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Effect of Polychlorinated Biphenyls (Phenoclor DP6) on *In Vivo* Protein and RNA Synthesis in Rat Liver

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The administration of polychlorinated biphenyls to rats increases a large number of liver microsomal enzyme activities (LITTERST et al. 1972, BRUCKNER et al. 1973, GRANT and PHILLIPS 1974). These changes have been correlated with an increase of total and microsomal liver proteins (NARBONNE and DAUBEZE 1978). The purpose of our work was to examine the mechanism of the PCB stimulation of protein synthesis. BERLIN and SCHIMKE (1965) have pointed out the importance of both enzyme synthesis and degradation in many studies on enzyme induction. Thus, a stabilization of an enzyme which prevents its degradation may mimic enzyme induction (GELBOIN 1972). A previous work (NARBONNE 1979 a) indicates that in the liver of rats ingesting a DP6 diet, the half lives of total and microsomal membrane proteins were significantly shorter than in the control group. Moreover a *in vitro* study (NARBONNE 1979 b) indicates that in rats fed a DP6 diet (10 ppm) the incorporation of amino acids into microsomal proteins was stimulated.

The present work was undertaken in order to determine the effect of Phenoclor DP6 ingestion on the *in vivo* rate of protein and RNA synthesis in liver cell fractions. ^{14}C-guanidino-arginine is used for protein labeling because this isotope minimizes the reincorporation of the labeled amino acids released by protein degradation (DALLMAN and MANIES 1973).

MATERIALS AND METHODS

Animals : Forty male rats of the Sprague-Dawley strain weighing 67 ± 2 g were maintained for 1 week on a standard diet containing 15 % (w/w) casein (NARBONNE and GILLET 1978). After the adaptation period, 20 rats were fed the DP6 diet containing 10 ppm (wet weight) for 2 days. The DP6 ingested by treated rats during the intoxication time was 242 µg ± 10/rat (2.72 mg/kg body weight). Twenty control rats were fed a standard diet during the experimental period. After a 16 hour overnight fast, rats were injected intra-

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peritoneally with a 0.5 ml solution of 9 3% NaCl containing 5 µci ^{14}C-guanidino-arginine and 50 µci ^{3}H-5-orotic acid purchased from C.E.A. France). The rats were killed by decapitation 5, 10, 30 and 60 min. after the isotope injection between 9 and 10 A.M.

Cell fractionation : Cell fractionation was carried out according to a modification of the methods described by DALLMAN and MANIES (1973) and ZILE and DE LUCA (1970). A 3 g sample of each liver was homogenized in a teflon glass homogenizer with 20 ml of cold medium A (0.25 M sucrose, 0.05 M tris pH 7.4, 0.025 M KCl, 0.005 $MgCl_2$) fractionation was carried out at 4°C. Four ml of the whole homogenate were mixed with 4 ml of 1.2 N perchloric acid (PCA) for total protein and RNA determination.

Nuclear fraction : the remaining homogenate was centrifuged for 10 min. at 800 x g. The pellet was rehomogenized two times in a hypotonic buffer consisting of 0.01 M NaCl, 0.01 M Tris chloride, pH 7.4, 0.0015 M $MgCl_2$ $6H_2O$ (PENMAN 1966). The final pellet was mixed with 5 ml of 0.7 N PCA and designated as the nuclear fraction.

Mitochondrial fraction : the 800 x g supernatant was centrifuged for 20 min. at 6,500 x g. The pellet was resuspended in 6 ml of medium A and centrifuged 20 min. at 6,500 x g. The final pellet was mixed with 5 ml of 0.7 N PCA and designated as the mitochondrial fraction.

Microsomal fraction : the 6,500 x g supernatant was centrifuged at 10,000 x g for 10 min and the pellet was discarded. The 10,000 x g supernatant was then centrifuged at 105,000 x g for 60 min. The pellet was resuspended in 10 ml of medium A and then 5 ml of this suspension were mixed with 5 ml of 1.2 N PCA and designated as the microsomal fraction.

Microsomal membrane fraction : the remaining 5 ml were mixed with 0.75 ml of 10 % sodium dodecyl sulfate and allowed to stand for 10 min. After centrifugation for 2 hours at 105,000 x g the supernatant, containing the non-ribosomal elements of the microsomal fraction, was mixed with 15 ml of 1.2 N PCA and designated as the microsomal membrane fraction.

Supernatant fraction: the 105,000 x g supernatant was mixed with 15 ml of 1.2 N PCA and designated as the supernatant fraction.

Measurement of RNA, protein and radioactivity : The RNA extraction was carried out by the method of SCHMIDT and THANNHAUSER (1945) modified by FLECK and MUNRO (1962). The cellular fractions mixed with PCA were centrifuged for 5 min. at 5,000 x g. The pellets were washed twice with 0.5 N PCA, resuspended in 0.33 N KOH, and then allowed to stand for 1 hr at 37°C. Samples of this solution were used for protein determination by LOWRY's method (1951).

