Table 3. Enzymic phosphorolysis of 3-deazaguanosine and 7-ribosyl-3-deazaguanine by cell-free extracts of Ehrlich ascites tumor cells

Compound	Reaction conditions	λmax*	Product identity	Analysis
3-Deazaguanosine	60°, 4 min +37°, 18 hr	270,298	3-Deazaguanosine	No reaction
3-Deazaguanosine	37°, 18 hr	258,300	3-Deazaguanine	Complete phosphorolysis
7-Ribosyl-3-deazaguanine	60°, 4 min + 37°, 18 hr	317,258	7-Ribosyl-3-deazaguanine	No reaction
7-Ribosyl-3-deazaguanine	37°, 18 hr	317,258	7-Ribosyl-3-deazaguanine	No reaction

* Refer to [1] for u.v. spectral properties of the nucleosides and the free base. The reaction conditions are described in the text.

drolytic) cleavage to release the free base followed by subsequent phosphoribosylation to form the 5'-nucleotide. When 3-deazaguanosine was incubated with a cell-free extract of Ehrlich ascites (Table 3), u.v. spectral analysis revealed that, indeed, the nucleoside was cleaved to the free base, and further that the activity is specific for the 9-ribosyl linkage to the base. Thin-layer chromatographic analysis of the reaction mixtures was consistent with the results reported in Table 3.

In summary, these studies show that 3-deazaguanine can interfere with the biosynthesis of purine nucleotides in Ehrlich ascites tumor cells, most probably through the inhibition of IMP dehydrogenase by the 5'-nucleotide. At the concentration used in these studies (1 mM), 3-deazaguanine is also about 50 per cent cytotoxic to KB tumor cell cultures. This, then, would offer one means of interfering with tumor cell growth through consequent inhibition of nucleic acid synthesis. However, direct interference with nucleic acid synthesis either by incorporation or interference with polymerase activities is also possible if metabolism to the triphosphate does occur. Further studies on the actual metabolism of 3-deazaguanine in tumor cells is obviously required to determine the primary mode of action of this compound.

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Allylisopropylacetamide preferentially interacts with the phenobarbital-inducible form of rat hepatic microsomal P-450

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Cytochrome P-450 is the terminal oxidase of a number of mammalian microsomal electron transport systems which metabolize a variety of compounds, including steroid hormones, fatty acids, and a variety of xenobiotics [1]. Several studies provide nearly overwhelming evidence that rat liver microsomes contain several distinct forms of this cytochrome [2–5], thus offering an explanation for the observable broad specificity of this enzyme system. How-

ever, it has not been demonstrated that distinct cytochrome P-450 forms confer substrate specificity within intact organisms.

Treatment of rats with 2-allyl-2-isopropylacetamide (AIA) produces a rapid loss of hepatic microsomal P-450 content with a concomitant appearance of ill-defined green pigments [6–10]. The destruction of P-450 *in situ* by AIA presumably results from activation of AIA to a highly reactive, destructive epoxide [10], inasmuch as the destruction of P-450 by AIA, secobarbital and other barbiturates is dependent upon the presence of an allyl function within the molecule [9, 11]. Sweeney and Rothwell [12] have presented spectroscopic evidence for an interaction between AIA and cytochrome P-450, suggesting that the initial step in the degradation must involve binding of AIA to the cytochrome.

In this report, we give spectral and electrophoretic evidence that AIA interacts preferentially with one of the multiple forms of hepatic microsomal cytochrome P-450 *in vivo*.

Male, albino CFN rats, 90 days of age, and weighing approximately 300 g, were divided into four groups. Animals in Group A received daily intraperitoneal injections of 0.9% saline for 3 days. Rodents in Group B received daily intraperitoneal injections of PB (75 mg/kg body wt, as 25 mg/ml in saline) for 3 days. Animals in Group C were injected daily, intraperitoneally with 3-methylcholanthrene (3-MC, 25 mg/kg body wt, as 10 mg/ml in corn oil) for 2 successive days. Finally, animals in Group D were injected once with the polychlorinated biphenyl (PCB) Aroclor 1254 (400 mg/kg body wt, as 400 mg/ml in corn oil).

