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CYTOCHROME P-450 INDUCTION IN GENETICALLY OBESE RATS

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SUMMARY Using two dimension iso-electric focusing sodium dodecyl sulphate polyacrylamide gel electrophoresis, we have shown that the lack of response by genetically obese Zucker rats to phenobarbital treatment is due to a decreased induction of cytochrome's P-450 especially cytochrome P-450-PB_3. We do not know whether this represents a defect in transcription or translation.

INTRODUCTION Previous studies have shown that genetically obese rats do not respond to phenobarbital (PB) treatment to the same extent as their lean littermates (1). In addition, untreated obese animals have lower basal cytochrome P-450 contents and drug metabolizing activities (1,2) than their lean siblings, and these differences increase as a result of PB treatment (1). At the present time it is not clear whether the reduced response is due to decreased liver protein synthesis, or whether there is a different pattern of the cytochrome P-450 multiple forms present in untreated obese rat livers. It is now clear that in other rat strains there are at least eight different forms of liver microsomal cytochrome P-450, each of which has different substrate preferences, and responds differently to a wide range of inducers (3-7). In an attempt to study this question, we treated lean Zucker rats and their obese littermates with PB, then measured in vitro liver protein synthesis, and also examined the microsomal cytochrome P-450's using two-dimension isoelectric-focusing sodium dodecyl sulphate polyacrylamide gel electrophoresis (ISF-SDS-PAGE).

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MATERIALS AND METHODS

Animals and Drug Treatment. Male Zucker rats obtained from a closed colony maintained at NEOUCOM, were housed in individual cages with free access to food and water, and maintained on a regular 12 hr light/dark cycle. Rats were either obese (fa/fa) or their lean littermates (Fa/- or Fa/Fa). Animals were put on 0.1% PB drinking water (w/v) for 4 days and water intake was measured daily; fluid intake of the two groups of rats was equivalent.

Food was removed for 24 hr prior to killing the animals by cervical dislocation. The livers were rapidly removed and weighed. One piece (5-7 g) was used to prepare microsomes, another portion (3-4 g) was frozen at -70° and used for the subsequent preparation of polysomes.

Preparation of Polysomes and In Vitro Protein Synthesis. Polysomes were prepared by Mg^{2+} precipitation (8), and translated using lysate prepared from wheat germ (9) purchased from a local health food store. *In vitro* protein synthesis was measured using the incorporation of [^{3}H]leucine into protein as described (10) and was linear over a range of 0 - 0.6 A_{260} polysomes/50 µl reaction volume.

Preparation and Analysis of Microsomes. The isolation of microsomes has been described (11). Samples were stored at -70° until use, and protein concentrations measured by the procedure of Lowry *et al* (12) with bovine serum albumin as the standard. Microsomal cytochrome P-450 was measured using the difference spectra of the reduced CO complex as described by Omura and Sato (13); the benzphetamine N-demethylase and 7-ethoxycoumarin-O-deethylase assays have been described (14,15).

Two Dimensional Electrophoresis of Microsomal Proteins. The procedure followed the method of O'Farrell (16) as modified by Vlasuk and Walz (17). A pH gradient was formed in polyacrylamide gels containing 1.5% pH 3-10 and 0.5% pH 6-8 ampholytes. Approximately 225 µg of protein was focused over a 13 hr period (17). The focused gels were either transferred to the top of 7.5% polyacrylamide slab gels containing 0.1% SDS and fixed in place with 1% agarose or stored at -70° until use (17).

After electrophoresis, proteins were detected by use of Coomassie blue staining (17). Conditions were established such that complete reproducibility of the protein staining patterns was obtainable when the same samples were rerun, or when different lean or obese microsomal protein samples were analyzed.

RESULTS AND DISCUSSION From Table 1, it can be seen that the obese animals are heavier than their lean littermates, and only the latter show a significant increase in liver weight with PB treatment. It can also be seen that the yield of polysomes from untreated lean and obese rats was approximately the same. In addition, there were no differences for polysome driven *in vitro* protein synthesis. Treatment with PB for 4 days increased both

Table 1
Effect of PB Treatment on Age Matched
Lean and Obese Zucker Rats

	Lean (Fa/- or Fa/Fa)		Obese (fa/fa)	
	H_2O	PB	H_2O	PB
Body weight (bw)[1]	368±67	376±27	537±98	629±148
Liver weight (% bw)	2.87±0.26	4.02±0.13[6]	3.63±0.49	3.78±0.30
Microsomal protein/gm	18.40±1.15	22.21±4.32	13.25±1.97	14.67±0.82
Polysomes (OD_{260}/gm)[2]	78.8	80.8	86.4	76.7
In vitro protein synthesis[2,3]	19.9	25.2	21.0	31.8
Cytochrome P-450[4]	0.73±0.12	1.33±0.29[6]	0.77±0.15	0.70±0.14
Cytochrome b_5[4]	0.23±0.05	0.58±0.09[6]	0.31±0.02	0.43±0.21
Benzphetamine N-demethylase[5]	6.63±1.81	20.17±5.50[6]	7.13±2.78	9.90±2.42
7-ethoxycoumarin O-deethylase[5]	1.14±0.27	2.14±0.40[6]	0.61±0.09	0.68±0.17

[1]Each group contained three animals, and all values are means ± S.E. of three estimations, except for the enzyme activity assays, where duplicate assays were carried out on each individual microsome preparation.
[2]Derived from three pooled livers.
[3]Represents stimulation of protein synthesis above background. Incorporation of [^{3}H] leucine into protein in the absence of added polysomes was 2750 cpm.
[4]nmol/mg microsomal protein.
[5]nmol product/min/mg microsomal protein.
[6]$p<0.02$ for PB compared to H_2O.

polysome yield and polysome driven in vitro protein synthesis, but again, there were no differences between lean and obese rats. Even after 10 days of PB treatment, there were no differences between lean and obese rats (data not shown).

