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Influence of Age and Diet on the Induction of Hexobarbital-Metabolizing Enzymes in the Mouse (32960)

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The rate of hexobarbital metabolism in the liver increases steadily from birth to adulthood (1). The microsomal enzymes responsible for such metabolism can be activated in the newborn or their activity can be increased in the adult by the administration of enzyme inducers such as phenobarbital or chlorcyclizine (2). The enzyme-inducing activity of such agents has been correlated with the synthesis of new enzyme protein in the liver (3). Factors which tend to decrease protein synthesis, such as starvation or the short-term feeding of a diet low in proteins, are known to decrease the rate of drug metabolism in the adult male rat (4). The weanling mouse maintained on a similar low protein diet, however, continues to synthesize drug-metabolizing enzymes until it reaches early maturity (5). The stimulus for protein synthesis present in such animals apparently overrides the influence of the low protein diet. Agents like phenobarbital which also stimulate protein synthesis have not been widely studied in such weanling animals. In the present study we have therefore examined the ability of such agents to induce the development of hexobarbital-metabolizing enzymes in adult and weanling mice maintained on various dietary intakes.

Materials and Methods. Adult and 21-day-old weanling CD-1 male mice obtained from the Charles River Mouse Farms, Inc. were used for this study. The induction of hexobarbital-metabolizing enzymes was promoted by the daily intraperitoneal administration of equimolar doses (0.01 ml/gm) of one of the following agents for 3 consecutive days: phenobarbital (25.4 mg/kg), chlorcyclizine (37.3 mg/kg), hexobarbital (25.8 mg/kg), antipyrine (18.8 mg/kg), metronidazole (17.2 mg/kg) or 3,4-benzpyrene (100 μg/kg). All inducers were given in H_2O except 3, 4-benzpyrene which was given in corn oil. Control groups were similarly injected with H_2O or corn oil (0.01 ml/gm). A hypnotic dose of hexobarbital (100 mg/kg) was injected 24 hours after the last injection of the inducer and the sleeping time was estimated by determination of the duration of the loss of the righting reflex.

TABLE I. The Effect of Microsomal Enzyme Inducers on Hexobarbital Sleeping Time (ST) in Mice.[a]

	Adult				Weanling			
	Fed		Starved		27% Casein		8% Casein	
Pretreatment	ST[b] (min)	(%)[c]	ST[b]	(%)[c]	ST[b]	(%)[c]	ST[b]	(%)[c]
Water control	49.6 ± 6.1	—	87.0 ± 4.0	—	142.5 ±14.3	—	160.5 ±12.7	—
Phenobarbital	15.1 ± 1.3	69[d]	21.5 ± 1.1	76[d]	9.5 ± 1.3	93[d]	12.9 ± 1.5	91[d]
Chlorcyclizine	7.1 ± 0.5	85[d]	8.3 ± 1.2	91[d]	9.4 ± 0.8	93[d]	6.5 ± 0.9	95[d]
Hexobarbital	35.7 ± 3.4	28[e]	41.3 ± 2.9	52[d]	41.9 ± 3.7	71[d]	77.2 ± 7.4	52[d]
Antipyrine	32.2 ± 2.3	35[e]	44.8 ± 4.5	48[d]	68.0 ± 4.0	52[d]	87.2 ± 6.9	45[d]
Metronidazole	47.2 ± 3.4	2[f]	62.1 ± 6.6	28[e]	65.9 ± 6.4	53[d]	78.2 ±16.0	51[d]
Corn oil control	37.2 ± 3.8	—	140.5 ± 9.2	—	95.8 ±22.0	—	126.9 ± 8.8	—
3,4-Benzpyrene	43.8 ± 4.3	—	144.8 ±14.9	—	23.8 ± 4.8	75[e]	84.0 ±12.5	34[e]

[a] Food withheld for 48 hours from adult starved group, weanling mice fed the experimental protein diets for 1 week.

[b] ST = Hexobarbital sleeping time in minutes ± SE of the mean.

[c] The percentage decrease in sleeping time between control hexobarbital sleeping time and the sleeping time observed after 3-day pretreatment with microsomal enzyme inducers. Ten or more mice were used per group.

[d] $p < 0.01$ as compared to control ST; [e] $p < 0.05$ as compared to control ST; [f] not significant by student's test.

