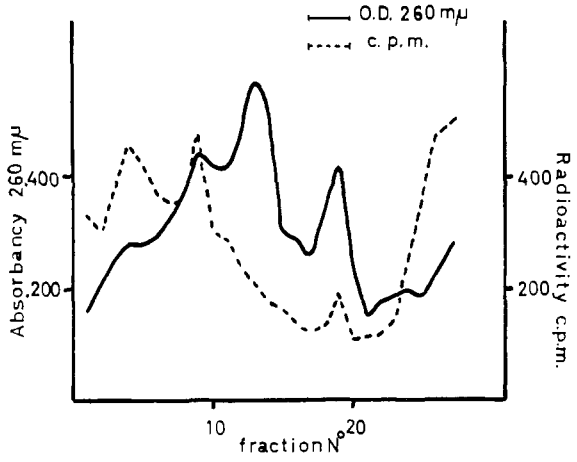

FIGURE 8: Sucrose density gradient centrifugation of polysomes from insects (Fc strain) injected with DDT-^{14}C. The insects were injected with DDT-^{14}C and after 20 min the polysomes were analyzed as in Figure 1. The radioactivity of each fraction was asessed as described under Experimental Section.

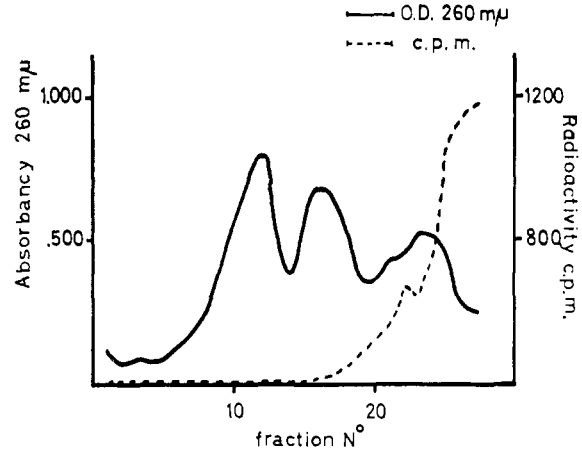

FIGURE 9: Effect of SDS on the sucrose density gradient pattern of polysomes obtained from insects given a 20-min pulse of DDT-^{14}C. Procedure as in Figure 8. SDS was used at a final concentration of 1% as indicated under Experimental Section.

sucrose density gradient analysis that DDT is associated with ribosomal aggregates, mainly with the 237S and 180S particles. Some radioactivity was also found at the top of the gradient, which apparently corresponds to association of DDT to nascent peptides, as it will be shown below.

It is known that SDS denatures ribosomal protein and releases newly synthesized peptides attached to tRNA, which provides a convenient method for following the peptides at the 4S region in sucrose density gradients (Gilbert, 1963). Polysomes labeled with DDT obtained as in Figure 8 were incubated with SDS at a 1% final concentration for 30 min at 22°. The polysomes were then analyzed in a 5–20% sucrose gradient as indicated in Figure 9. Two absorbancy peaks at 260 mμ were found with sedimentation coefficients of 18 and 28 S, which indicates their ribosomal origin. No radioactivity was associated with these two peaks but with a 4S peak, indicating that DDT is tightly bound to nascent peptides. These results are supported by the fact that radioactivity of DDT-^{14}C-labeled polysomes was partially released (45%) by the addition of puromycin. Again this figure of 45% which is higher than for amino-^{14}C acid labeled polysome peptide release (21%), suggests that DDT may affect translational mechanisms at the ribosomal level.

Following the above line of thought, it was then possible to contemplate some *in vitro* effects of DDT on the ability of housefly polysomes to incorporate labeled amino acids. As shown in Figure 10, DDT at a final concentration of 2 μg/ml increases the incorporation of L-phenylalanine-2-^{14}C into polysomal protein in about 20% in the Fc strain. However, higher concentrations were inhibitory. Similar results were obtained with the P_2/sel strain. The kinetics of the stimulatory effect of DDT is presented in Figure 11. Maximal incorporation with 2 μg/ml is observed at not more than 5-min incubation, while in the system without DDT the optimal is reached after 10-min incubation. These results were consistently obtained in several experiments.

