Aberrant Animals Showing Disparate Liver and Kidney β-Glucuronidase Activities in a Genetic Study.* (30482)

RICHARD H. DORSAY, MICHAEL J. ROBINSON AND WILLIAM H. FISHMAN
(Introduced by W. P. Anslow, Jr.)
Department of Pathology (Oncology), Tufts University School of Medicine and Cancer Research Laboratory, New England Center Hospital, Boston, Mass.

The purpose of the present study has been to investigate the inheritance of liver and kidney β-glucuronidase in high (A/Jax) and low (C3H/HeJ) tissue β-glucuronidase mice in order to increase our understanding of the biologic basis of the renal β-glucuronidase response to androgenic hormone(1,2,3). The study has also included assays of acid phosphatase activities of both tissues.

The inheritance of liver β-glucuronidase in the F1 and F2 generations corresponds well with the data of Law(4) which established GG as the dominant genes in mice exhibiting the "high" liver β-glucuronidase trait and, gg, as the recessive alleles for the strain showing "low" liver β-glucuronidase. However, the backcross generation of F1 hybrid mice to the recessive C3H/HeJ parents yields a number of offspring expected to show low levels of β-glucuronidase in both liver and kidney but which instead had a high activity in liver and a low value in kidney. An account is given of these findings, the experiments which preceded them, and an effort is made to explain them.

Materials and methods. One male and 2 female A/Jax mice (pedigree number 26191-31421) of the 113th filial generation and one male and 2 female C3H/HeJ (pedigree number 29386-1321) of the 90th filial generation† were inbred until sufficient offspring accumulated to provide the Parent (P1) generation for the proposed crosses.

The animals were kept in metal cages in a temperature controlled room at 20°C and the cages were cleaned and changed weekly. Laboratory chow and water were fed *ad libitum*. Most of the matings were made with 2 females and 1 male in each cage. The resulting pregnant female was isolated in a separate cage until her young were weaned 4 weeks after birth. At that time, the mother was mated again. Some of the young were taken for the designated matings (Fig. 1) while others were separated according to sex and maintained under normal laboratory conditions. At 5 ± 0.5 months of age, these animals were sacrificed by cervical fracture. A kidney was removed, decapsulated, weighed and homogenized in acetate buffer and made up to 10 ml. A small specimen of liver was similarly prepared. The homogenates were kept cold at all times before assaying for β-glucuronidase(5) and acid phosphatase(6). These assays were performed on each organ and the β-glucuronidase expressed as Fishman units and the acid phosphatase as micrograms phenol, both per gram of wet tissue. "High" β-glucuronidase values were defined as 700 units/gram and over and "low" β-glucuronidase values as 699 and less. The 700 units/gram dividing point was chosen after inspection of the data in Fig. 2 and 3 since it separated the bimodal distributions in kidney as well as liver β-glucuronidase.

Results and discussion. The first impression of the results of liver β-glucuronidase (Table I, Fig. 2) is that they fit the Mendelian inheritance of a single pair of genes as found by Law. Thus, the first filial generation all show high liver β-glucuronidase and the second filial generation exhibits the anticipated ratio of 3 high to 1 low. However, in the mice which represent the backcross of the F1 generation to the C3H/HeJ, the ratio is 1.6 high to 1 low, not 1 to 1. The 1 to 1 ratio was expected because of the genotype, (Gg,gg) resulting from this mating.

The righ ratio in this particular backcross

* Supported in part by Grants P-106, P-107 from Am. Cancer Soc., Inc., New York; Research Grants CS-9733 and CA 07538-01 and Career Award CA-K6-18,453, from Nat. Cancer Inst., N.I.H., U.S.P.H.S., Bethesda, Md.

† Obtained from Jackson Laboratory, Bar Harbor, Maine on Nov. 15, 1961.

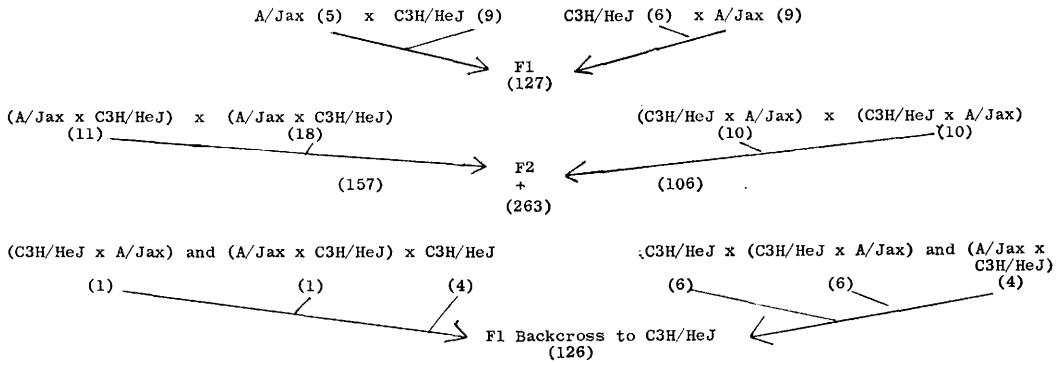


FIG. 1. Summary of matings conducted in present experiments. Number of animals in parenthesis. In any given cross, the male is identified first, thus A/Jax × C3H/HeJ represents A/Jax♂ × C3H/HeJ ♀.

generation is not an isolated finding. The ratio of 2.5 to 1.0 for liver β-glucuronidase was reported by Paigen(7) for the progeny derived from backcrossing the F1 generation from DBA (high) and C3H/HeJ (low) to the recessive C3H/HeJ parents.

