the result of, breathing since it began before the head was above the surface. It would seem to be associated with a planned course of action, not an automatic reflex from the breathing itself.

An example of bradycardia due to a conditioned response is seen in the sea lion, which can be trained to slow its heart whether or not it is diving (*9*). With sufficient training this rewarded bradycardia will develop many times faster than that seen in actual diving. These observations give the impression that heart rate is affected by volitional as well as autonomic factors.

With a few exceptions (*2-4,9*), very few investigators have suggested that either emotion or learned behavior plays any part in accounting for the observed physiological details of diving; in fact, Jones and West (*10*) even suggest that the different heart rate response during free diving is a modification of that seen in forced submergence. We feel that this relation should be inverted. We suggest that from now on the word "diving" be reserved for the voluntary underwater activity of aquatic animals. "Forced submersion" is a more accurate title for studies on restrained animals. Our telemetry results indicate that stress-induced artifacts account for a large part of the "diving" bradycardia reported in laboratory studies.

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Exposure of Newborn Rats to Pharmacologically Active Compounds May Permanently Alter Carcinogen Metabolism

Abstract. *Administration of phenobarbital to mother rats during early lactation causes long-term, perhaps permanent, alteration of hepatic microsomal mixed-function oxidase activity and aflatoxin B_1 adduct formation in the adult male offspring. These findings suggest that perinatal exposure to pharmacologically active compounds may be a determinant of cancer risk.*

Environmental factors modulate the microsomal mixed-function oxidase (MFO) enzyme system, which metabolizes xenobiotics, endogenous steroids, and fatty acids (*1*). Modulation of the activity of the MFO system by exposure to foreign compounds occurs primarily through enzyme induction (*1, 2*). This induction generally requires the presence of the inducer, results in an increase in enzyme protein, and is associated with increased synthesis or decreased degradation of the enzyme or both. Since most carcinogens are either metabolically activated or detoxified through the MFO system, the biological activity of these compounds can be altered by factors that modulate their metabolism (*3*). However, for enzyme induction to affect carcinogen metabolism and neoplastic sequelae, temporal coincidence is required between induced enzyme activities and the presence of the carcinogen substrate since enzyme induction is a transient mechanism.

If the basal enzyme activities were more permanently altered, their impact on carcinogen metabolism and tumorigenicity would be more significant. Such would be the case if the perinatal environment were to modify long-term enzyme development. This is supported by the observation that steroid metabolism in the adult male rat is imprinted or programmed by neonatal testicular androgen (*4*).

The present study was designed to determine whether neonatal exposure to foreign compounds confers an altered capacity for carcinogen metabolism later in life. It was surmised that such an alteration might be the result of a mechanism other than that responsible for the transient MFO induction.

Phenobarbital (PB) has a powerful and comprehensive effect on the classical MFO induction process. Therefore, a protocol was designed to examine the influence of PB administration during the neonatal period on (i) the metabolism of the hepatocarcinogen aflatoxin B_1 (AFB_1) in the adult rat and (ii) the adult MFO activities for *N*-demethylation of ethylmorphine. Phenobarbital decreases AFB_1 tumorigenicity (*5*) and macromolecular adduct formation in rats (*6*) but increases the *N*-demethylation of ethylmorphine (*1*).

Mature male and female Long-Evans hooded rats (200 to 225 g) (Blue Spruce Farms) were randomly paired and bred in our facility. Upon procurement, the animals were acclimated for 10 days in a room with controlled temperature and 12-hour photoperiodic cycle. Relative humidity was maintained between 40 and 60 percent. All the animals were given free access to purified AIN-76 diet (Dyets, Inc.) and tap water. During gestation and lactation, the dams were housed in polycarbonate cages containing hardwood shavings (American Excelsior Co.). At parturition they were randomly assigned to a treatment or control group, and the newborn pups were pooled, sexed, and randomly assigned to dams with the same genetic background. Litter size was maintained at ten pups (five males and five females) per dam.

