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EFFECTS OF 3,4-BENZPYRENE, PHENOBARBITAL, AND CHLORDANE ON THE NUCLEIC ACID AND PROTEIN CONTENT OF SUBFRACTIONS OF RAT LIVER HOMOGENATES*

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Abstract—3,4-Benzpyrene, phenobarbital, and chlordane are three compounds that produce marked increases in the activities of hepatic microsomal drug-metabolizing enzyme systems. They were compared with regard to their effects on the nucleic acid and protein content of various subfractions of rat liver homogenates. Pretreatment of rats with chlordane and phenobarbital produced similar changes. The changes produced by pretreatment with 3,4-benzpyrene, however, differed from those of phenobarbital and chlordane. The differences between the effects of 3,4-benzpyrene and those of chlordane and phenobarbital were noted primarily in the subfractions of rat liver homogenates which contained largely smooth endoplasmic reticulum (SER). Chlordane and phenobarbital caused significantly greater increases than 3,4-benzpyrene in the RNA and protein content of the fraction containing the SER. Pretreatment with each of the three compounds produced statistically significant decreases in DNA content of liver homogenates when expressed as mg DNA/g liver. Considered in the light of previously accumulated evidence, the results provided further support for the hypothesis that 3,4-benzpyrene may exert its stimulatory effects on hepatic microsomal drug-metabolizing enzyme systems through a mechanism different from that of phenobarbital and chlordane.

SEVERAL investigators have shown that there is a marked increase in activity of microsomal drug-metabolizing enzymes of the liver after the administration of a variety of drugs and/or chemicals. Reviews have appeared in recent years which describe these phenomena.[1–5] Among the agents that are most potent in producing such activity increases are the polycyclic aromatic hydrocarbons, the long-acting barbiturates, and the chlorinated hydrocarbon insecticides. Conney *et al.*[6] proposed that increased drug-metabolizing enzyme activity resulting from administration of stimulatory agents was due to increased enzyme synthesis *de novo*. The long-acting barbiturates and chlorinated hydrocarbon insecticides[7] appear to cause a nonspecific type of adaptation by the liver which results in the accelerated metabolism of a wide variety of lipid-soluble compounds. The polycyclic aromatic hydrocarbons, on the other hand, produce increases in the metabolism of comparatively few compounds. Associated with the increases in enzymic activity produced by phenobarbital and chlordane pretreatment are increases in the amount of the smooth endoplasmic reticulum (SER), as observed by Remmer and Merker[8] and Fouts and Rogers.[9]

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Remmer and Merker[10] also noted increases in the phosphorus, nitrogen, and lipid content of the SER, as well as smaller increases in protein and RNA content of the same fraction when animals were pretreated with phenobarbital. Fouts and Rogers[9] reported that little change occurs in the SER of livers of animals pretreated with the polycyclic aromatic hydrocarbons 3-methylcholanthrene and 3,4-benzpyrene. Hart and Fouts[7] noted an increase in the nitrogen content of the 9000 *g* supernatant fraction of livers from animals pretreated with chlordane.

The present study compares the effects of 3,4-benzpyrene, phenobarbital, and chlordane on the protein and nucleic acid content of various subfractions of rat liver homogenates from rats pretreated with these enzyme-stimulating agents. Special attention is given to the protein and RNA content per cell (where DNA is used as a measure of cellularity), per g liver tissue (wet weight) and per total liver. Considered in the light of previous studies pertaining to mechanisms of induction of hepatic microsomal drug-metabolizing enzyme systems, the present studies appear to present further evidence that phenobarbital and chlordane exhibit their enzyme-stimulating properties through similar mechanisms which differ from that of 3,4-benzpyrene.

MATERIALS AND METHODS

Preparation of animals

Male Long-Evans rats weighing 60–80 g were used for all experiments. They were maintained on regular Purina lab chow and water *ad libitum* until immediately preceding each experiment. All stimulators of hepatic microsomal enzyme activity were injected intraperitoneally (i.p.). Phenobarbital sodium (dissolved in saline) was injected at a dose of 40 mg/kg twice a day for 3 days. Protein and nucleic acid assays were run 24 hr after the last dose of phenobarbital. Chlordane and 3,4-benzpyrene were administered as solutions in corn oil. Chlordane, as the γ-isomer (98·9 $\pm$ 0·3% pure) was given once a day at a dose of 50 mg/kg for 3 days, with assays run 24 hr after the last dose. 3,4-Benzpyrene was injected only once at a dose of 20 mg/kg, with assays run 48 hr later. All animals, regardless of the stimulator used, were weighed at the beginning of injections for the phenobarbital-treated group and immediately before sacrifice.