Although the *in vitro* effect of DDT was consistently obtained, the degree of stimulation obtained could not explain the *in vivo* enhancing of the incorporation of labeled amino acids into polysomes by pretreatment of the insects with DDT. Therefore, it was concluded that in addition to the possible translational mechanism, there should be a modification on gene expression, mediated through the DNA-dependent synthesis of mRNA. The ratio of RNA to DNA in nuclei preparations of the Fc strains was found to be 2.2. This ratio is much higher than that of rat liver nuclei (Gelboin and Bates, 1966) and *Triatoma infestans* (S. Litvak, unpublished results). However, these results are in line with those reported by Price (1965) for total nucleic acids of *Calliphora* larvae. When the insects were treated for 60 min with 1 μg of DDT/fly, the ratio of RNA/DNA in nuclei increased to 3.7. Since the amount of nuclear DNA was practically unchanged, as determined by the diphenylamine method (Dische, 1930), this is indicative that nuclear RNA is increased by DDT. When nuclear RNA,

TABLE II: Intracellular Distribution of DDT-^{14}C in Houseflies.[a]

Fraction	Radioactivity (cpm/fraction)
Nuclei	2240
Sarcosomes	140
Microsomes	720
Soluble	75
Sum	3175

[a] Groups of 40 flies each of the Fc strain were injected with 1 μl of ^{14}C-ring-labeled DDT (8000 cpm/fly). After 20-min standing at 27°, the fractions were obtained and their radioactivity was determined as described under Experimental Section. The figures represent the average of three closely agreeing experiments.

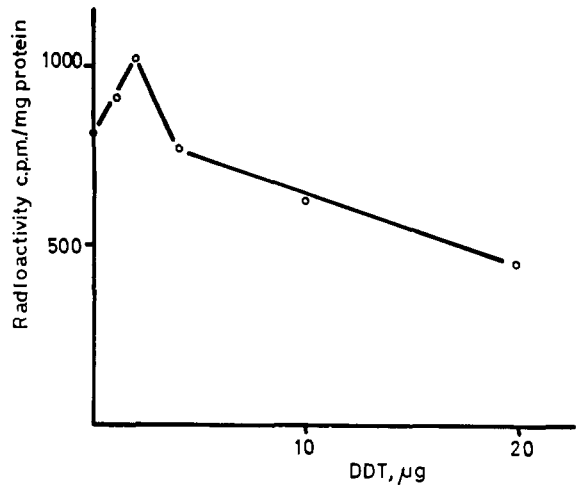

FIGURE 10: The *in vitro* effect of DDT on the incorporation of L-phenylalanine-2-^{14}C into protein by the cell-free system. The reaction mixtures contained in a final volume of 1.0 ml: creatine phosphate, 2 μmoles; creatine phosphokinase, 60 μg; adenosine triphosphate, 5 μmoles; guanosine triphosphate, 0.2 μmole; Tris-HCl buffer (pH 8.0), 50 μmoles; β-mercaptoethanol, 5 μmoles; $MgCl_2$, 10 μmoles; KCl, 25 μmoles; polysomes obtained from the Fc strain, 0.2 mg; pH 5 fraction, 0.1 mg; amino-^{14}C acid, 2.5 μmoles; (300,000 cpm/μmole); and DDT as indicated, dissolved in 10 μl of ethanol. Incubation was for 20 min at 37°. Other conditions as described by Litvak *et al.* (1967). The zero DDT value corresponds to the control. Each point corresponds to the average of four closely agreeing experiments.

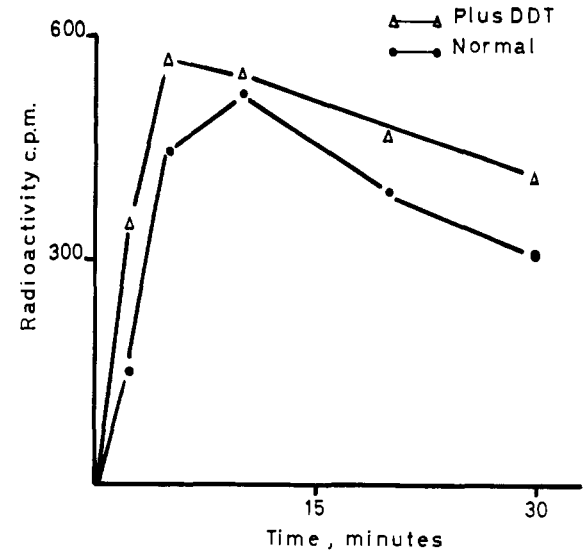

FIGURE 11: Kinetics of the *in vitro* effect of DDT on the incorporation of L-phenylalanine-2-^{14}C into protein by the cell-free system. Conditions as in Figure 10, with 2 μg of DDT/ml.

isolated by the phenol procedure from control and DDT-treated insects, was added to the cell-free protein-synthesizing system, it was found that the DDT–RNA had a much higher template activity than the control RNA (Table III). It is interesting to note that polysomes isolated from DDT-treated insects are more active in incorporating L-phenylalanine-2^{14}-C than control polysomes, indicating that the former have higher mRNA contents. It is further evident that polysomes obtained from DDT-treated insects seem to be almost fully saturated with mRNA. The differences cannot be attributed to the pH 5 enzymes which were obtained in all cases from normal insects.

TABLE III: Template Activity of Nuclear RNA Isolated from Control and DDT-Treated Insects.

