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Research Article

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Shortening of Plasma Antipyrine Half-Lives in Man

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A B S T R A C T   The mean half-life of antipyrine in the plasma of four sets of identical and four sets of fraternal twins after a single oral dose of 16 mg/kg of antipyrine was 12.7 ±SD 3.3 hr. After 2 wk on sodium phenobarbital (2 mg/kg daily) the half-life of antipyrine in the plasma of these twins was reduced to 8.0 ±SD 1.5 hr. Shortening of the plasma antipyrine half-life occurred in all but one of these 16 normal, adult volunteers, but there was considerable variation in the extent of reduction which ranged from 0 to 69%. Phenobarbital administration decreased individual variations in antipyrine metabolism as indicated by the smaller standard deviation of the plasma antipyrine half-lives after phenobarbital than observed initially and by the narrowed range of variation in plasma antipyrine half-lives from 2.8-fold initially to 1.8-fold after phenobarbital. These results suggest that some inducing agents may be used to minimize individual variations in drug metabolism where such variations create therapeutic problems by exposing patients who slowly metabolize certain drugs to toxicity and other patients who rapidly metabolize some drugs to undertreatment.

During the course of phenobarbital administration blood levels were determined. Phenobarbital blood levels correlated neither with the final values for plasma antipyrine half-lives nor with the per cent reduction in plasma antipyrine half-life produced by phenobarbital treatment. There was a direct relationship between initial antipyrine half-lives and the per cent shortening of antipyrine half-life produced by phenobarbital administration: the shorter the initial antipyrine half-life, the less the reduction caused by phenobarbital treatment. Larger intrapair variances in fraternal than in identical

twins indicate genetic, rather than environmental, control of phenobarbital-induced alterations in plasma antipyrine half-life.

INTRODUCTION

Genetic control of induction in man. By changing rates of drug metabolism in hepatic microsomes, numerous factors may substantially modify the duration and intensity of action of various therapeutic agents. Although genetic, dietary, hormonal, and nutritional causes of alterations in the drug-metabolizing activities of hepatic microsomes have been investigated, particular attention has focused on certain foreign compounds that augment these activities (1–11). Various agents may shorten not only their own duration of action, but also that of other drugs administered simultaneously. Such drug interactions have important clinical consequences.

Phenobarbital has been employed therapeutically in combatting hyperbilirubinemia in children with congenital nonhemolytic jaundice (12–14). However, simultaneous administration of several agents may be hazardous, as when patients on Dicumarol or warfarin receive phenobarbital, thereby raising the dosage requirement of the anticoagulant, or conversely, when patients on Dicumarol or warfarin and phenobarbital have the latter drug discontinued, thereby decreasing the dosage requirement of the anticoagulant.

The newly recognized role of phenobarbital to alter the effectiveness and dosage requirements of other drugs makes individual variations in response to phenobarbital a clinically significant problem. It might be assumed that subjects achieving comparable blood levels of phenobarbital would have their blood levels of other drugs changed to similar extents. However, this study reveals genetically determined differences among individuals in the extent of phenobarbital-induced alterations of plasma antipyrine half-lives. These individual variations in the

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degree of shortening of antipyrine half-life by pheno-
barbital appear to be independent of absolute blood
levels of phenobarbital.

Large variations in the metabolism of certain drugs
occur in man (3). Significant individual differences in
the metabolism of antipyrine, Dicumarol, and phenyl-
butazone have been demonstrated and recently attributed
to genetic, rather than to environmental, factors (3).
These individual variations in rates of drug elimination
from plasma presumably arise from polygenically deter-
mined differences among normal subjects in the activities
of their hepatic microsomal drug-metabolizing enzymes.
Evidence is now presented to suggest that large indi-
vidual differences in the phenobarbital-induced shorten-
ing of plasma antipyrine half-lives are also genetically,
rather than environmentally, controlled.

METHODS

Eight pairs of twins from the Washington, D. C., area
volunteered for this study. All were 21 yr of age or over,
Caucasian, and in good health, as determined by history,
physical examination, and routine laboratory studies. None
were medicated for 1 month before the study. Typing of the
volunteers for approximately 30 blood groups helped deter-
mine that four sets were identical, or monozygotic, and four
sets were fraternal, or dizygotic.