Preparation of liver homogenates

All rats were killed by cervical dislocation; livers were quickly and completely removed and rinsed thoroughly in ice water. They were then blotted, weighed, and homogenized in ice-cold 0·88 M sucrose in a Potter homogenizer with a plastic pestle. Pooled homogenates of three livers were employed for each determination. Each 3 ml of the whole homogenate contained 1 g liver. After having taken appropriate aliquots for nucleic acid and protein determinations, the whole homogenate was centrifuged at 9000 *g* for 20 min at 1–3°. Both the supernatant and the pellet were reconstituted with ice-cold distilled water to the original volume of the whole homogenate, and aliquots were again taken for nucleic acid and protein assays. The 9000 *g* supernatant presumably contained the soluble fraction and the microsomal elements of the disrupted cells, whereas the pellet contained nuclei, mitochondria, and some undisrupted cells.

Smooth endoplasmic reticulum and rough endoplasmic reticulum (RER) membranes were separated according to a modification of the procedure of Fouts.[11]

In this modification, 2 ml of 1·33 M sucrose solution was layered under exactly 8 ml of the reconstituted (in approximately 0·6 M sucrose solution) 9000 *g* supernatant fraction in ultracentrifuge tubes. This was centrifuged for 8 hr in a Spinco ultracentrifuge at 40,000 rev/min (104,000 *g*) at 1–3°. The pellet, containing the rough endoplasmic reticulum, was resuspended in 0·88 M sucrose solution such that the final volume was 4 ml. The entire supernatant was reconstituted to 10 ml, mixed thoroughly, and centrifuged for 1 hr at 104,000 *g* at 1–3°. This pellet, containing smooth endoplasmic reticulum, was resuspended in 0·88 M sucrose such that the final volume was 4 ml. Aliquots from these suspensions of rough- and smooth-surfaced membranes and from the 104,000 *g* supernatant were then assayed for nucleic acid and protein content.

Assay procedures

The protein content of homogenates of each subfraction was assayed by a microbiuret method described by Itzhaki and Gill.[12] Actual values were determined from a standard curve; bovine serum albumin was used as the reference standard. RNA and DNA determinations were made on each subfraction according to a modified method of Scott *et al.*[13] The modifications consisted of an adaptation of the method to accommodate analyses of 0·5- to 1·0-ml aliquots of tissue homogenates. Reagent volumes were increased by a factor of 80. With the exception of the incubations, all parts of the procedure, including centrifugations and stirrings, were carried out in the cold (1–3°). All reagents used in the assay were also kept refrigerated. Actual values of RNA and DNA were obtained from standard curves, with yeast RNA, type XI, and the sodium salt of calf thymus DNA, type 1, as respective standards. (RNA and DNA were obtained from Sigma Chemical Co.). Yeast RNA and calf thymus DNA were carried through the same assay procedure and plotted against the 260-mμ O.D. readings. In assays of homogenates, dilutions were made such that all O.D. readings were in the linear range of the standard curve.

Statistics

A randomized complete block was employed in the experimental design. The results were evaluated by analysis of variance and Duncan's new multiple-range test. The level of significance chosen was $P < 0{\cdot}05$. All statistical procedures are described by Steel and Torrie.[14]

RESULTS

Comparison of the effects of stimulating agents on liver weights

The liver weights of the chlordane- and phenobarbital-treated animals were significantly greater than those of the controls, as indicated in Table 1. However, the ratio of the liver weights to the animal weights was significantly increased only in the chlordane-treated rats. The differences in weight gain of the animals were not statistically significant. Weights of all the animals were measured immediately prior to the first injections of phenobarbital-treated group and again just prior to sacrifice.