Control Polysomes	DDT Polysomes	Control RNA	DDT–RNA	L-Phenylalanine-2-^{14}C Incorp (cpm/mg of protein)
+	−	−	−	275
+	−	+	−	385
+	−	−	+	700
−	+	−	−	355
−	+	+	−	650
−	+	−	+	800

[a] The insects were topically exposed to DDT (1 μg/fly) for 60 min. RNA, as well as polysomes from control and intoxicated insects, was obtained as indicated under Experimental Section. System as in Figure 10. RNA (200 μg) was added to each flask as indicated. The figures represent the average of three closely agreeing experiments.

Discussion

It is evident from our results that polysomes in the classical sense are present in our preparations. It is difficult to ascertain at present whether the differences observed between the Fc strain and the other ones (Figure 1) have physiological implications. However, in all the strains examined, with the exception of the Fc, ribosomal aggregates larger than trimers predominate. This is in contrast to the observation of other authors (Tsiapalis *et al.*, 1967) who reported a predominance of monomers, dimers, and trimers with the apparent absence of larger aggregates. Strain differences do not seem to account for their results, but probably the procedure used to isolate polysomes. The method of Wettstein *et al.* (1963) used by Tsiapalis *et al.* (1967) did not prove to be convenient for isolating polysomes from our material. Even after centrifugation of a 15,000*g* (10 min) supernatant for 4 hr at 40,000 rpm through a discontinuous sucrose gradient from 17.1 to 68.4% a pellet was not obtained.

That the pattern obtained in Figure 1 corresponds to polysomes is clearly evidenced by the effect of RNase (Figure 2B). The radioactivity patterns after the *in vivo* injection of ^{14}C-labeled amino acids indicate that these polysomes are the main site of protein synthesis (Figure 2A). It may then be concluded that polysomes prepared from housefly tissue are similar to those described for mammalian tissues.

The 79S monomer of Figure 1 corresponds to the

ribosomes described for rat spleen by Talal (1966). Reports from our laboratory show that two ribosomal components are present in housefly RNA, with sedimentation coefficients of 28 and 18 S, respectively (I. Balazs and M. Agosin, unpublished results). These values agree very well with the presence of the 79S monomer. Our results do not confirm the presence of RNA fractions of 23 and 15 S in housefly tissues (Tsiapalis *et al.*, 1967) although our 79S monomer seems to be equivalent to the 78S one described by these authors.

It has been fairly well established that DDT is a powerful enzyme inducer (Agosin *et al.*, 1967). This is an indication of possible changes in the gene action. This is supported by the fact that nuclear RNA increases by the effect of DDT, and that this DDT–RNA has a high template activity (Table III). Further evidence implicating DNA-dependent RNA synthesis as an early effect of DDT has been obtained in our laboratory (I. Balazs and M. Agosin, unpublished results). How this DNA-dependent RNA synthesis is brought about by the effect of DDT is difficult to interpret at this time. However, the results of the DDT intracellular distribution studies indicate that the compound is concentrated in the nuclei (Table II). DDT is apparently bound to protein in the nuclei since the protein purification procedure used (Schneider, 1957) eliminates lipids, carbohydrates, and nucleic acids. It is then possible that DDT may be bound to certain histones forming the so-called charge-transfer complexes described for insect axon (O'Brien and Matsumura, 1964). As a result, the ionic bonds holding together these histones with DNA are weakened. The latter, in turn, may bring about a derepression of genes with an increased synthesis of mRNA. Derepression of genes for pea seed globulin synthesis by removal of histones has been already reported (Bonner, 1965).

Part of the injected DDT is also found associated with 180S and 240S polysomes (Figure 8). Apparently, in this case DDT is bound to nascent proteins as suggested by the effects of SDS (Figure 9) and puromycin. It is also possible that DDT may be forming charge-transfer complexes with these nascent proteins. It has been reported that compounds that are substrates for microsomal mixed-function oxidases interact with a microsomal haemoprotein causing spectral changes (Remmer *et al.*, 1966). Whether this type of binding is applicable to our system remains to be established, specially since ferritin has been found in certain types of polysome preparations (Wilson and Hoagland, 1965). In any case, DDT is modifying the *in vivo* labeling polysome pattern, since most of the radioactivity from amino-^{14}C acids is found associated not only with the 180S as in control insects, but mainly with the 240S particles (Figure 8).

Work from this laboratory (I. Balazs and M. Agosin, unpublished results) suggests that DDT induces the synthesis of a mRNA of approximately 23 S. This observation supports the existence of polysome aggregates higher than pentamers in our preparations. The 23S mRNA would be holding together about eight ribosomes, a size that could correspond to our 240S particles. Furthermore, this indicates that the upper size for proteins synthesized by polysomes in houseflies would be around a molecular weight of 35,000, which is double than the value previously reported (Tsiapalis *et al.*, 1967).

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