After treatment with saline or with inducers of the microsomal enzyme system (Group A, 3 days; Group B, 3 days; Group C, 2 days; and Group D, 5 days), the animals in each group were injected subcutaneously either with saline or AIA (400 mg/kg body wt, as 20 mg/ml in saline). Five hr later the animals were killed and liver microsomes were prepared as described by Welton and Aust [4]. Cytochrome P-450 content was determined according to the method of Omura and Sato [13], while protein content was determined according to the method of Lowry *et al.* [14], using bovine serum albumin as standard. Microsomal proteins were electrophoresed in 0.1% SDS-acrylamide gels according to the method of Welton and Aust [4]. The gels were stained with Coomassie blue [15] and scanned at 550 nm with a Gilford 2400S spectrophotometer. Microsomal hemoproteins were localized on the gels by staining for the peroxidase activity of the heme moiety [4].

Microsomal cytochrome P-450 content of control animals was 1.38 nmoles/mg of microsomal protein (Table 1). Pretreatment of animals with PB, 3-MC or Aroclor 1254 produced marked increases in hemoprotein content. In addition, there was a blue shift in the Soret maximum of the CO-difference spectrum of P-450 after treatment with either 3-MC or Aroclor, as has been well-documented by others [16–18].

Treatment with AIA produced a decline in P-450 content in all groups (Table 1), with the greatest change in animals which had been pretreated with either PB or PCB. Unexpectedly, the Soret maximum of the reduced CO-difference spectrum for the hemoprotein complex shifted from 450 to 453 nm in PB-treated animals after treatment with AIA, while a similar shift from 446 to 449 nm occurred in PCB-treated animals after treatment with AIA.

The shift in Soret maximum is not species specific, since similar results were obtained with male Holtzman rats of corresponding age and body weight (unpublished results). Also, the appearance of the shift depends upon treatment with a specific inducer, since treatment with AIA after pretreatment with 3-MC produces a decline in total hemoprotein content but no shift in the absorption maximum from 448 nm.

It may be argued that the observed spectral shift represents interaction of metabolites of PB or Aroclor with the reduced CO–AIA hemoprotein complex, as has been shown to occur following interaction of the CO–hemoprotein complex with metabolites of a variety of compounds [19–22]. However, the shift does not occur when animals are injected with PB and AIA simultaneously and sacrificed 5 hr later (unpublished observations), although it would be expected that metabolites of PB would be present in animals so treated. Furthermore, the shift in the absorption maximum of the reduced CO adduct occurs in animals which have been pretreated with Aroclor.

We hypothesized that the shift observed in AIA-treated rats after pretreatment with PB or Aroclor results from preferential interaction of AIA with one of the several forms of microsomal P-450. The CO-difference spectrum observed for microsomes which exhibit an absorption maximum at 450 nm is, in fact, the sum of the contribution of multiple forms of the hemoprotein. Treatment with 3-MC apparently alters the ratios of the multiple hemoproteins, resulting in a shift from 450 to 448 nm in the reduced CO adduct. Destruction of a specific form of P-450 may permit similar alterations in the absorption maximum of the CO-difference spectrum. It has been demonstrated that one of the multiple forms of P-450 which is induced by PB [4] is also induced by polychlorinated biphenyls [2]. The present results demonstrate that the shift in absorption maximum only occurs after treatments which are known to increase the level of this form of P-450. If this hypothesis is correct, preferential destruction of one form of the hemoprotein by AIA should be accompanied by a loss of the PB-inducible form of P-450 in microsomal material from AIA-treated rats. Accordingly, microsomal protein from such animals was subjected to SDS-polyacrylamide gel electrophoresis and the results are shown in Fig. 1. It is apparent from a comparison of panels A and B that the 50,000 dalton component interacts preferentially with AIA