Comparison of the effects of stimulating agents on the nucleic acid content of subfractions of liver homogenates

When expressed as mg DNA/g liver (wet weight), small but statistically significant decreases in DNA content were observed in the whole homogenates of livers from

TABLE 1. DIFFERENCES IN THE LIVER WET WEIGHTS OF ANIMALS AND DNA CONTENT OF LIVERS

Treatment	Liver weights (g)				Liver weight/animal weight				Mg DNA/g liver (whole homogenate)			
	Mean liver weight	(% of control)	Significance*	N†	(g liver/ g animal)	(% of control)	Significance	N	(mg DNA/ g liver)	(% of control)	Significance	N
Control	4·38			26	0·196			21	3·79			8
3,4-Benzpyrene	4·89	+11		26	0·216	+11		21	3·54	−7		8
Phenobarbital	5·22	+19		26	0·224	+14		21	3·33	−14		8
Chlordane	5·31	+21		26	0·259	+32		21	3·33	−14		8
	CV‡ = 17·6%				CV = 18·3%				CV = 6·4%			

* For all tables, in each column, any values joined by a straight line are not significantly different ($P > 0.05$); any values not joined by a straight line are significantly different ($P < 0.05$).

† N = number of values used to compute the mean, in all tables.

‡ Coefficient of variability, in all tables.

animals pretreated with the stimulating agents (Table 1). The decreases in DNA content produced by phenobarbital and chlordane appeared to be of a greater magnitude than that produced by 3,4-benzpyrene but, when analyzed by Duncan's new multiple range test, the differences between stimulators were not statistically significant.

Expressed as mg DNA/total liver, the mean values of the control homogenates and homogenates from animals pretreated with stimulating agents did not differ significantly. Measurements of DNA were made on the other subfractions of homogenates, but significant quantities were detected only in the whole homogenate and in the pellet obtained from the 9000 *g* centrifugation. The stimulating agents also caused decreases in the DNA content of the 9000 *g* pellet.

With the exception of the fraction containing the SER, no significant changes in the RNA content of any subfraction could be detected when the results were expressed as mg RNA/g liver (wet weight) (Table 2). Phenobarbital and chlordane pretreatment produced 30–35 per cent increases, whereas administration of 3,4-benzpyrene resulted in no detectable change in the RNA content of the SER. However, if the results were expressed as mg RNA/mg DNA (DNA values were obtained from determinations of the whole homogenate), significant increases were produced by phenobarbital and chlordane in the whole homogenate and in the smooth pellet (Table 3). Chlordane also caused significant increases in RNA/mg DNA in the 9000 *g* and 104,000 *g* supernatants. 3,4-Benzpyrene produced a significant increase in mg RNA/mg DNA only in the 9000 *g* pellet. When expressed as mg RNA/total liver (Table 4), increases produced by the stimulating agents were comparable to those detected when the results were expressed as mg RNA/mg DNA. Significant differences, however, were fewer, owing to an increase in experimental variability. It should be noted that, regardless of the method of expressing the results, the RNA content of the pellet containing the SER was always increased by pretreatment with phenobarbital and chlordane but not by 3,4-benzpyrene. Phenobarbital and chlordane produced changes in the RNA content which were not significantly different from each other in any subfraction.

Comparison of the effects of stimulating agents on the protein content of subfractions of liver homogenates

Increases in protein/g liver (wet weight) were slight in all homogenate subfractions (Table 5.) However, significant increases were produced by all stimulating agents in the 9000 *g* supernatant fraction and in the 104,000 *g* supernatant fraction. In addition, pretreatment with phenobarbital and chlordane resulted in significant increases in the protein content of the pellet containing the SER. Increases were more pronounced when the results were expressed as mg protein/unit DNA (Table 6) or per total liver (Table 7). With the exception of the fraction containing the RER, phenobarbital and chlordane caused significant increases in protein per unit DNA or per total liver in every subfraction. Pretreatment with 3,4-benzpyrene, on the other hand, resulted in increases in only the 9000 *g* supernatant fraction. When results were calculated as mg protein/mg RNA, no significant differences were produced by chlordane, phenobarbital, or 3,4-benzpyrene.

In addition, the wet weights of the 104,000 *g* pellets were obtained by weighing the empty ultracentrifuge tubes and reweighing after pouring off the supernatant. The results obtained further corroborated results obtained in the assay procedures (Table 8). Phenobarbital and chlordane pretreatment resulted in a significant increase in weights