At 9 a.m. each subject received a single oral dose of anti-
pyrine (18 mg/kg) dissolved in water. At 3, 6, 9, and 12 hr
thereafter, blood (12 ml) was drawn, anticoagulated in
0.1 ml of 40% sodium citrate and the plasma assayed in
duplicate for antipyrine by the method of Brodie, Axelrod,
Soberman, and Levy (15). According to this method, which
is based on the formation of the nitroso compound, 4-nitroso-
antipyrine, the following are added to 2 ml of plasma: dis-
tilled water (2 ml), a solution (2 ml) composed of 0.33 M
$ZnSO_4$, and 0.25 N H_2SO_4, and finally, 0.75 N NaOH (2 ml
dropwise, with continuous agitation). After centrifugation
at 3300 g for 15 min, one drop of 4 N H_2SO_4 is added to
the supernatant. The optical density (OD) is read in a
Beckman DU spectrophotometer at 350 mμ against a blank
composed of distilled H_2O (3 ml) plus one drop 4 N
H_2SO_4. Then to each specimen of acidified plasma, as well
as to the blank, is added two drops of $NaNO_2$ (2%). After
20 min the OD at 350 mμ is redetermined and the differences
in OD at T_0 and T_{20} are measured and compared to stan-
dards prepared from plasma to which known amounts of
antipyrine have been added.

On each of 14 successive days thereafter every twin took
a single oral dose of sodium phenobarbital (2 mg/kg) at
11 p.m. During this period blood levels of phenobarbital
were determined at regular intervals by the method of
Butler, Mahaffee, and Waddell (16). In this method 2 ml
of plasma and 1 ml of Na_2HPO_4–NaH_2PO_4 buffer, pH 8.0,
1 mole/liter, are extracted with 12 ml of ether. 10 ml of the
ether layer, obtained after shaking for 20 min and centri-
fuging at 2800 g for 5 min, is added to 4 ml of buffer,
pH 11, composed of 0.1 N $NaHCO_3$ and 0.09 N NaOH.
After shaking and centrifugation are performed as before,
the ether is removed and the aqueous layer is rewashed
with 2 ml of ether. After shaking and centrifuging as
above, the ether is removed. The OD of the aqueous layer
is then measured in a Beckman DU spectrophotometer at
both 240 and 260 mμ and from the difference the amount of

phenobarbital can be estimated by comparison with standards
composed of plasma to which known amounts of pheno-
barbital have been added.

On a daily dose of 2 mg/kg phenobarbital, drowsiness and
lethargy developed in almost all volunteers after a few days,
but in only a few were these symptoms sufficiently severe
to interfere with performance at work. Several reported
abdominal discomfort and nausea; two complained of diar-
rhea. A generalized drug rash occurred in two individuals,
one of whose identical twin had no symptoms while on the
same regimen. The identical twin who suffered the rash
denied previous exposure to phenobarbital, but gave a
history, unlike her sib, of hay fever and allergy to certain
foods. Phenobarbital was discontinued, and this set of twins
was dropped from our study. No other twins starting the
study failed to complete it, although in another twin
without a history of allergy or skin eruption what appeared
to be a drug rash occurred on forearms, elbows, and trunk
after 1 wk of phenobarbital ingestion. She insisted on con-
tinuing phenobarbital; and the erythematous, scaly, pruritic
rash receded while she remained on the same dose of
phenobarbital.

At the end of the 2 wk course of phenobarbital, each
volunteer received a single oral dose of antipyrine (18
mg/kg) at 9 a.m. and blood specimens were drawn at 2, 4,
6, and 8 hr thereafter to determine the rate of elimination
of the drug from plasma.

