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PRELIMINARY COMMUNICATION

REDUCTION IN BINDING OF [^{14}C] AFLATOXIN B_1 TO RAT LIVER MACROMOLECULES BY PHENOBARBITONE PRETREATMENT

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Various dietary regimens have been shown to alter the acute and chronic toxicity of aflatoxin B_1 (AFB_1) in rats[1-4]. Phenobarbitone (PB), an inducer of liver mixed function oxidases[5] reduces the liver carcinogenicity of AFB_1 contaminated peanut meal[6], decreases the LD_{50} after an intraperitoneal injection of AFB_1 from 1 to 5 mg/kg (Garner, Miller and Miller, unpublished) and reduces the inhibitory action of AFB_1 on rat liver ribonucleic acid synthesis[7]. The protective effect of PB contrasts with <u>in vitro</u> findings that AFB_1 metabolism is increased by this enzyme-inducing agent, both to detoxification products such as aflatoxin B_{2a}[8] and aflatoxin M_1[9] as well as to AFB_1 2,3-oxide, the probable ultimate carcinogenic form of this compound[10]. To investigate this apparent discrepancy further the macromolecular binding of [^{14}C] AFB_1 after a single intraperitoneal injection has been studied in control and PB pretreated animals.

MATERIALS AND METHODS

Preparation of [^{14}C] AFB_1 was as previously described[11]. 250–300g male Wistar rats (A. Tuck & Son, Rayleigh, Essex) maintained on Diet 41B (Oxoid Ltd., London) were injected intraperitoneally with 40 μg/100g [^{14}C] AFB_1 dissolved in dimethylsulphoxide (1mCi/millimole, 40 μg/0.1 ml). PB pretreated animals received 1 mg/ml sodium phenobarbitone in the drinking water for 7 days prior to carcinogen administration[12]. Animals were killed 6 hours after being given the labelled compound and liver and kidney radioactivity determined[13].

RESULTS AND DISCUSSION

The percentage of [^{14}C] AFB_1 remaining in the liver and kidneys 6 hours after carcinogen injection was the same for control and PB treated rats.

Table 1

Percentage of [^{14}C]AFB$_1$ remaining 6 hours after administration to control or phenobarbitone pretreated rats

	Percentage of dose remaining	
	Liver	Kidney
Control	9.7 ± 1.4	0.6 ± 0.1
Phenobarbitone	12.2 ± 1.5	0.5 ± 0.1

Each result is the mean ± S.D. for 3 rats

This time point was chosen because previous results had shown that macromolecular binding in rat liver had reached its maximum by this time. In agreement with earlier findings[13] and those of other workers[14] binding of carcinogen to liver nucleic acids was higher than that to protein in control animals.

PB treatment decreased carcinogen binding to nucleic acids so that on a per milligram basis binding to all liver macromolecules was similar. Differences were also seen in kidney macromolecular binding for the two groups of animals, there again being less nucleic acid-bound carcinogen in enzyme-induced rats. Interestingly protein binding in the liver and kidney was unaffected by prior PB administration.

These results confirm the greater reactivity of nucleic acid, compared with protein towards a metabolite of AFB$_1$ in control rats[13, 14]. The decreased binding of AFB$_1$ to nucleic acids in PB-induced animals suggests that induction reduces the amount of reactive carcinogen formed during metabolism by liver mixed function oxidases. This reduction in nucleic acid binding may account for the protective effect of PB on AFB$_1$

Table 2

Binding of [^{14}C] AFB$_1$ to control and phenobarbitone pretreated liver and kidney macromolecules

	ng[^{14}C]AFB$_1$ bound / milligram			
	Control		Phenobarbitone	
	Liver	Kidney	Liver	Kidney
DNA	40.7 ± 13.7	14.0	11.0 ± 2.8	9.0
rRNA	44.8 ± 5.1	27.0	9.0 ± 1.9	16.0
Protein	6.3 ± 2.6	1.0	7.7 ± 2.5	1.0

Liver results are the mean ± S.D. for 3 rats: kidney data are for pooled organs from 3 rats.

toxicity and carcinogenicity. Furthermore, since protein binding is the same for normal and induced animals this could indicate the relative unimportance of such binding for the biological effects of AFB_1.

The data reported in this paper and that published previously on the binding of AFB_1 to hamster liver macromolecules[13] highlight an apparent discrepancy between *in vivo* and *in vitro* AFB_1 activation. The *in vitro* results using microsomes from either PB pretreated animals[10] or hamsters[15] show an efficient conversion of AFB_1 to its 2,3-oxide compared with control rats and yet *in vivo* one finds lower levels of liver carcinogen binding than in untreated animals. This difference between the *in vitro* and *in vivo* findings could be due to a fundamental change in the pharmokinetics of metabolism of AFB_1, more metabolism proceeding via detoxification pathways in the animal than in the extremely artificial microsomal system with its excess of both substrate and cofactors. Such differences may have an important bearing on attempts to develop *in vitro* assays using liver microsomes in conjunction with bacteria to detect potential carcinogenic substances[16].

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