TABLE 2. DIFFERENCES IN RNA CONTENT PER g WET WEIGHT LIVER

Treatment	Whole homogenate		9000-g Supernatant		9000 g Pellet		104,000 g Supernatant		104,000 g Smooth pellet		104,000 g Rough pellet	
	(mg RNA/ g liver)	(% change)	(mg RNA/ g liver)	(% change)	(mg RNA/ g liver)	(% change)	(mg RNA/ g liver)	(% change)	(mg RNA/ g liver)	(% change)	(mg RNA/ g liver)	(% change)
Control	9·02		3·17		4·82		0·301		0·151		1·59	
3,4-Benzpyrene	9·01	0	2·99	−5	5·20	+8	0·293	−3	0·147	−3	1·57	−2
Phenobarbital	8·64	−4	3·03	−4	4·87	+1	0·274	−9	0·197	+31	1·57	−2
Chlordane	9·09	+1	3·43	+8	4·71	−2	0·316	+5	0·203	+34	1·61	+1
CV	13·2%		17·6%		11·3%		12·5%		18·4%		13·8%	
N	8		6		6		7		7		7	

TABLE 3. DIFFERENCES IN RNA CONTENT PER mg DNA*

Treatment	Whole homogenate		9000 g Supernatant		9000 g Pellet		104,000 g Supernatant		104,000 g Smooth pellet		104,000 g Rough pellet	
	(mg RNA/ mg DNA	(% control)	(mg RNA/ mg DNA)	(% control)	(mg RNA/ mg DNA)	(% control)	(mg RNA/ mg DNA)	(% control)	(mg RNA/ mg DNA)	(% control)	(mg RNA/ mg DNA)	(% control)
Control	2·64		0·92		1·47		0·078		0·041		0·418	
3,4-Benzpyrene	2·99	+13	0·98	+ 7	1·69†	+15	0·082	+ 5	0·042	+ 3	0·440	+ 5
Phenobarbital	3·17	+20	1·01	+10	1·50	+ 2	0·081	+ 4	0·060	+48	0·478	+14
Chlordane	3·17	+20	1·19	+29	1·52	+29	0·095	+21	0·062	+52	0·483	+16
CV	14·4%		20·6%		10·5%		13 6%		21·8%		16·2%	
N	8		6		6		7		7		7	

* DNA values obtained from determinations on whole homogenates.
† Significantly different from control.

TABLE 4. DIFFERENCES IN RNA CONTENT OF VARIOUS HOMOGENATE SUBFRACTIONS PER TOTAL LIVER

Treatment	Whole homogenate		9000 *g* Supernatant		9000 *g* Pellet		104,000 *g* Supernatant		104,000 *g* Smooth pellet		104,000 *g* Rough pellet	
	mg RNA/ liver)	(% control)	(mg RNA/ liver)	(% control)	mg RNA/ liver)	(% control)	(mg RNA/ liver)	(% control)	(mg RNA/ liver)	(% control)	(mg RNA/ liver)	(% control)
Control	39·4		12 7		19·1		1·29		0·655		6·67	
3,4-Benzpyrene	43 6	+ 11	12·5	− 2	22 1	+16	1·37	+ 6	0·742	+13	7·37	+11
Phenobarbital	44·2	+12	14·1	+11	23·1	+21	1·38	+ 7	1·03	+57	7 65	%14
Chlordane	47·6	+20	15·5	+21	22·3	+17	1·55	+20	1·06	+62×	8·11	+ 21
CV	13·8%		20·7%		16·8%		22·3%		23·1%		20·7%	
N	8		6		6		7		7		7	

TABLE 5. DIFFERENCES IN PROTEIN CONTENT PER g WET WEIGHT LIVER

Treatment	Whole homogenate		9000 *g* Supernatant		9000 *g* Pellet		104,000 *g* Supernatant		104,000 *g* Smooth pellet		104,000 *g* Rough pellet	
	(mg protein/ g liver)	(% control)	(mg protein/ g liver)	(% control)	(mg protein/ g liver)	(% control)	(mg protein/ g liver)	(% control)	(mg protein/ g liver)	(% control)	(mg protein/ g liver)	(% control)
Control	203		96		114		48·6		16·6		21·3	
3,4-Benzpyrene	207	+2	106	+10	120	+5	52·1	+ 8	17·2	+ 4	22·6	+6
Phenobarbital	219	+ 8	106	+ 10	125	+9	53·2	+ 19	19·4	+17*	21·7	+2
Chlordane	207	+2	111	+15	120	+5	54·2	+12	19·2	+16	20·6	−3
CV	7·19%		6·42%		8·74%		5·90%		9·57%		8·62%	
N	8		8		8		7		7		7	

* Chlordane and 3,4-benzpyrene values are not significantly different.