The experimental dams received their first PB treatment within 12 hours after giving birth. They were gavaged with PB (40 mg per kilogram) for seven successive days. It was expected that the pups would receive PB and its metabolites through the milk (*7*). We did not measure the amount of PB or its metabolites which may have been transmitted through the milk to the pups, although we did observe a higher incidence of deaths in these pups than in the control pups. The control dams received an equal volume of 0.9 percent saline. Progeny were weaned at 21 days. Only the data for males are presented in this report.

The effect of PB exposure on AFB_1 disposition in the offspring was examined when they were 37 weeks of age. Before killing the rats, we gave them a secondary treatment of PB (80 mg/kg, intraperitoneally) or 0.9 percent saline daily for 5 days. Hence there were four groups of animals based on the primary neonatal treatment (saline or PB) and the secondary adult treatment (saline or PB). Thus the groups were designated control-control, control-PB, PB-control, and PB-PB. To evaluate the AFB_1 disposition effect, we injected animals in

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Table 1. Hepatic AFB_1 adducts and microsomal ethylmorphine-*N*-demethylase activity for 37-week-old male rats exposed to PB neonatally or as adults. The data are means ± standard deviations for three to five animals per treatment group. Significance was established at $P < .05$ by using Student's *t*-test for two independent sample means. Significant differences between data are indicated by different superscripts.

Treatment (neonatal-adult)	AFB_1 adducts			Ethylmorphine-*N*-demethylase* (nmole/min-mg protein)
	ng/mg DNA	ng/mg RNA	ng/mg protein	
Control-control	46.4 ± 4.6[a]	196.9 ± 33.6[b]	11.7 ± 4.9[a]	1.05 ± 0.25[a]
Control-PB	38.9 ± 5.5[a]	116.2 ± 23.0[a]	7.9 ± 4.3[a]	4.87 ± 0.42[c]
PB-control	64.3 ± 5.8[b]	225.5 ± 23.5[b]	16.0 ± 5.0[a]	2.73 ± 0.32[b]
PB-PB	38.3 ± 10.7[a]	125.8 ± 25.4[a]	9.7 ± 2.0[a]	5.37 ± 0.77[c]

*Formaldehyde.

each group with ^3H-labeled AFB_1 (1.0 mg/kg in 0.2 ml of dimethylformamide; 365 dpm/ng) 24 hours after their fifth secondary injection of PB or saline. The presence of labeled macromolecular AF-DNA adducts in the liver was determined after 6 hours by a perchloric acid extraction procedure adapted (*8*) from one used by Glazer and Weber (*9*), which in turn was a modification of the method of Rowland (*10*). This method also permits the measurement of RNA and protein carcinogen adducts. A comparison of this method with the Marmur procedure (*11*) showed that, although the absolute quantities of adducts were slightly different, the trends between treatments were not altered.

Early exposure to PB increased the formation of AF-DNA adducts by approximately 40 percent (Table 1). Similar effects for RNA and protein adducts were observed, although these data are not statistically significant. On the other hand, PB administered as the secondary inducer (adult treatment) decreased the formation of AF-DNA adducts in both groups, in agreement with the results of Garner (*6*); however, this decrease was only significant in animals exposed to PB during lactation. The percentage of decrease noted by Garner (*6*) was greater than that observed here, probably because his animals were much younger than ours.

Hepatic microsomal MFO enzyme activity for the *N*-demethylation of ethylmorphine was also determined (Table 1). Hepatic microsomes were isolated from the 37-week-old animals that did not receive AFB_1 (*12*); enzyme activity was measured as described in (*13*).

Early exposure to PB increased the basal level of ethylmorphine-*N*-demethylase by 160 percent ($P < .01$). Similar results were reported by Yanai (*14*), who demonstrated that mice prenatally exposed to PB had elevated *p*-nitroanisol *N*-demethylase activity at 45 to 50 days of age. Also, Salganik *et al.* (*15*) showed that adult SWR/y mice treated neonatally with 16-α-isothiocyanopregnenolone-3-acetate had double the hepatic arylhydrocarbon hydroxylase activity at 8 months.