Table 1. Effect of 2-allyl-2-isopropylacetamide on hepatic microsomal P-450 content in CFN male rats after pretreatment with various compounds

Group	Treatment	Soret maximum (λ, nm)	P-450 content (nmoles/mg protein)	% P-450 AIA sensitive
A	Saline	450	1.38	
	Saline + AIA	450	0.65	53
B	PB	450	2.57	
	PB + AIA	453	0.46	82
C	3-MC	448	2.33	
	3-MC + AIA	448	1.08	57
D	PCB	446	3.42	
	PCB + AIA	449	1.19	65

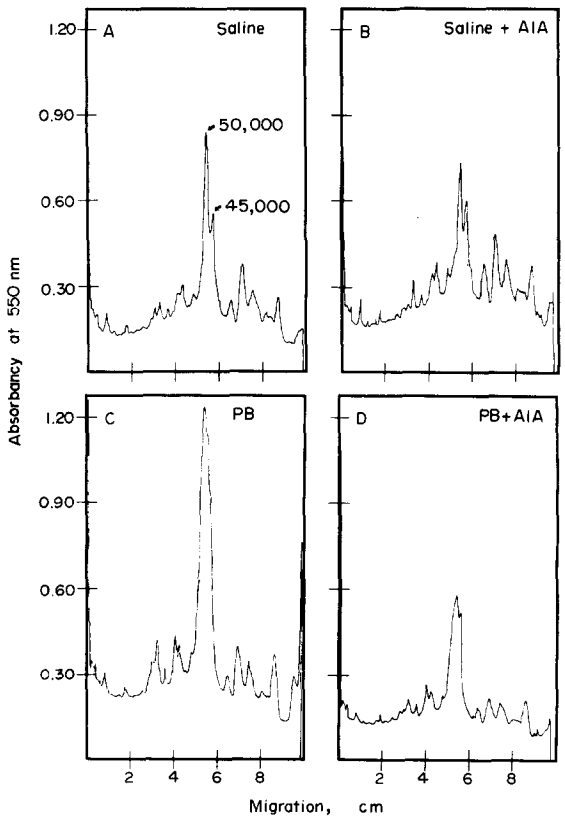

Fig. 1. SDS-polyacrylamide gel electrophoresis of rat hepatic microsomal proteins. Ten μl protein was applied to each 5 × 100 mm gel (μg protein/gel: A, 4.86; B, 5.06; C, 5.23; and D, 3.27). Four milliamperes was applied to each gel, and electrophoresis was carried out in the dark, at 5°, for 3 hr. Standards for molecular weight calibration of the gels included phosphorylase A, bovine serum albumin, pyruvate kinase, aldolase, lactate dehydrogenase, lysozyme and cytochrome *c*.

and is subsequently destroyed by metabolism of the allyl-containing compound. Comparison of the figures in panels C and D supports this finding by showing that the relative amount of 45,000 dalton component in animals treated with AIA and PB is nearly identical to that in saline-treated animals, while the AIA interacts preferentially with the 50,000 dalton component.

Additional support for our hypothesis is obtained from a consideration of the microsomal hemoprotein content after treatment with AIA (Table 1). The microsomal P-450 content in PB-treated animals, after treatment with AIA, is very nearly the same as that in control animals after similar treatment. In contrast, P-450 content in 3-MC-treated animals after treatment with AIA is much higher, and similar to that found in animals pretreated with Aroclor. These findings indicate that the form of hemoprotein induced by 3-MC, and also by Aroclor, is not greatly affected by treatment with AIA. However, the PB-inducible component, which is present in 3-MC-treated animals (prior to induction) and induced in Aroclor-treated animals, is preferentially destroyed by treatment with AIA.

These results indicate that AIA preferentially binds to one of several forms of hepatic microsomal P-450 *in vivo*. During the preparation of this manuscript, it was shown that metapyrone and SKF 525-A interact selectively with different fractions of microsomal P-450 *in vitro* [23]. The present results demonstrate that the different forms of rat hepatic cytochrome P-450 possess distinct substrate specificity within intact organisms.

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