TABLE 6. DIFFERENCES IN PROTEIN CONTENT PER mg DNA*

Treatment	Whole homogenate		9000 *g* Supernatant		9000 *g* Pellet		104,000 *g* Supernatant		104,000 *g* Smooth pellet		104,000 *g* Rough pellet	
	(mg protein/ mg DNA)	(% control)	(mg protein/ mg DNA)	(% control)	(mg protein/ mg DNA)	(% control)	(mg protein/ mg DNA)	(% control)	(mg protein/ mg DNA)	(% control)	(mg protein/ mg DNA)	(% control)
Control	54·8		26·0		31·1		12·7		4·26		5·35	
3,4-Benzpyrene	60·3	+10	31·3	+20	35·7	+15	14·9	+17	4·73	+11	6·20	+16
Phenobarbital	68·4	+25*†	33·2	+28	39·3	+27	15·9	+25	5·84	+37	6·45	+20
Chlordane	63·5	+16	34·6	+33	37·6	+21	15·9	+25	5·72	+34	6·10	+14
CV	10·7%		11·3%		13·2%		10·5%		12·6%		12·1%	
N	8		8		8		8		7		7	

* DNA values obtained from determinations on whole homogenates.
† Chlordane and 3,4-benzpyrene values not significantly different.

TABLE 7. DIFFERENCES IN PROTEIN CONTENT OF VARIOUS HOMOGENATE SUBFRACTIONS PER TOTAL LIVER

Treatment	Whole homogenate		9000 *g* Supernatant		9000 *g* Pellet		104,000 *g* Supernatant		104,000 *g* Smooth pellet		104,000 *g* Rough pellet	
	(mg protein/ liver)	(% control)	(mg protein/ liver)	(% control)	(mg protein/ liver)	(% control)	(mg protein/ liver)	(× control)	(mg protein / liver)	(% control)	(mg protein/ liver)	(% control)
Control	888		429		495		212		70		91	
3,4-Benzpyrene	1008	+13	510	+22	584	+18	251	+18	81	+15	107	+17
Phenobarbital	1120	+26	536	+25	632	+28	276	+30%	98	+40	108	+19
Chlordane	1075	+21	572	+33	616	+25	283	+34	101	+44	103	+13
CV	10·1%		11·7%		12·3%		15·5%		21·0%		16·3%	
N	8		8		8		7		7		7	

of the pellets which contained the SER. No significant differences were observed in the weights of the pellets which contained the RER. 3,4-Benzpyrene caused no significant changes in SER or RER pellet weights.

TABLE 8. WET WEIGHTS OF 104,000 *g* ROUGH AND SMOOTH PELLETS FROM FRESH LIVER*

Treatment	Smooth				Rough			
	Wet weight of pellet (mg)	(% of control)	Significance	N	Wet weight of pellet (mg)	(% of control)	Significance	N
Control	313			4	582			5
3,4-Benzpyrene	317	+1·1		4	611	+5		5
Phenobarbital	378	+20·6		4	581	0		5
Chlordane	377	+20·3		4	519	−11		5
CV		9·1%				13·2%		

* 2·67 g.

DISCUSSION

The results indicated that injections of phenobarbital and chlordane, when administered in quantities sufficient to cause maximal increases in the activities of several microsomal, drug-metabolizing enzyme systems, caused significant increases in the total wet weight of livers and significant decreases in the DNA content per unit of liver (wet weight). The changes produced by 3,4-benzpyrene on these parameters were in the same direction but of a lesser magnitude.

In many cases, the changes produced by 3,4-benzpyrene lacked statistical significance. Such changes in DNA content are ordinarily associated with cellular hypertrophy rather than hyperplasia. However, if there were a change in ploidy, e.g. a decrease in relative numbers of triploid cells, there still could be hyperplasia. This aspect should be further investigated.