The total, nuclear and soluble supernatant RNA nucleotides were extracted from KOH hydrolysate aliquots of the corresponding fractions by PCA extraction and measured at 260 nm in a I.S.C.O. flow cell spectrophotometer. For measurement of radioactivity, 0.2 ml of the hydrolysate was transferred to a counting vial and mixed with 1 ml of soluen 100 and 10 ml of a toluene scintillation fluid containing 0.4 % 2,5-diphenyloxazole and 0.01 % 2,2'-p-phenylen-bis (5-phenyl oxazole). Radioactivity was measured in a SEARLE liquid scintillation counter model delta 300. The channels were calibrated for a ^{3}H and ^{14}C dual label. Quenching and efficiencies were calculated using the external standard. The results were expressed as dpm ^{14}C/milligram protein (apparent specific radioactivity, A.S.R. of protein) dpm ^{3}H/milligram RNA (apparent specific radioactivity of RNA).

RESULTS

Effect of DP6 treatment on in vivo incorporation labeled amino acid into the proteins from liver cell fractions : As shown in figure 1, the incorporation of ^{14}C arginine into liver proteins is maximum at 30 min in most of the studied fractions. At this experimental time we found a significant difference ($P < 0,001$) in the protein A.S.R. of microsomal and microsomal membrane fraction between control and treated rats. At time 60 min. the protein A.S.R. was siginficantly higher in microsomal and microsomal membrane from DP6 fed rats than from controls. The higher A.S.R. found in total and cytosoluble fractions from treated rats was little significative ($P < 0,01$).

Effect of DP6 treatment on in vivo incorporation of ^{3}H-5-orotic acid into the RNA of various liver cell-fractions : As shown in figure 2 (a and b) the RNA A.S.R. were lower in total and nuclear fractions from the treated group than in the control. However, the incorporation of ^{3}H radioactivity was significantly increased in the supernatant fraction from DP6 fed rats (figure 2 C).

DISCUSSION

Protein synthesis : The present data indicate that in the liver of Phenoclor DP6 treated rats there is an increased rate of incorporation of the labeled amino acid studied into microsomal proteins. In this protein fraction, the increased synthesis rate may be due to an increased rate of incorporation into membrane proteins. In the nuclear and mitochondrial fractions the protein synthesis is not affected by DP6 treatment. KATO et al. (1965) have studied the effect of phenobarbital on the in vivo incorporation of ^{14}C-leucine in the various subcellular fractions of rat liver. These authors showed that phenobarbital affected only the rate of