Phenobarbital administered as the secondary inducer increased the *N*-demethylation of ethylmorphine in both groups to the same extent, although the relative increase was greater in the animals not exposed to PB during lactation—primarily because of the lower basal activities.

It should be emphasized that, in contrast to the decrease in the formation of AF-DNA adducts in 37-week-old rats given secondary PB treatment, primary PB treatment during the neonatal period resulted in an increase in these adducts in adult males. Classical MFO induction by PB (secondary treatment) not only increases MFO-catalyzed reactions, it increases other enzyme activities (glucuronyl transferases, glutathione *S*-transferases) that are involved in the detoxification of carcinogens (*1*, *3*). Therefore, the observed decrease in AF-DNA adduct formation after secondary PB treatment may have been determined by these auxiliary enzymes. On the other hand, primary PB treatment may result in altered MFO activities later in life without affecting these auxiliary enzymes if their ontogenetic critical periods are not coincident. Thus, if neonatal exposure to PB increases the production of the ultimate carcinogen in the adult animal, one would expect to find a concomitant increase in carcinogen-DNA adducts.

The effects seen here are not due to long-term retention of the induced and transient MFO activity. We previously showed (*16*) that neonatal exposure to PB increased AF-DNA adduct formation in 8-day-old male pups but had no effect on AFB_1 adduct formation in 23-day-old weanlings. Thus, it is evident that altered carcinogen metabolism in adult male rats is neither due to the transient PB induction effects nor to a residual PB effect since AFB_1 disposition is not altered in male weanlings.

It is almost axiomatic that the induction of cancer by carcinogens results from the covalent interaction of the carcinogen with one or more tissue macromolecules (*17*). It is also well established that many carcinogens require metabolic activation by microsomal MFO's to an electrophilic form as a prerequisite for such a reaction (*18*). Although the target site is unclear, the covalent interaction of a carcinogen and DNA is generally considered necessary to initiate the malignant lesion (*19*). Moreover, the quantity of covalent carcinogen-DNA adducts is assumed to be indicative of the carcinogenic potential of a compound (*20*). Thus perinatal exposure to MFO enzyme inducers that alter the formation of carcinogen DNA adducts may be an important determinant of cancer risk. However, whether or not an increase in the amount of AF-DNA adducts in the adult animal after a neonatal exposure to PB results in an increased tumor response is not known, although these findings suggest that such an effect should exist. The increase in AF adduct formation caused by neonatal treatment directly contrasts with the decrease in AF adducts caused by adult exposure to PB. These effects appear to be distinctly different phenomena. Although the mechanism is not known, it is interesting to note that MFO activities in adult male rats can be imprinted by neonatal testicular androgen (*4*).

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Sex Ratio Manipulation and Selection for Attractiveness

Abstract. *Laboratory experiments performed on a monogamous estrildid, the zebra finch (*Poephila guttata*), indicate that sex ratio of offspring is affected by nongenetic markers (colored plastic leg bands) that vary in attractiveness to birds. Results suggest that natural selection favors individuals that produce offspring of the sex of the more attractive parent within a breeding pair.*

Trivers and Willard (*1*) advanced the provocative hypothesis that natural selection favors parental control over the sex of offspring, but empirical support for their idea is limited (*2*). I now report that the sex ratio of progeny is affected by nongenetic (human-made) "attractive" traits in a social estrildid, the zebra finch (*Poephila guttata*).

Zebra finches are nomadic opportunistic breeders that reach sexual maturity quickly (*3*) and form long-term pair bonds (*4*). They are principally granivorous and breed in loose colonies of variable size (*5*). Both sexes contribute to all phases of offspring care. Size dimorphism is slight, but plumage dimorphism is striking, with males much more colorful than females.