Based on the assumption that DNA content is a measure of cellularity, it appeared that chlordane and phenobarbital were both capable of stimulating cells to synthesize more protein per cell. This was particularly evident in the fraction associated with the smooth endoplasmic reticulum (SER) but was also evident in both the 9000 *g* and 104,000 *g* supernatant fractions. These results are in accord with the data obtained by Gelboin and Sokoloff,[15] who showed that previous treatment of rats with phenobarbital stimulated the incorporation of amino acids into microsomal proteins of cell-free liver preparations. Gelboin and Sokoloff[16] also showed that the administration *in vivo* of 3-methylcholanthrene or 3,4-benzpyrene resulted in a stimulatory effect on the rate of amino acid incorporation *in vitro* into protein in rat liver homogenates. Our results tend to indicate that, if pretreatment with 3,4-benzpyrene results in an increased biosynthesis of cellular protein, as the data of Gelboin and Sokoloff suggest, increased synthesis proceeds more rapidly than protein catabolism. The increased synthesis is thereby reflected in significant increases in the protein content of the 9000 *g* and 104,000 *g* supernatant fractions.

Associated with the increases in protein content per cell produced by pretreatment with phenobarbital and chlordane were corresponding increases in the RNA content of various subfractions of liver homogenates. Several laboratories have reported increases in microsomal RNA content associated with phenobarbital pretreatment.[10, 17–19] Remmer and Merker reported that the increase of RNA in the SER after phenobarbital pretreatment was not significant. Our data indicated significant increases in this fraction irrespective of the method of calculation. The percentage increases, however, were similar to those obtained by Remmer and Merker. The results obtained in this work do not appear to agree with those obtained by Von der Decken and Hultin[17] or Orrenius and Ernster.[18] Von der Decken and Hultin reported increases in the RNA/protein ratio of total microsomes after 3-methylcholanthrene pretreatment. Orrenius and Ernster reported an increase in the RNA/protein ratio in the SER after phenobarbital pretreatment. Our results indicate that the RNA/protein ratio is decreased in both SER and RER after treatment with phenobarbital or benzpyrene. The observed decreases were not significant. The data obtained by Remmer appear to be in agreement with ours, since he obtained larger increases in the protein content than in the RNA content of SER after phenobarbital pretreatment.

Increased RNA content after benzpyrene pretreatment was significant only in the 9000 *g* pellet. This result is in agreement with the work of Gelboin,[20] who suggested an increase in available messenger RNA associated with the 3-methylcholanthrene-stimulated increases of microsomal amino acid incorporation. (3-Methylcholanthrene and 3,4-benzpyrene behave similarly with regard to stimulation of enzyme systems, and thus it might be expected that they would produce similar effects on RNA and protein.)

One of the most striking features of the study was that phenobarbital and chlordane did not differ from each other in their effects on RNA, DNA, or protein content of homogenate subfractions, regardless of the method of expressing these values. In addition, no statistically significant differences were shown in weight gain of the animals, liver weights, mg RNA/mg protein, liver weight/animal weight, or wet weights of the rough or smooth pellets from animals which had been treated with chlordane vs. phenobarbital. Pretreatment with benzpyrene, however, resulted in changes of several of the aforementioned parameters, which were significantly different from those produced by phenobarbital or chlordane. Such observations correspond to observed differences of these agents with regard to stimulation of microsomal enzyme systems; i.e. phenobarbital and chlordane appear to enhance hepatic microsomal drug metabolisms by similar mechanisms which differ from the mechanism by which benzpyrene stimulates drug metabolisms.

Some question may arise with regard to the presence of RNA in the smooth microsomal fraction. This may seem to be a paradox since, by definition, the smooth microsomes are devoid of ribonucleoprotein particles. As previously mentioned, several laboratories have reported RNA associated with SER. It is possible that some of this RNA is due to contamination of the SER fraction with RER. However, the RNA that is involved in the synthesis of proteins of the SER may be closely associated with the smooth membranes *per se*; i.e. certain proteins in the SER may be synthesized by RNA which is associated with the SER. Current evidence suggests that the ribosomal particles synthesize plasma proteins for export, but much less is known concerning the biosynthesis of proteins found in the SER—i.e. are they

transported there or synthesized at this site? This question should be further investigated.

An alternative explanation for the observed increases in the RNA content of the SER after treatment with phenobarbital or chlordane should be considered. It is possible that pretreatment with phenobarbital or chlordane may have resulted in an increased contamination of the SER with RER relative to control livers. However, it should be noted that if the SER of livers from phenobarbital- or chlordane-treated rats contained a greater contamination of RER than is true of SER from livers of control rats, the RNA/protein ratios would have been expected to increase, since RER contains much more RNA than does SER. The analysis of variance of our data revealed that pretreatment of rats with any of the stimulating agents employed did not significantly alter the RNA/protein ratios. In the cases of phenobarbital and benzpyrene, slight decreases were noted. The fact that our results accord with those obtained by Remmer and Merker, yet apparently conflict with those of Orrenius and Ernster (as previously discussed) may reflect a difference in the method of separation of rough and smooth microsomes. Orrenius and Ernster employed a method described by Dallner *et al.*,[21] whereas Remmer and Merker employed the method of Fouts.[11] This discrepancy has been further investigated and discussed.[22] It should finally be noted that in our experiments the RNA/protein ratios obtained in the SER were very low (approximately 0·01) which indicates a relatively clean separation of the SER when compared to results obtained in other laboratories.