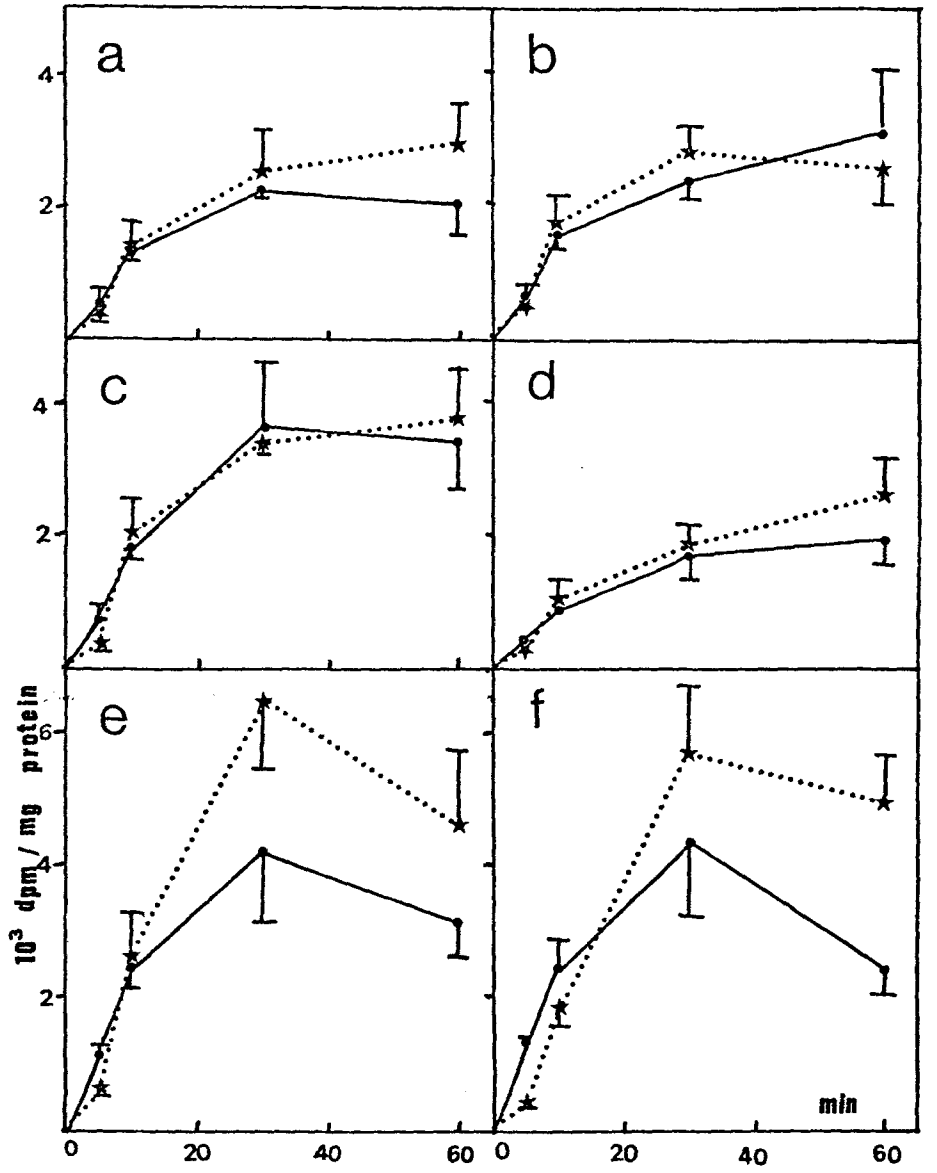

Figure 1. ^{14}C guanidino-arginine incorporation into the proteins of various cellular fractions of liver from controls (•———•) and DP6 fed rats (★·····★).
a- total liver, b- nuclear fraction, c- mitochondrial fraction, d- soluble fraction of the cell sap, e- microsomal fraction, f- microsomal membrane fraction

