4. Epstein, F. H., Rivera, M. J., Carone, F. A., ibid., 1958, v37, 1702.
5. Yoffee, H. F., Dingman, J. F., Metabolism, 1960, v9, 995.
6. Manitius, A., Levitin, H., Beck, D., Epstein, F. H., J. Clin. Invest., 1960, v39, 693.
7. Beck, D., Levitin, H., Epstein, F. H., Am. J. Physiol., 1959, v197, 1118.
8. Wallach, S., Carter, A. C., ibid., 1961, v200, 359.
9. Epstein, F. H., J. Chronic Dis., 1960, v11, 255.
10. Deitrick, J. E., Whedon, G. D., Shorr, E., Am. J. Med., 1948, v4, 3.
11. Howard, J. E., Parson, W., Bigham, R. S., Jr., Bull. Johns Hopkins Hosp., 1945, v77, 291.
12. Whedon, G. D., Shorr, E., J. Clin. Invest., 1957, v36, 966.

Received December 4, 1964. P.S.E.B.M., 1965, v118.

Differences in Hepatic Drug Metabolism in Various Rabbit Strains Before and After Pretreatment with Phenobarbital.* (29994)

RICHARD L. CRAM, MONT R. JUCHAU AND JAMES R. FOUTS
Department of Pharmacology, College of Medicine, University of Iowa, Iowa City

Species variation in drug response is a well recognized pharmacological phenomenon. Strain variations within a given species, however, have been characterized to a much lesser degree. These problems assume importance not only when attempting to extrapolate results of animal experiments to man, but also in basic biological research.

Law *et al*(1) have shown variations in beta-glucuronidase activity in various strains of mice. Jay(2) has also shown strain differences in mice with regard to hexobarbital sleeping time. The experiments of Yaffe(3) indicate that strain variation in drug response in mice is a genetic factor which is manifest soon after birth. Strain variation in drug response and hepatic drug metabolism in rats have been reported by Quinn *et al*(4). However, to our knowledge, no studies on strain differences in drug response have been reported using rabbits, a species commonly employed in research.

We have attempted to determine whether the activity of several hepatic microsomal drug metabolizing enzyme systems varies in different rabbit strains. Studies were carried out on strains of rabbits commonly employed in research as well as on wild rabbits. We have also attempted to determine whether the stimulation of the hepatic drug metabolizing enzyme activity by phenobarbital differed in these strains.

Materials and methods. Dutch, New Zealand, English, and California strains were studied as representatives of commonly employed research animals. In addition, wild Jack rabbits were obtained from the Durant Animal Co., Ft. Sumner, New Mexico and wild Cottontails were obtained from the Earl Johnson Farms, Rago, Kansas. Rabbits were maintained on regular Purina Lab Chow and water *ad libitum* during the experiment, and constant conditions were maintained as nearly as possible. In all cases young adult bucks were employed.

To study the effect of pre-treatment with phenobarbital, rabbits were injected intraperitoneally twice daily with 15 mg/kg phenobarbital sodium dissolved in normal saline. Injections were made for 3 days, on the 4th day the animals were not injected, and on the fifth day the animals were sacrificed and livers removed. Control animals were injected with normal saline on the same schedule. The livers were placed immediately on ice, blotted, weighed, and ground in the cold with a Potter homogenizer (teflon pestle) in 2 volumes of ice cold 1.15% KCl solution. This homogenate was then centrifuged at 1-3°C (International portable refrigerated centrifuge, model PR-2) at 9,000 *g* for 30 minutes.

The following metabolic pathways were studied: Side-chain oxidation of hexobarbital

* This research was supported by a grant from Nat. Inst. Health (GM-06034).

TABLE I. Determination of Basal Levels of Drug Metabolism by Hepatic Enzyme Systems of Various Rabbit Strains. Results expressed as μmoles substrate metabolized per g of 9000 *g* supernatant nitrogen per hour with standard deviations. Numbers in parentheses represent number of animals exployed in each determination.