RESULTS

Figs. 1 and 2 show the decay of antipyrine from the
plasma of four sets of identical twins and four sets of
fraternal twins, respectively. They illustrate the decline
of antipyrine in the plasma of each subject after two sepa-
rate doses of antipyrine were administered, the first be-
fore and the second after 2 wk of medication with sodium
phenobarbital (2 mg/kg daily). In all but one of the
16 twins, phenobarbital treatment effected a decrease in
the plasma half-life of antipyrine, but the extent of the
reduction varied considerably and ranged from 0 to 69%
of the initial plasma antipyrine half-life. Table I shows
the twin's age, sex, plasma half-life of antipyrine before
and after phenobarbital administration, per cent change
in plasma half-life of antipyrine, blood levels of pheno-
barbital at various times during the 14 day period of its
administration, and habits with respect to cigarette
smoking, coffee and tea ingestion, and alcohol con-
sumption. The mean half-life of antipyrine in the plasma
of four sets of identical and four sets of fraternal twins
was 12.7 $\pm$SD 3.3 hr. After 2 wk on a daily dose of 2
mg/kg sodium phenobarbital, the plasma antipyrine half-
life was redetermined and observed to be shortened to
8.0 $\pm$SD 1.5 hr. In these 16 subjects phenobarbital nar-
rowed the variation in plasma antipyrine half-life from
2.8-fold (range of 6.5–18.2 hr) initially to 1.8-fold
(range of 5.5–10.3) after 14 days on phenobarbital
(Table I). Another indication of the narrowing in indi-
vidual differences produced by phenobarbital administra-
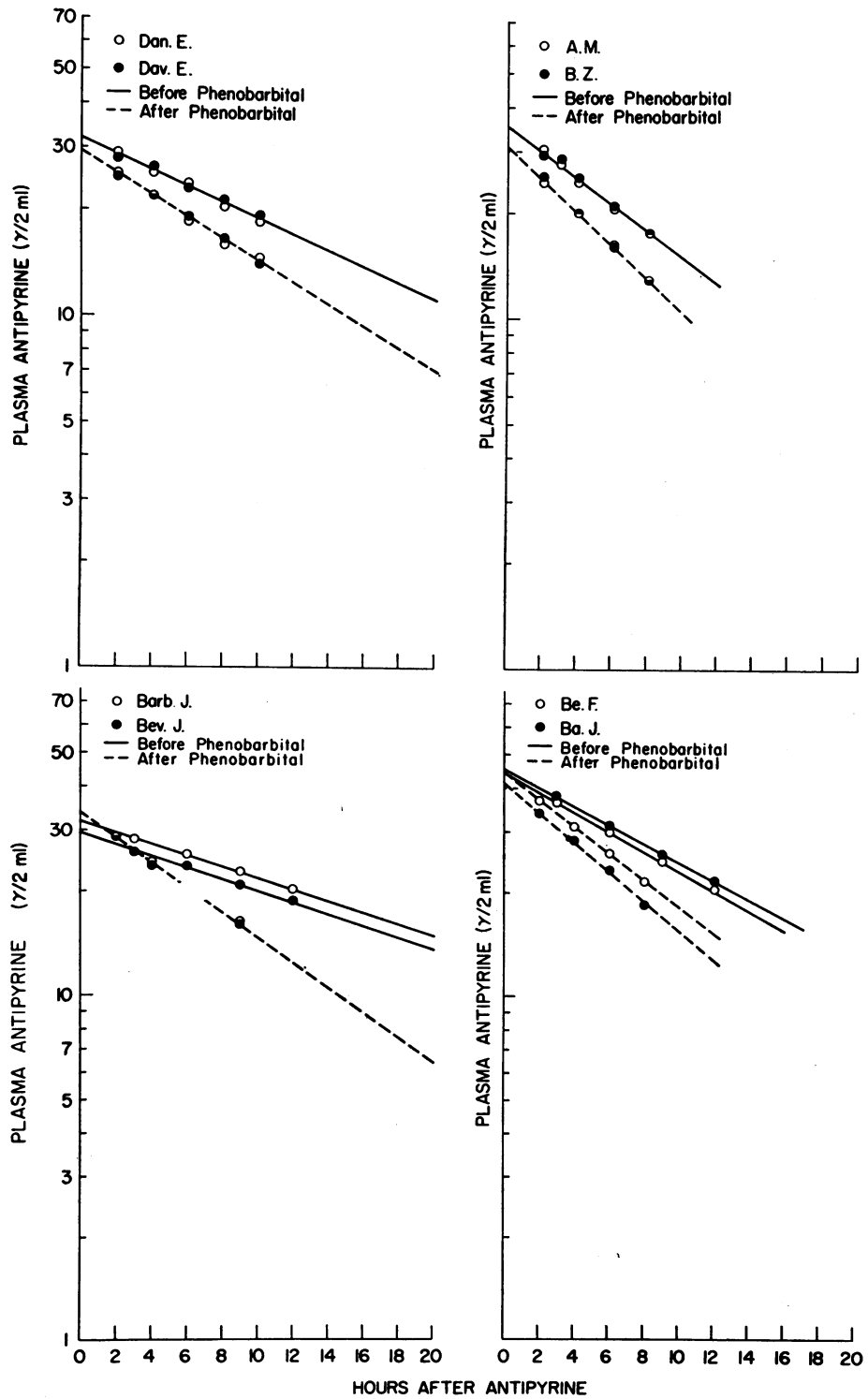tion is the reduction in the standard deviation of the

FIGURE 1 Decline of antipyrine from the plasma of four sets of identical twins after a single oral dose of antipyrine (16 mg/kg). Plasma decay of antipyrine was determined before and after 14 consecutive days on sodium phenobarbital (2 mg/kg daily).

mean plasma antipyrine half-life from 3.3 initially to 1.5 after phenobarbital treatment.