All mice were kept in air-conditioned animal quarters for 24 hours prior to use. Adult mice were maintained on Purina laboratory chow. The starved adult group had food, but not water, withheld for the 48-hour period prior to the administration of the hypnotic dose of hexobarbital. Weanling mice were maintained on isocaloric, complete protein diets of either 8 or 27% casein for 7 days before the beginning of the experimental study (5).

Results. In Table I it can be seen that the administration of phenobarbital or chlorcyclizine prior to the hypnotic dose of hexobarbital significantly decreased hexobarbital sleeping time from control levels in all groups studied (69–95%). Pretreatment with hexobarbital caused a 28% decrease in sleeping time in the fed adult group and significantly greater decrease in the other three groups studied (52–71%). Pretreatment with antipyrine decreased sleeping time 35% in the fed adult groups and caused a greater decrease in hexobarbital sleeping time both in the starved adult and in the weanling groups (45–52%). Although metronidazole did not alter the hexobarbital sleeping time in the fed adult group, it did decrease sleeping time in the starved adult mice (28%) and in the weanling groups (51-53%).

The 3,4-benzpyrene is a relatively specific inducer of hepatic drug-metabolizing enzymes, since it increases the detoxication of zoxazolamine, but has no effect on the metabolism of hexobarbital (6). In agreement with this, no alteration in hexobarbital sleeping time was observed in fed or starved adult mice pretreated with 3,4-benzpyrene. However, this compound caused a considerable reduction (34–75%) in hexobarbital sleeping time in both of the weanling groups of mice.

Discussion. The duration of hexobarbital sleeping time is primarily dependent on the rate of microsomal detoxication of the drug in the liver (3). Decreases in hexobarbital sleeping time were therefore assumed to result from increased microsomal metabolism of the drug and were used as the criterion for evidence of induction of hexobarbital-metabolizing enzymes. The various agents used in this study were grouped in Table I, according to the relative potency they exhibited as enzyme inducers. Significant decreases in hexobarbital sleeping time were obtained in all mice with the more potent enzyme inducers, phenobarbital and chlorcy-

clizine. Thus it was apparent that enzyme induction occurred in both the adult and weanling mice, regardless of dietary intake. Since it is believed that the induction of increased microsomal enzyme activity involves the synthesis of new protein (3), it was interesting to note that such protein synthesis appeared to be as efficient in the starved adults and weanlings fed the low protein diet as it was in the adult and weanling control mice stimulated by these potent inducers.

It was also apparent from the studies with the weaker inducers that the degree of enzyme induction by these inducers was always greater in the starved adult and weanling mice than it was in the control adult mice. The greater activity of these weaker inducers may result from the fact that the hepatic enzymes are at a subadult level in these animals and there is therefore a greater inherent stimulus for enzyme synthesis.

The observation that 3,4-benzpyrene caused a decrease in hexobarbital sleeping time in both weanling groups, but not in the adult mice again points out the susceptibility of the weanling mouse to enzyme induction. It also suggests that the specificity of action of this compound in inducing only certain drug-metabolizing enzymes may be dependent on the presence of a more stabilized state of protein synthesis that exists in the adult mouse.

Summary. Potent inducers of drug-metabolizing enzymes such as phenobarbital and chlorcyclizine are as effective in fed and in starved adult mice as they are in weanling mice maintained on 8 or 27% casein diets. Weaker enzyme inducers such as hexobarbital, antipyrine, or metronidazole are more effective agents in starved adults and weanling mice than they are in the well-fed adult mice. Compounds such as 3,4-benzpyrene which do not induce increased hexobarbital-metabolizing activity in adult mice are capable of inducing such activity in weanling mice.

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A Difference in Erythropoietin Production between Anemic and Hypoxic Mice* (32961)

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Genetic variations in various species of mice have shown a number of interesting abnormalities including hematological disturbances. The WWv mouse is anemic with an elevated plasma level of erythropoietin and a markedly diminished response to exogenous erythropoietin (1). However, the strain is capable of adequate, if not excessive, erythropoietin production in response to hypoxia. That the defect is one of erythropoietic cells seems confirmed by improvement following transplantation with normal coisogenic cells (2). In like manner, the Steel mouse is characterized by a macrocytic anemia with adequate erythropoietin production. The pluripotential cells of this strain have adequate transplant potential in irradiated normal coisogenic and WWv mice. However, the irradiated SL/SL mice do not adequately support colony formation even with parabiotic normals (3). This implies a tissue rather than an erythropoietic cellular defect.

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