	Strain					
Substrate	Dutch (5)	English (6)	Jacks (3)	California (6)	New Zealand (6)	Cottontails (5)
Hexobarbital	141 ± 52	173 ± 36	220 ± 51	248 ± 125	254 ± 92	19 ± 23
Aniline	35 ± 13	129 ± 27	121 ± 60	64 ± 20	95 ± 28	72 ± 20
1-Amphetamine	154 ± 20	87 ± 28	102 ± 2	55 ± 22	82 ± 14	8 ± 8
p-Nitrobenzoic acid	89 ± 44	67 ± 20	92 ± 59	22 ± 9	122 ± 58	50 ± 12
Zoxazolamine	155 ± 56	153 ± 28	71 ± 61	200 ± 71	162 ± 39	139 ± 84
Chlorpromazine	152 ± 4	139 ± 19	100 ± 33	159 ± 10	143 ± 19	147 ± 32
Codeine	148 ± 23	85 ± 36	79 ± 50	232 ± 69	84 ± 55	161 ± 83
3,4-Benzpyrene*	24 ± 7	35 ± 4	9 ± 13	48 ± 19	30 ± 56	51 ± 19
Aminopyrine	73 ± 36	221 ± 24	—	122 ± 51	78 ± 24	15 ± 16

* All incubations were for one hour except benzpyrene which was for 30 min. Values for benzpyrene metabolism are nevertheless expressed as μmoles/g N/hour.

determined by the method of Cooper and Brodie(5), deamination of 1-amphetamine determined by the method of Axelrod(6), reduction of aromatic nitro group of p-nitrobenzoic acid determined by the method of Fouts and Brodie(7), ring sulfur oxidation of chlorpromazine determined by the method of Salzman and Brodie(8), o-dealkylation of codeine determined by the method of Snell and Snell(9), N-dealkylation of aminopyrine determined by the method of Brodie and Axelrod(10), aromatic ring p-hydroxylation of aniline determined by the method of Gillette as described by Dixon *et al*(11), ring oxidation of zoxazolamine and polycyclic aromatic ring hydroxylation of 3,4-benzpyrene determined by methods described by Juchau *et al*(12). The results were expressed as μmoles substrate metabolized per g 9,000 *g* supernatant nitrogen per hour. Conditions of incubation, cofactors, and concentrations thereof were the same as those reported by McLuen and Fouts(13) except that incubation time was cut to one hour for all metabolisms except benzpyrene where it was 30 minutes. Nitrogen determinations were made on the supernatant fractions by a modified Kjeldahl method described by Juchau *et al* (12). Comparisons of hepatic microsomal activity of phenobarbital treated rabbits to the saline injected controls were made by Student's "t" test.

Results. Wide variations in basal hepatic microsomal drug-metabolizing activity of the supernatant fraction were observed among the various rabbit strains as indicated in Table I. California, Cottontail, and Jack rabbits exhibited the most striking differences when compared with other strains. The microsomal supernatant fraction from livers of California rabbits showed a relatively marked inability to metabolize 1-amphetamine and p-nitrobenzoic acid but metabolized more aminopyrine, zoxazolamine, and 3,4-benzpyrene when compared with other strains of rabbits. Nine-thousand *g* supernatants from livers of Cottontail rabbits were deficient in hexobarbital, amphetamine, and aminopyrine metabolizing activity but were comparatively more active in the hydroxylation of 3,4-benzpyrene. Zoxazolamine, chlorpromazine and 3,4-benzpyrene metabolisms were comparatively low in Jack rabbit liver supernatants. Similar levels of drug metabolizing activity were seen in liver supernatants from Dutch, New Zealand, and English rabbits. However, in comparing these 3 strains, Dutch rabbit liver supernatants appeared to metabolize less aniline and more codeine, English rabbit livers exhibited somewhat higher activity in the metabolism of aminopyrine, and New Zealand rabbit livers appeared to metabolize more hexobarbital and p-nitrobenzoic acid.

Response of these enzyme systems to previous *in vivo* injections of phenobarbital also varied among strains (Tables II and III). Again, Jacks, Cottontails, and California rabbits were quite different from other strains. Jack rabbit liver supernatants displayed large

TABLE II. Effects of Phenobarbital Pretreatment on Levels of Drug Metabolism by Hepatic Enzyme Systems of Various Rabbit Strains. Rabbits were injected twice daily for 3 days with 15 mg/kg phenobarbital, untreated on 4th day and sacrificed on 5th day. Results expressed as μmoles substrate metabolized per g 9000 *g* of supernatant nitrogen per hour with standard deviations. Numbers in parentheses represent number of animals employed in each determination.