Fig. 3 shows that the fraternal twins F. D. and P. D. and E. E. and E. W. had little intrapair differences in their stepwise increments in plasma phenobarbital levels at various times during its administration, although Table I reveals that they exhibited the greatest differences in their antipyrine response to phenobarbital of all the twins in the study. Further evidence dissociating the absolute values of blood phenobarbital from the antipyrine response is the lack of significant correlation (0.46) between the per cent shortening in plasma antipyrine half-life and the plasma levels of phenobarbital almost 14 days after initiation of the drug. Furthermore, no significant correlation (0.25) occurred between the value of plasma phenobarbital almost 14 days after initiation of the drug and the antipyrine half-life in plasma at this time. Similar results were obtained at an earlier time when phenobarbital was measured in plasma almost 7 days after administration of the drug

was begun. The correlation coefficient between plasma phenobarbital and final antipyrine half-life was 0.19; the correlation coefficient between plasma phenobarbital and per cent change in antipyrine half-life was 0.22. Fig. 4 shows a significant correlation of 0.84 between the initial antipyrine half-life in plasma and the per cent shortening of antipyrine half-life in plasma produced by phenobarbital administration: the longer the initial half-life of antipyrine in plasma, the greater the per cent decrease caused by phenobarbital. Conversely, the shorter the initial half-life of antipyrine in plasma, the less the per cent shortening produced by phenobarbital administration.

The contribution of heredity to the trait under study (degree of phenobarbital-induced shortening of plasma antipyrine half-life) was estimated from the formula (17): (variance within pairs of fraternal twins minus variance within pairs of identical twins)/variance within pairs of fraternal twins. This formula permits values from zero, indicating an insignificant hereditary and a
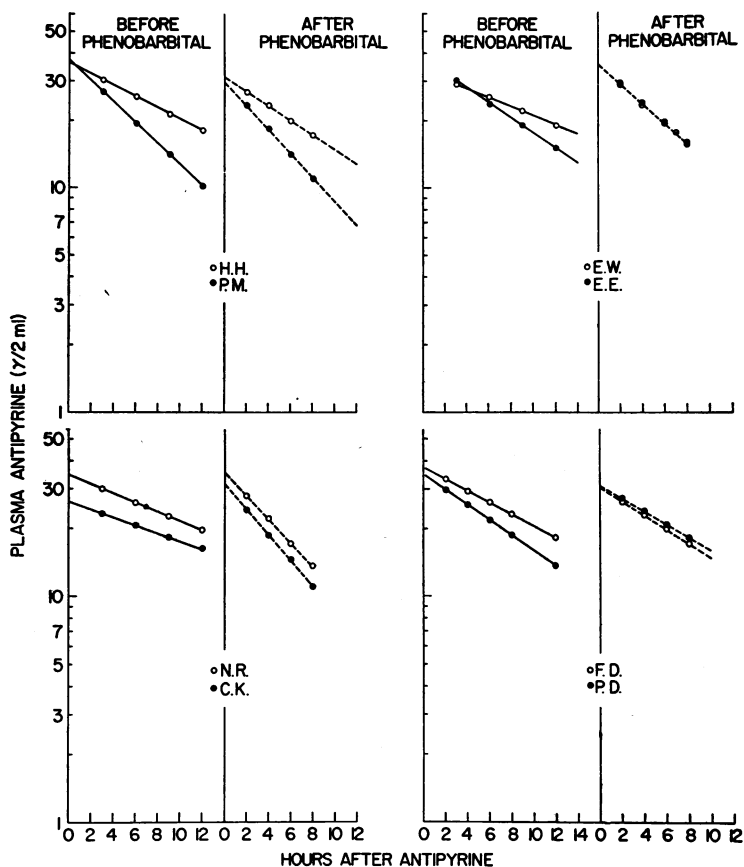

FIGURE 2   Decline of antipyrine from the plasma of four sets of fraternal twins after a single oral dose of antipyrine (16 mg/kg). Plasma decay of antipyrine was determined before and after 14 consecutive days on sodium phenobarbital (2 mg/kg daily).