Experiments have indicated that zebra finches are sensitive to the color of plastic leg bands worn by opposite sex conspecies (*6*). To measure preferences for bands, individuals are permitted to perch next to any of four birds, three of which wear color bands, while the fourth is unbanded. Preference is measured as relative time spent with each bird during a 30-minute test interval. In an experiment in which males had red (R), orange (O), or light green (g) bands, females perched most often in view of R males and least often in view of g males. In a sequence in which females had black (Bl), pink (P), or light blue (b) bands, males preferred to perch in view of Bl and P and spent least time perching near b females. Thus Bl, P, and R are attractive colors (preferred to bandless) to at least one sex, whereas b and g are unattractive (bandless preferred). The natural leg color is orange.

To determine whether band color influences reproductive success, I released 30 adults of each sex into an aviary (8 by 5 by 2 m) containing abundant food and nesting sites. Ten females each were banded Bl (attractive), b (unattractive), and O (intermediate). Ten males each were banded R (attractive), g (unattractive), and O (intermediate). Birds were permitted to select mates and reproduce freely. Data were collected on the reproductive patterns of each color type.

The nine possible pair combinations produced 125 offspring that reached sexual maturity between December 1979 and July 1980 (*7*). Mortality during the interval between fledgling and molting into adult plumage was about 6 percent, so the figures presented below approximate relative parental investment and secondary sex ratios.

By 1 January 1980, 49 offspring had hatched that survived to adulthood. Of these, 29 were fathered by R males ($\chi^2 = 17.8$, $P < .005$), and 36 had O mothers ($\chi^2 = 36.6$, $P < .005$). This trend continued through July, so that, overall, R males and O females achieved disproportionate reproduction (Table 1) (male $\chi^2 = 8.9$, $P < .05$; female $\chi^2 = 26.9$, $P < .005$) (*8*).

Sex ratio varied according to pair combination (Table 1). Attractive (Bl) females that were mated with less attractive O and g males produced a lower fraction of male offspring than did attractive (R) males mated with less attractive (O and b) females (6 males, 11 females versus 30 males, 16 females; $P = .03$ by the Fisher exact test) (Table 1). This trend is more striking when only the extremes are considered (g males with Bl females versus R males with b females; $P = .003$). Overall, R males produced a higher proportion of sons than g males ($P = .03$), and Bl females produced a higher proportion of daughters than b females ($P = .007$). Thus, individuals wearing colors that were found to be attractive in preference experiments were more likely to produce offspring of that sex than were those judged unattractive. Pairs with traits of similar attractiveness produced a balanced sex ratio (Table 1).

A determination of the overall association between disparity in attractiveness within pairs and sex ratio of the clutches produced was made with a rank-order correlation test (*9*), performed by ranking individuals from 1 (unattractive) to 3 (attractive). For each pair, the female's rank was then subtracted from the male's, with resulting pair rankings that varied from + 2(R × b) through −2(g × Bl). The correlation between this measure and the sex ratio for all clutches is significant (gamma = .334; $P = .03$ by a two-tailed test) and reinforces the conclusion that birds adjust the sex ratio to reflect differences in attractiveness within pairs. At present the mechanism of sex ratio manipulation is unknown, but observations suggest that adults may recognize the sex of young shortly after hatching.

These results suggest that epigamic selection (mate choice) (*10*, *11*) has contributed to the evolution of dimorphism in zebra finches, but they do not exclude the possibilities that intrasexual competition (*10*, *12*) and ecological specialization (*13*) by the sexes have also occurred. A possible function of preferences for attractive traits is enhanced confidence of appropriate mate selection when related species occur sympatrically (*14*). Zebra finches breed in several habitats and have by far the widest distribution of any Australian estrildid (*15*). The array of species encountered by individuals is variable and somewhat unpredictable (*16*).

Quantitative models of the evolution

Table 1. Number of offspring of each pair combination to reach maturity by 30 June 1980. Band colors: R, red; O, orange; g, light green; Bl, black; and b, light blue.

Female parent	Male parent					
	R (attractive)		O		g (unattractive)	
	Male	Female	Male	Female	Male	Female
Bl (attractive)	5	6	5	3	1	8
O	21	14	4	4	13	13
b (unattractive)	9	2	9	6	1	1

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