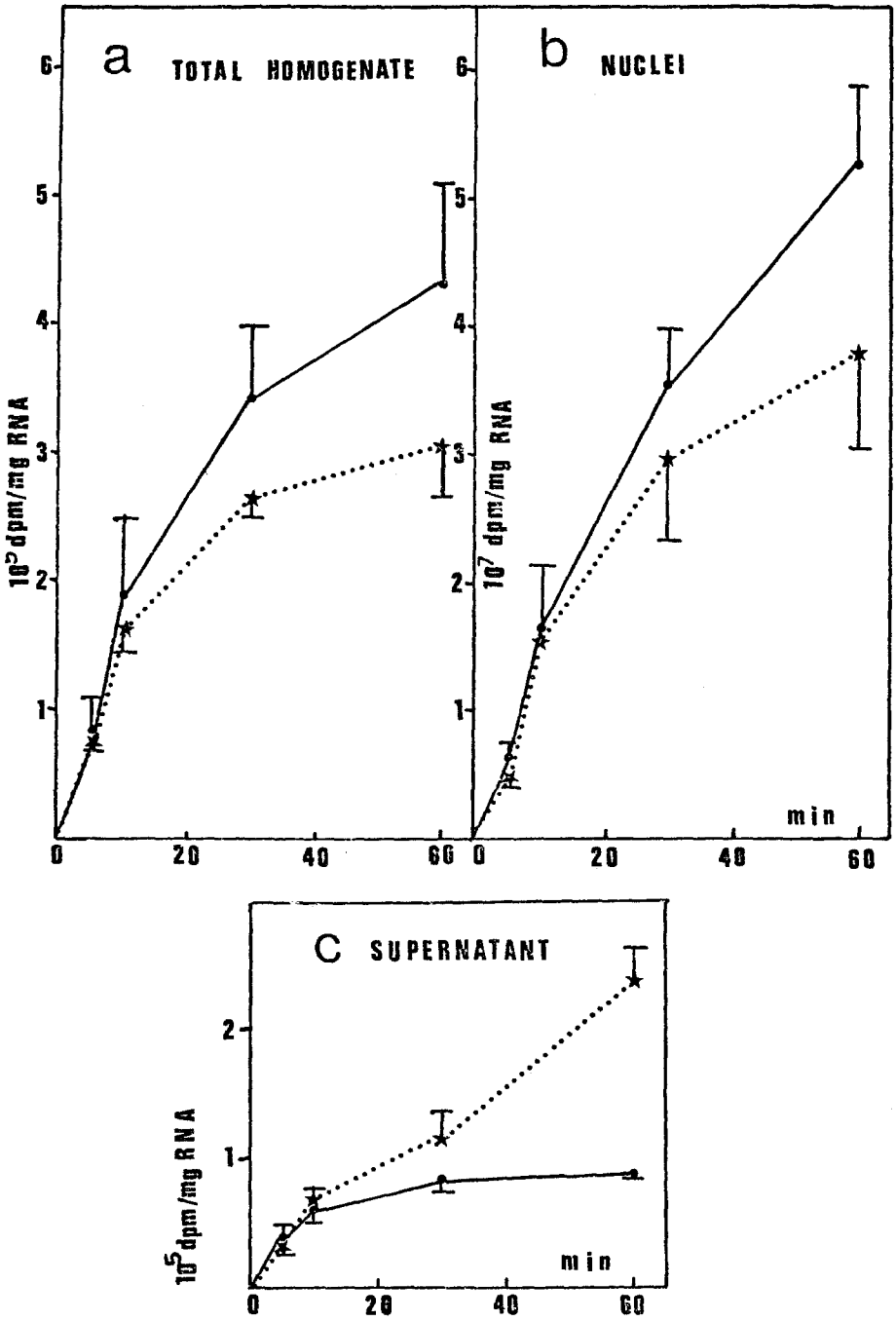

Figure 2. ^{3}H-5-orotic acid incorporation into the RNA of various cellular fractions of liver from controls (• ——— •) and DP6 treated rats (★·····★)

incorporation of amino acids into the microsomal fraction. The protein from nuclear, mitochondrial, or the soluble fraction of the cell sap showed a similar incorporation rate in control and in phenobarbital-treated rats. Moreover, this increase was observed in the membrane component of rat liver microsomes. Several authors have reported an increase in the in vitro incorporation of ^{14}C-leucine into microsomal proteins from phenobarbital or methycholanthrene (M.C.) treated rats (DECKEN and HULTIN 1960, GELBOIN and SOKOLOFF 1964). The increased incorporation of ^{14}C-lysine in vitro observed after 30 min. incubation time into an acellular system from rats fed a 10 ppm DP6 diet was previously reported (NARBONNE 1979 b). Moreover, the DP6 treatment increases the rate of protein breakdown (NARBONNE 1979 a). Our results indicate that protein metabolism (synthesis and breakdown) in the liver microsomal fraction is enhanced by DP6 ingestion.

RNA synthesis : In our experimental conditions the ingestion of DP6 had no stimulatory effect on the synthesis of total and nuclear RNA. At the 60 min. incorporation time the RNA A.S.R. was significantly lower in DP6 treated rats than in controls. Although, our experimental procedure does not permit us to conclude that the synthesis of one type of RNA may not be increased by DP6 treatment. Thus, the incorporation rate of ^{3}H orotic acid into soluble RNA was higher in the soluble fraction of the cell sap from DP6 fed rats. In an in vitro study we have demonstrated than the activating factor of protein synthesis in amino acid incorporating systems from livers of DP6 treated rats was located in the post-polysomal supernatant (NARBONNE 1979 b). These results suggest that a part of the stimulatory effect of DP6 amino acid incorporation is due to soluble factors which may influence the formation of tRNA-bound amino acid. However GELBOIN and SOKOLOFF (1961) also observed the stimulatory effect of M.C. when tRNA-^{14}C leucine and tRNA-^{14}C proline were used as precursors in place of the amino acid. In a subsequent study GELBOIN and SOKOLOFF (1964) found an increased level of GTP in rat liver supernatant of animals treated with M.C.

In considering the "induction" effect of a Phenoclor DP6 treatment on liver microsomal enzyme systems it must be noted that in the microsomal membrane some enzymes remain little affected by DP6 treatment (e.g. ATPase, NARBONNE 1978 a; or G6Pase NARBONNE 1978 b) while drug metabolizing systems were increased by DP6 ingestion (e.g. aminopyrine demethylase, aniline hydroxylase, cyt. P450, NARBONNE 1978 b). Thus, although totally different messenger RNA's may be involved in these cases, striking quantitative changes in the synthesis of total RNA in response to DP6 may not be observed. This reasoning best explains our data which demonstrate

a moderate difference (< 30 %) in nuclear RNA synthesis between the DP6 fed rats and control rats. In this regard, it is interesting to note that DP6 administration actually decreases the (^{3}H) 5-orotic acid incorporation into nuclear RNA. It is likely that DP6 is acting in some fashion other than an inducer of one or more genes and is probably playing a somewhat more general role in nuclear activity. Thus, it would be desirable to study the nature of RNA formed in response to DP6 rather than merely measuring total RNA synthesis.

CONCLUSION

The results obtained from this study indicate that DP6 ingestion increases the protein synthesis in microsomal membrane fraction from rat liver. However, the nature of the relation between DP6 and RNA metabolism is still not clear. Our experimental procedure does not study all types of RNA involved in the protein synthesis mechanism. However, the increased incorporation of ^{3}H-orotic acid into the soluble RNA from DP6 fed rats suggests that this fraction participates in the stimulatory effect of DP6 ingestion.

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