TABLE I

Response of Plasma Antipyrine Half-Life to Phenobarbital Administration with Smoking, Coffee, Tea, and Alcohol History in 16 Twins

Twin	Age, Sex	Plasma antipyrine half-life		Decrease in half-life produced by phenobarbital	Percentage difference between sibs in response to phenobarbital	Plasma phenobarbital levels at		Smoking	Coffee	Tea	Alcohol		
		Before phenobarbital	After phenobarbital			156 hr	212 hr				Beer	Wine	Hard liquor
		hr	hr	%				pack/day	cup/day	cup/day	bottles/day	glasses/day	oz/day
Identical twins													
Dan. E.	22, M	13.6	9.6	29.4	0	20.0	23.0	0	2	0	1/4	0	1/4
Dav. E.	22, M	13.6	9.6	29.4		20.0	23.2	1/4	0	2	1/4	0	1/4
A. M.	35, F	8.0	6.3	21.2	0	17.0	18.0	1/2	0	3	0	0	0
B. Z.	35, F	8.0	6.3	21.2		15.0	16.0	2	6–7	0	0	0	1
Bar. J.	23, F	18.2	8.4	53.8	0	16.8	17.1	0	0	0	0	0	0
Bev. J.	23, F	18.2	8.4	53.8		17.9	25.0	0	0	0	0	0	0
B. F.	26, F	10.8	7.3	32.4	2.6	16.0	23.4	0	0	0	0	0	0
B. J.	26, F	11.4	7.4	35.0		17.4	24.4	0	0	1	0	0	1/4
Fraternal twins													
F. D.	49, M	12.0	10.3	14.2	14.2	14.8	19.4	0	1	2	0	0	0
P. D.	49, M	9.3	9.3	0		15.2	18.3	1 1/2	5	0	0	0	1
C. K.	49, M	17.5	5.5	68.6	8.6	—	23.0	1 1/2	2	0	1	0	18
N. R.	49, F	14.5	5.8	60.0		—	19.0	1 1/2	4	0	0	0	0
H. H.	47, F	12.3	9.2	25.2	9.8	17.8	16.6	2	2	0	1/2	0	1/2
P. M.	47, F	6.5	5.5	15.4		16.0	20.8	1 1/2	2	0	1/2	0	0
E. W.	54, F	15.0	6.9	54.0	30.7	20.0	29.0	0	4	0	0	0	1/2
E. E.	54, F	9.0	6.9	23.3		20.0	30.0	1	2	2	0	0	1/4

predominantly environmental component, to 1, indicating a strong hereditary and a negligible environmental component. Variance within pairs was determined from the formula (17): Σ (difference between twins)^2/2n. A value of 0.99 was obtained when these formulae were applied to the per cent alteration in plasma antipyrine half-life produced by phenobarbital administration. This value indicates that hereditary, rather than environmental, factors control the extent to which plasma antipyrine half-lives were shortened by phenobarbital administration under the conditions of this investigation.

DISCUSSION

These experiments have several new and potentially useful therapeutic implications. The first new result is the discovery of large individual variations in the extent of phenobarbital-induced shortening of plasma antipyrine half-life. In 16 subjects the range in reduction of plasma antipyrine half-life after phenobarbital administration was from 0 to 69%. Previously, in seven individuals,

variation in the response of plasma Dicumarol levels to phenobarbital administration was noted (8). Because phenobarbital is increasingly used to accelerate the elimination of toxic substances (12–14), large individual differences in the magnitude of phenobarbital-induced decreases of drug half-life have clinical applications. Therefore, our results indicate that uniformity in the extent of phenobarbital-induced reductions of drug half-life should not be expected when phenobarbital is employed to treat toxic accumulations of various compounds.

The present studies help to identify mechanisms responsible for individual differences in the phenobarbital-induced shortening of antipyrine half-life. In healthy adults not receiving other drugs, these variations in response to phenobarbital are genetically, rather than environmentally, determined, as indicated by much smaller intrapair differences in identical than in fraternal twins. Moreover, the present studies establish a direct relationship between the magnitude of the initial plasma

antipyrine half-life and the extent of phenobarbital-induced shortening (Fig. 4). This relationship, which permits prediction of the extent to which phenobarbital will depress the initial antipyrine half-life, has potential clinical application. Since phenobarbital shortens the antipyrine half-life of slow metabolizers more than it shortens the antipyrine half-life of fast metabolizers, use of phenobarbital to reduce toxic blood levels of various drugs would appear to aid preferentially those individuals most in need of such therapy, namely, the slow metabolizers. On the other hand, if high blood levels of a certain drug are therapeutically desirable but difficult to attain, maximum effectiveness for such an agent will be easier to achieve in a slow than in a rapid drug metabolizer. Theoretically, in this situation the simultaneous administration of agents such as phenobarbital will impede attainment of adequate blood levels of the drug more in slow than in rapid drug metabolizers (Fig. 4).