	Strain					
Substrate	Dutch (5)	English (6)	Jacks (3)	California (6)	New Zealand (6)	Cottontails (5)
Hexobarbital	329 ± 24	339 ± 73	540 ± 50	490 ± 54	520 ± 58	490 ± 34
Aniline	56 ± 12	156 ± 29	247 ± 35	151 ± 28	181 ± 19	128 ± 10
l-amphetamine	154 ± 33	104 ± 14	104 ± 36	48 ± 26	90 ± 22	14 ± 4
p-nitrobenzoic acid	249 ± 48	148 ± 26	689 ± 44	15 ± 8	314 ± 47	170 ± 32
Zoxazolamine	253 ± 25	219 ± 39	274 ± 76	321 ± 126	308 ± 52	312 ± 50
Chlorpromazine	141 ± 7	140 ± 22	137 ± 21	161 ± 26	158 ± 11	170 ± 13
Codeine	202 ± 30	84 ± 32	266 ± 80	315 ± 113	117 ± 69	269 ± 83
3,4-benzpyrene*	27 ± 7	29 ± 10	50 ± 4	44 ± 7	39 ± 10	98 ± 13
Aminopyrine	160 ± 31	235 ± 67	—	308 ± 42	190 ± 60	66 ± 19

* See footnote Table I.

TABLE III. Determination of Effect of Phenobarbital (injected *in vivo*) on Levels of Drug Metabolism by Hepatic Enzyme Systems of Various Rabbit Strains. Results expressed as the ratio: μmoles substrate metabolized per g nitrogen per hour by 9000 *g* supernatant fractions of livers obtained from phenobarbital-treated animals/μmoles substrate metabolized per g nitrogen per hour by 9000 *g* supernatant fractions of livers obtained from animals injected with normal saline. Numbers in parentheses represent number of animals employed in each determination.

	Strain					
Substrate	Dutch (10)	English (12)	Jacks (6)	California (12)	New Zealand (12)	Cottontails (10)
Hexobarbital	2.33†	1.96†	2.45†	1.98†	2.05†	25.8†
Aniline	1.62*	1.22	2.04*	2.36†	1.89†	1.77†
Amphetamine	1.00	1.19	1.02	.87	1.10	1.76
p-nitrobenzoic acid	2.80†	2.21†	7.45*	.68	2.58†	3.41†
Zoxazolamine	1.63†	1.43†	3.88†	1.60	1.90†	2.24
Chlorpromazine	.93	1.01	1.37	1.01	1.10	1.16
Codeine	1.37†	.98	3.37†	1.36	1.39	1.67
3,4-benzpyrene	1.10	.83	5.37†	.92	1.29	1.91†
Aminopyrine	2.19*	1.06	—	2.52†	2.44†	4.46†

* Significantly different from controls ($P < .05$).
† " " " " ($P < .01$).

increases in activity with regard to metabolism of p-nitrobenzoic acid, zoxazolamine, benzpyrene and codeine. These increases appeared to be greater than in other strains. Large increases in hexobarbital, benzpyrene, and aminopyrine metabolism occurred in Cottontail liver supernatants. Unlike other strains, California rabbit liver supernatants showed no significant increase in metabolism of p-nitrobenzoic acid or zoxazolamine. English rabbit hepatic microsomal metabolism of aniline and aminopyrine was not significantly increased, in contrast to that of other strains, all of which showed significant increases. Hepatic microsomal metabolism of l-amphetamine and chlorpromazine was not significantly affected in any strain and benzpyrene hydroxylation was stimulated only in liver from Jack rabbits and Cottontails. Dutch, English, and New Zealand strains were quite similar with regard to the levels finally reached of those pathways which were stimulated by phenobarbital.

Discussion. These experiments show that a marked variation in the basal rate of hepatic metabolism of several drugs and chemicals occurred among 6 strains of rabbits. In some cases, this variation in metabolic rate for a single drug was 10-fold or greater (*e.g.* —amphetamine, hexobarbital, and aminopyrine). In marked contrast, the rate of metabolism of chlorpromazine was almost identical in all rabbits studied. One is tempted to predict that variability among rabbits in drug response might be least with a drug like chlorpromazine and most for drugs like am-

phetamine, hexobarbital, and aminopyrine. This could be true only if metabolism were the major factor in determining drug response and we know this is not the case with some drugs.