Taken together, these results provide further support for currently existing theories pertaining to the mechanism of induction of hepatic microsomal drug-metabolizing enzyme systems by drugs and chemicals. Such theories suggest that the induction is mediated via DNA-directed RNA synthesis, with subsequent increases in cellular RNA and in active enzyme protein population. Such increases might be expected to be observed in the SER, where drug-metabolizing enzymes are presumably predominantly located. Such changes might also be expected to be more pronounced in pretreatment with phenobarbital or chlordane than with benzpyrene, since the former agents stimulate a larger number of enzyme systems.

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REFERENCES

1. H. REMMER, in *Factors Affecting Drug Toxicity; Proceedings of the Cambridge Conference*, vol. 4, p. 57. Proceedings of the European Society for the Study of Drug Toxicity, Excerpta Medica Foundation, Amstrdam (1964).
2. K. J. NETTER, *Metabolic Factors Controlling the Duration of Drug Action* (Eds. B. B. BRODIE and E. G. ERDÖS), vol. 6, p. 213. Proceedings of the First International Pharmacological Meeting, Stockholm. Pergamon Press, New York (1962).
3. H. REMMER, in *Metabolic Factors Controlling the Duration of Drug Action* (Eds. B. B. BRODIE and E. G. ERDÖS), vol. 6, p. 235. Proceedings of the First International Pharmacological Meeting, Stockholm. Pergamon Press, New York, (1962).
4. A. H. CONNEY and J. J. BURNS, in *Advances in Pharmacology* (Eds. S. GARATTINI and P. A. SHORE), vol. 1, p. 31. Academic Press, New York (1962).
5. J. R. GILLETTE, *Prog. Drug Res.* **6**, 11 (1963).
6. A. H. CONNEY, C. DAVISON, R. GASTEL and J. J. BURNS, *J. Pharmac. exp. Ther.* **130**, 1 (1960).
7. L. G. HART and J. R. FOUTS, *Biochem. Pharmac.* **14**, 263 (1965).

8. H. REMMER and H. J. MERKER, *Klin. Wschr.* **41**, 276 (1963).
9. J. R. FOUTS and L. A. ROGERS, *J. Pharmac. exp. Ther.* **147**, 112 (1965).
10. H. REMMER and H. J. MERKER, *Science, N.Y.* **142**, 1657 (1963).
11. J. R. FOUTS, *Biochem. biophys. Res. Commun.* **6**, 373 (1961).
12. R. F. ITZHAKI and D. M. GILL, *Analyt. Biochem.* **9**, 410 (1964).
13. J. F. SCOTT, A. P. FRACCASTORO and E. B. TAFT, *J. Histochem. Cytochem.* **4**, 1 (1966).
14. R. G. D. STEEL and J. H. TORRIE, *Principles and Procedures of Statistics*, McGraw-Hill, New York (1960).
15. H. V. GELBOIN and L. SOKOLOFF, *Science, N.Y.* **134**, 611 (1961).
16. H. V. GELBOIN and L. SOKOLOFF, *Biochem. biophys. Acta* **91**, 122 (1964).
17. A. VON DER DECKEN and T. HULTIN, *Archs Biochem.* **90**, 201 (1961).
18. S. ORRENIUS and L. ERNSTER, *Biochem. biophys. Res. Commun.* **16**, 60 (1964).
19. H. REMMER and H. J. MERKER, *Ann. N.Y. Acad. Sci.* **123** ,79 (1965).
20. H. V. GELBOIN, *Biochim. biophys. Acta* **91**, 130 (1964).
21. G. DALLNER, S. ORRENIUS and I. BERGSTRAND, *J. cell. Biol.* **16**, 426 (1963).
22. J. R. FOUTS, T. E. GRAM and L. A. ROGERS, *Exp. molec. Path.* in press (1966).