Related to these observations are the reduction of large individual differences in plasma antipyrine half-life from 2.8-fold to 1.8-fold after 2 wk of phenobarbital administration and the decline of the standard deviation of the mean plasma antipyrine half-life from 3.3 to 1.5. These results suggest that where large individual differences in drug metabolism create problems in clinical management, relatively innocuous inducing agents could be administered to minimize such variations, thereby
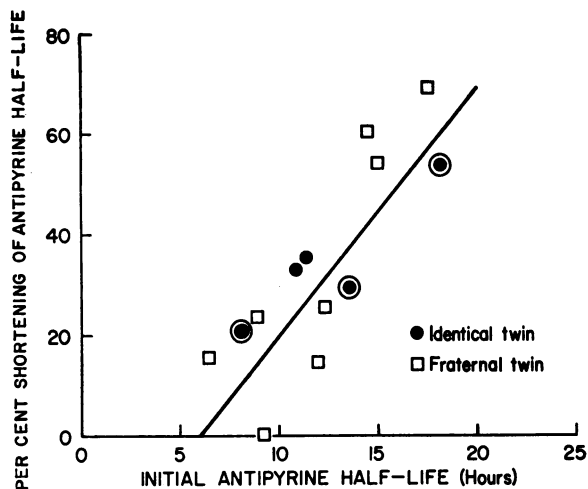
FIGURE 4 Positive correlation (0.84) between the initial antipyrine half-life in plasma and the phenobarbital-induced shortening of antipyrine half-life.

facilitating achievement of uniform and optimum blood levels of drugs and reduction in the incidence of toxicity on the one hand and of undertreatment on the other. It might be suggested that phenobarbital administration stabilizes drug half-lives, which might be considered to fluctuate under many nutritional, hormonal, and physiologic conditions. However, as we (3, 18) and others (19, 20) have demonstrated, values for antipyrine half-life are stable, highly reproducible, genetically controlled traits in normal adults not receiving other drugs. Explanations are not currently available for the marked reduction in the extent of individual variations in antipyrine half-life after phenobarbital administration or for the related observation that phenobarbital shortens antipyrine half-life more in individuals with high than with low initial values. It is not known how much further plasma antipyrine half-lives could be reduced by giving larger doses or longer courses of phenobarbital.

Antipyrine was selected as a model drug to investigate because it is bound to the extent of only 10% to plasma proteins (19) and is not excreted unchanged by the kidney (20), but handled in the body almost exclusively by hydroxylation in hepatic microsomes (20). Antipyrine has been employed effectively to measure total body water (19). It is not secreted into the stomach in significant amounts nor removed by the biliary circulation. Theoretical calculations according to volumes of distribution reveal that after equilibrium is attained, less than 1% of the dose in the body would be expected to be in the stomach. Figs. 1 and 2 demonstrate that phenobarbital administration has apparently not altered the volume of distribution of antipyrine. Previous studies revealed a threefold difference among 18 pairs of identical and fraternal twins in antipyrine metabolism (18)
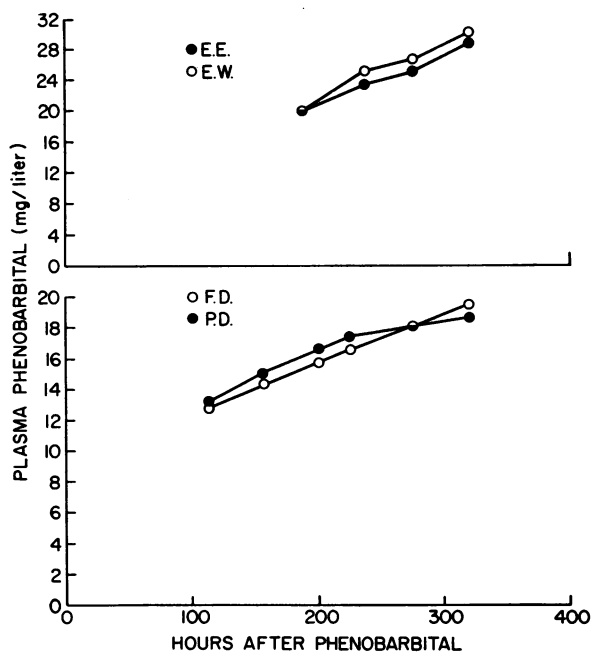