Hepatic microsomal drug metabolism was stimulated by phenobarbital to different levels in the rabbits studied. Both qualitative and quantitative differences between strains seemed to be present. The wild rabbits (Cottontail and Jack) seemed most responsive of all strains studied in that more metabolic pathways were significantly increased and the ratios of phenobarbital-treated to untreated rabbits were greater than in the other rabbits. Of the drug metabolic pathways investigated, some were not stimulated by phenobarbital in any strain (1-amphetamine deamination and chlorpromazine sulfoxidation), some were stimulated in only one or two strains (codeine dealkylation and benzpyrene hydroxylation), while others were increased in all or nearly all rabbits studied (the side chain oxidation of hexobarbital, the N-dealkylation of aminopyrine, the hydroxylation of zoxazolamine, the hydroxylation of aniline, and the reduction of the nitro group of p-nitrobenzoic acid). It would be interesting to know if such strain variations in response of the hepatic microsomal drug metabolizing enzymes to inducers like phenobarbital is seen in other species.

Thus, both heredity and environment seem to influence not only the basal level of hepatic microsomal drug metabolism, but also the response of these enzymes to enzyme inducers like phenobarbital. The use of mongrel animals (dogs, cats) or undesignated strains (albino rats, mice, or rabbits) may make comparisons of data about drug disposition from one laboratory to another even more difficult than it now is.

Summary. The results of these experiments indicate that basal levels of activity of hepatic microsomal drug metabolizing enzyme systems from various rabbit strains can vary as much as 20-fold in the strains studied. This is particularly true of hexobarbital, amphetamine, and aminopyrine metabolism where the widest variations were observed. Relatively little strain variation was seen in the basal metabolism of chlorpromazine. The wild rabbits and California rabbits exhibited the greatest differences from other rabbit strains in basal hepatic drug metabolism.

A wide range of variability was also observed in the response of hepatic drug metabolizing enzyme systems to *in vivo* pretreatment with phenobarbital. Especially noteworthy were the 26-fold increase in hexobarbital metabolism seen with the hepatic enzyme systems of Cottontails, the 7.5-fold increase in metabolism of p-nitrobenzoic acid in Jack rabbits, and the significant increases in 3,4-benzpyrene metabolism observed only in the wild rabbits. Also interesting was the lack of phenobarbital stimulation of p-nitrobenzoic acid and zoxazolamine metabolizing systems in California rabbits. Jack rabbits, Cottontails, and California rabbits again exhibited the widest deviations from the general pattern, whereas Dutch, English, and New Zealand strains showed fair agreement among themselves in response to phenobarbital treatment.

The authors wish to acknowledge the valuable technical assistance of Mrs. Roberta Pohl, Mrs. Barbara Purdie, and Mrs. Diana Lewiston, Dept of Pharmacology, Univ. of Iowa.

1. Law, L. W., Morrow, A. G., Greenspan, E. M., J. Nat. Cancer Inst., 1952, v12, 909.

2. Jay, G. E., Jr., Proc. Soc. Exp. Biol. and Med., 1955, v90, 378.

3. Yaffe, S. J., Perinatal Pharmacol., 1962, Report of 41st Ross Conference on Pediatric Research, May, C. D., ed., Columbus, Ross Laboratories, 48.

4. Quinn, G. P., Axelrod, J., Brodie, B. B., Biochem. Pharmacol., 1958, v1, 159.

5. Cooper, J. R., Brodie, B. B., J. Pharmacol. and Exp. Therap., 1955, v114, 409.

6. Axelrod, J., ibid., 1954, v110, 315.

7. Fouts, J. R., Brodie, B. B., ibid., 1957, v119, 197.

8. Salzman, N. P., Brodie, B. B., ibid., 1956, v118, 46.

9. Snell, F. D., Snell, C. T., Colorimetric Methods of Analysis, D. Van Nostrand, New York, 1937, 510.

10. Brodie, B. B., Axelrod, J., J. Pharmacol. Exp. Therap., 1950, v99, 171.

11. Dixon, R. L., Hart, L. G., Rogers, L. A., Fouts, J. R., ibid., 1963, v132, 12.

12. Juchau, M. R., Cram, R. L., Plaa, G. L., Fouts, J. R., Biochem. Pharmacol., 1965, in press.

13. McLuen, E. F., Fouts, J. R., J. Pharmacol. Exp. Therap., 1961, v131, 7.

Received December 17, 1964. P.S.E.B.M., 1965, v118.