PB treatment of lean rats significantly increases cytochrome P-450 and cytochrome b_5 contents, and both demethylase and deethylase activities; in contrast, similar treatment of obese rats resulted in a slight but not significant increase in cytochrome b_5 content, and of demethylase activity only. Overall, the results suggest that the impaired response of obese rats to PB is not due to decreased hepatic protein synthesis, even though this was measured indirectly.

Our results are difficult to compare with those of Litterst who never reported the effect of PB on lean Zucker rats, only on lean Sprague-Dawley rats (SD) (1). We find a slightly higher

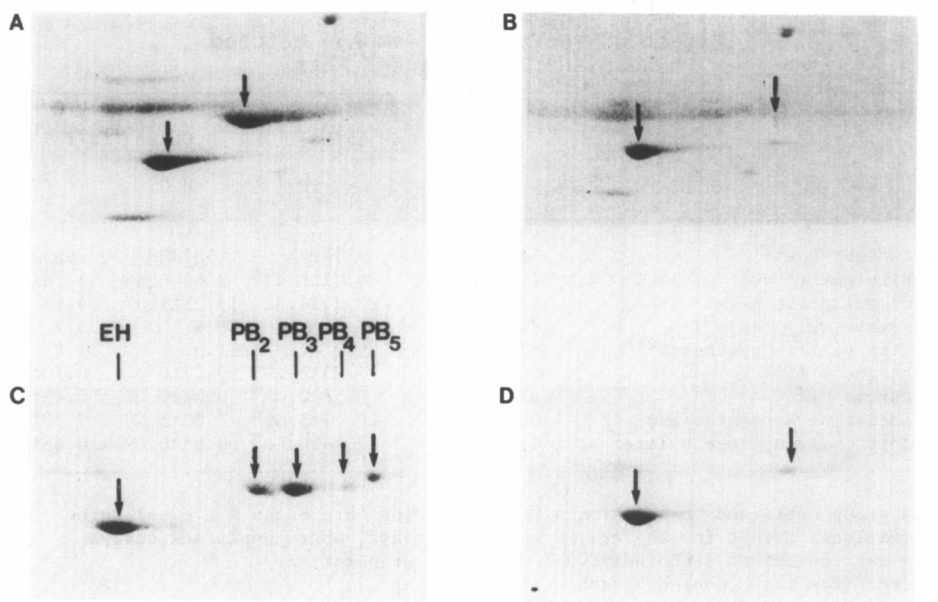

Figure 1. The effect of PB treatment on rat liver microsomal cytochrome P-450's.

A. Part of a two dimensional ISF-SDS-PAGE separation of microsomal proteins from an untreated lean rat.

B, C, and D represent the same regions from gels from individual untreated obese, PB treated lean and PB treated obese Zucker rat microsomes respectively. The nomenclature is taken from Vlasuk et. al. (17), e.g. EH = epoxide hydrolase, PB_2 the phenobarbital induced cytochrome $P\text{-}450_2$.

endogenous cytochrome P-450 content for obese rats, and we get no increase with PB treatment. We do get a slight increase in demethylase activity, as did Litterst although he measured demethylase activity with a different substrate (1).

In an effort to determine whether the lean rats had multiple forms of cytochrome P-450's similar to those described for other rat strains (7), and whether the lack of response by obese rats was due to a lack of, or a reduced amount of, one or more of these multiple forms, we chose to examine the microsomal cytochrome P-450's using ISF-SDS-PAGE. The two-dimensional electrophoretic profile of the microsomal cytochrome P-450's present in control and PB treated lean rats is shown in Fig. 1A and 1C. The nomenclature for describing the cytochrome-P450's follows that of

Vlasuk et. al. (7). Only the region of the gel which contains epoxide hydrolase (EH) and the cytochrome P-450's is shown (7,17). The microsomes from control lean rats (Fig. 1A) contain epoxide hydrolase, cytochrome P-450-PB_2 and possibly cytochrome P-450-PB_1 (shown as a streak above EH), after PB treatment (Fig. 1C), cytochromes P-450-PB_3, PB_4 and PB_5 are induced, and PB_1 and PB_2 are decreased. This is similar to studies on SD and other rat strains (7,17). In Fig. 1B, no cytochrome P-450-PB_2 is seen in untreated obese rat microsomes, only a diffuse Coomassie blue staining streak (possibly PB_1), similar to that seen in Fig. 1A. PB treatment of obese rats leads to the appearance of some cytochrome P450-PB_3 (Fig. 1D). The amount of induction of cytochrome P-450-PB_3 in obese rats varied somewhat between the different individual microsomal samples examined; however, the amount was always considerably less than that of similarly treated lean rats. On one occasion, a small induction of cytochrome P-450-PB_5 was observed.

Overall, our results indicate that the lean Zucker rats respond normally to PB treatment and decreased response of obese Zucker rats to this treatment is due to an inability to induce specific forms of cytochrome P-450. Even allowing for animal-animal variation, it is clear that obese rats have different quantitative levels of the separate cytochrome P-450's. It will be interesting to determine the molecular basis for this problem, i.e., whether it is a transcriptional or selective translational defect, especially as obese rats seem to respond to 3-methylcholanthrene treatment (1). Based on our data, the problem cannot to be due to a gene deletion, but may be due to an altered RNA processing system.

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