FIGURE 3 Plasma levels of phenobarbital in two sets of fraternal twins (F. D. and P. D. and E. E. and E. W.) at intervals from the start of sodium phenobarbital administration (daily dose of 2 mg/kg for 2 wk).

but no sex difference in plasma half-lives of antipyrine (18). Since phenobarbital treatment elevates the activity of hepatic microsomal drug-metabolizing enzymes, stimulates incorporation of amino acids into protein, and causes increased synthesis of protein (5), shortened antipyrine half-lives in all but 1 of 16 normal adult volunteers and the properties of the drug described above strongly suggest that phenobarbital accelerates the metabolism of antipyrine by inducing an hepatic microsomal hydroxylase. Although measurement of hepatic microsomal drug-metabolizing activity would be required to prove this hypothesis, no adequate alternative explanation is available for our observations on the effects of phenobarbital administration on plasma antipyrine half-lives. Greater similarity of the inductive response in identical than in fraternal twins implicates a genetic factor in the regulation of the hypothesized inductive process. Under the conditions of this investigation, the contribution of heredity to the trait in question was calculated to be 99%. Our data do not permit selection among the multiple possible regulatory sites at which the genetic factor may operate.

No attempt was made to control dietary or fluid intake in the volunteers; however, none received medications for 1 month before initiation of the study. Failure of plasma phenobarbital levels either at 156 or at 276 hr after initiation of medication to correlate with the plasma antipyrine half-life at 276 hr or with the percentage change in antipyrine half-life suggests that the effect of phenobarbital in inducing an hepatic microsomal antipyrine hydroxylase is not entirely dependent on blood levels of phenobarbital. Fig. 3 strengthens this conclusion by revealing that in the two sets of fraternal twins with largest intrapair differences in phenobarbital-induced shortening of plasma antipyrine half-lives, intrapair differences in plasma phenobarbital levels were negligible during the 2 wk of phenobarbital administration. Differences in plasma phenobarbital levels during the period of phenobarbital administration were anticipated; for phenobarbital, renal excretion plays a more prominent role in the elimination process than does metabolism; and diet, by affecting urinary pH, can alter appreciably the rate of renal excretion and hence the blood levels of the drug (21). Without controlling diet, we were surprised that greater individual differences in the plasma phenobarbital levels did not occur (Fig. 4, Table I).

During a year several twins not receiving other drugs had their plasma antipyrine half-lives determined on three separate occasions. Reproducibility of antipyrine half-lives in plasma within 10% of initial values indicates relative constancy of the rate at which individuals metabolize this drug. Reproducibility of individual plasma half-lives of antipyrine has been described pre-

viously (18). Wide fluctuations might have been anticipated because the quantity ingested of the numerous inducing agents to which many expose themselves, such as alcohol (11), caffeine (22), nicotine (23), 3,4-benzpyrene, and 3-methylcholanthrene (5), probably varies appreciably from day to day. Histories of coffee ingestion and cigarette smoking did not correlate with the rate of Dicumarol metabolism in a previous study (3), nor do these habits and alcohol consumption appear to be related to the phenobarbital-induced reduction of antipyrine half-life in this investigation (Table I). However, more extensive and detailed studies will be required to determine the effect of these habits on drug metabolism.

In a previous study sibs from five of seven sets of identical twins lived apart (3), so that similarity in their rates of Dicumarol metabolism could not be attributed to those environmental factors arising from cohabitation and consumption of identical food. In the present investigation two of the four sets of identical twins live apart, but several sets of fraternal twins who live together (see in particular H. H. and P. M.) had markedly different initial plasma antipyrine half-lives and strikingly different responses to phenobarbital. Such differences would be unexpected if similarity of environment significantly influenced rates of antipyrine metabolism or responsiveness to inducing agents under the conditions of our experiments.

REFERENCES

1. Vesell, E. S. 1968. Factors altering the responsiveness of mice to hexobarbital. *Pharmacology.* 1: 81.
2. Page, J. G., and E. S. Vesell. 1969. Hepatic drug metabolism in ten strains of Norway rat before and after pretreatment with phenobarbital. *Proc. Soc. Exp. Biol. Med.* 131: 256.
3. Vesell, E. S., and J. G. Page. 1968. Genetic control of Dicumarol levels in man. *J. Clin. Invest.* 47: 2657.
4. Gillette, J. R. 1967. Individually different responses to drugs according to age, sex and functional or pathological state. *In* CIBA Foundation Symposium on Drug Responses in Man. G. E. W. Wolstenholme and R. Porter, editors. J. & A. Churchill Ltd., London. 24.
5. Conney, A. H. 1967. Pharmacological implications of microsomal enzyme induction. *Pharmacol. Rev.* 19: 317.
6. Conney, A. H. 1969. Drug metabolism and therapeutics. *N. Engl. J. Med.* 280: 653.
7. Cram, R. L., M. R. Juchau, and J. R. Fouts. 1965. Differences in hepatic drug metabolism in various rabbit strains before and after pretreatment with phenobarbital. *Proc. Soc. Exp. Biol. Med.* 118: 872.
8. Cucinell, S. A., A. H. Conney, M. Sansur, and J. J. Burns. 1965. Drug interactions in man. I. Lowering effect of phenobarbital on plasma levels of bishydroxy-

coumarin (Dicumarol) and diphenylhydantoin (Dilantin). *Clin. Pharmacol. Ther.* **6**: 420.

9. Dayton, P. G., S. A. Cucinell, M. Weiss, and J. M. Perel. 1967. Dose-dependence of drug plasma level decline in dogs. *J. Pharmacol. Exp. Ther.* **158**: 305.

10. Hunninghake, D. B., and D. L. Azarnoff. 1968. Drug interactions with warfarin. *Arch. Intern. Med.* **121**: 349.

11. Rubin, E. and C. S. Lieber. 1968. Alcohol, other drugs and the liver. *Ann. Intern. Med.* **69**: 1063.

12. Yaffe, S. J., G. Levy, T. Matsuzawa, and T. Baliah. 1966. Enhancement of glucuronide-conjugating capacity in a hyperbilirubinemic infant due to apparent enzyme induction by phenobarbital. *N. Engl. J. Med.* **275**: 1461.

13. Crigler, J. F., Jr., and N. I. Gold. 1969. Effect of sodium phenobarbital on bilirubin metabolism in an infant with congenital, nonhemolytic, unconjugated hyperbilirubinemia, and kernicterus. *J. Clin. Invest.* **48**: 42.

14. Ramboer, C., R. P. H. Thompson, and R. Williams. 1969. Controlled trials of phenobarbitone therapy in neonatal jaundice. *Lancet.* **1**: 966.

15. Brodie, B. B., J. Axelrod, R. Soberman, and B. B. Levy. 1949. The estimation of antipyrine in biological materials. *J. Biol. Chem.* **179**: 25.

16. Butler, T. C., C. Mahaffee, and W. J. Waddell. 1954.

Phenobarbital: studies of elimination, accumulation, tolerance, and dosage schedules. *J. Pharmacol. Exp. Ther.* **111**: 425.

17. Osborne, R. H., and F. V. De George. 1959. Genetic Basis of Morphological Variation, an Evaluation and Application of the Twin Study Method. Harvard University Press, Cambridge. 17, 60.

18. Vesell, E. S., and J. G. Page. 1968. Genetic control of drug levels in man: antipyrine. *Science (Washington).* **161**: 72.

19. Soberman, R., B. B. Brodie, B. B. Levy, J. Axelrod, V. Hollander, and J. M. Steele. 1949. The use of antipyrine in the measurement of total body water in man. *J. Biol. Chem.* **179**: 31.

20. Brodie, B. B., and J. Axelrod. 1950. The fate of antipyrine in man. *J. Pharmacol. Exp. Ther.* **98**: 97.

21. Waddell, W. J., and T. C. Butler. 1957. The distribution and excretion of phenobarbital. *J. Clin. Invest.* **36**: 1217.

22. Mitoma, C., T. J. Sorich, II, and S. E. Neubauer. 1968. The effect of caffeine on drug metabolism. *Life Sci.* **7**: 145.

23. Wenzel, D. G., and L. L. Broadie. 1966. Stimulatory effect of nicotine on the metabolism of meprobamate. *Toxicol. Appl. Pharmacol.* **8**: 455.