The inheritance of renal β-glucuronidase is not entirely as expected from the genotypes. Thus, all 66 F1 male offspring possess unmistakably high renal β-glucuronidase activity whereas all 60 female animals are in the lowest percentile of the high category, being concentrated in a narrow range, *intermediate* between the parental C3H/HeJ and A/Jax distributions (Fig. 3). Moreover, the females exhibit a compact modal distribution, in contrast to the broader range of values found in the male mice.

In the bimodal distributions observed in the males of F2 generation, the "low" enzyme values are identical to the corresponding parental ones, while the "high" figures fall short of matching those of the A/Jax parents. By contrast, the high females of the second filial generation have levels comparable to those of the first generation. In both generations, female renal β-glucuronidase activity registers in the middle of extremes found in C3H and A/Jax kidneys (Footnote to Table I).

The backcross of F1 mice to the recessive produces a bimodal distribution of renal β-glucuronidase with a ratio of 1.3 high to 1 low. In this generation the β-glucuronidase level of the *males* (mean 1110) is lower than that of the high males in the F1 (1450),

TABLE I. Summary of Matings and Distribution of "High" and "Low" β-Glucuronidase Traits in the Progeny.

			Expected phenotype		Observed phenotype			
			Relative No. of animals		No. of animals			
					Liver		Kidney	
Cross	Matings	Genotype	High	Low	High	Low	High	Low
F1	♂ GG x gg	Gg	All	0	67	0	66	0
	♀ GG x gg	Gg	All	0	60	0	60*	0
	♂ + ♀ GG x gg	Gg	All	0	127	0	126*	0
F2	♂ Gg x Gg	GG,Gg,Gg,gg	3	1	105	29	102	31
	♀ Gg x Gg	GG,Gg,Gg,gg	3	1	91	35	89	37
	♂ + ♀ Gg x Gg	GG,Gg,Gg,gg	3	1	196	64	191	68
F1 backcross to C3H/HeJ	♂ Gg x gg	Gg,gg	1	1	38	22	33	27
	♀ Gg x gg	Gg,gg	1	1	41	26	39	28
	♂ + ♀ Gg x gg	Gg,gg	1	1	79	48	72	55

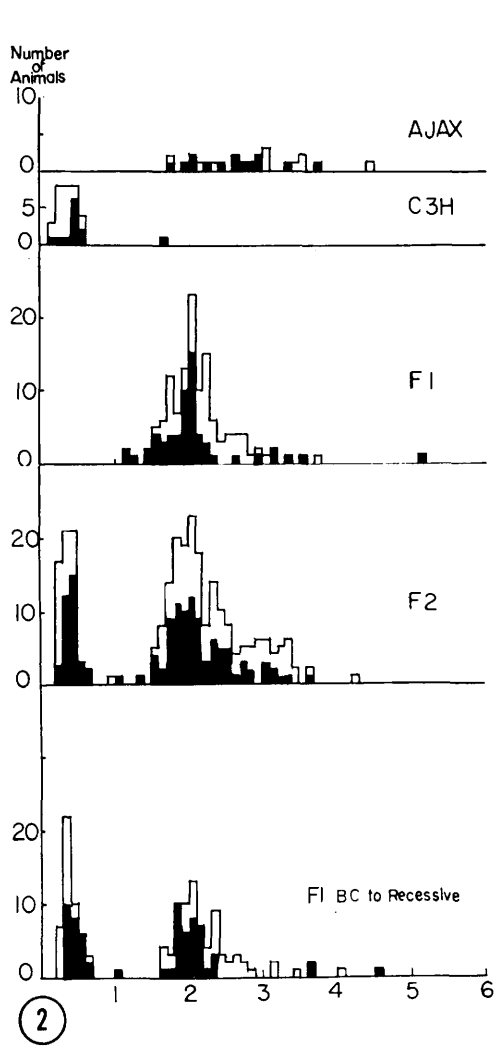
* The mean β-glucuronidase of the 60 female mice was 694 ± 92 F.U./g which was close to the average (864) between the parent A/Jax value (1530 ± 530) and the parent C3H value (149 ± 67).

in the F2 (1735) and in the parental (3045). On the other hand, the corresponding backcross *female* values show no similar diminution as seen from Fig. 3.

The inheritance of liver and kidney acid phosphatase (Fig. 4 and 5) resembled each other and differed altogether from β-glucuronidase.

Aberrant animals with regard to liver and kidney β-glucuronidase. The existence of animals whose liver β-glucuronidase is a trait of the "high" strain while their kidney β-glucuronidase is a characteristic of the "low" strain is observed (Table II) in the F2 (2.7%) and in the F1 backcross to recessive (7.1%) and not at all in the F1 generation. Hitherto, liver and kidney β-glucuronidase levels have either been both "low" or both "high"(8).

Among the possible causes of this phenomenon are a true separation of 2 loci controlling liver and kidney enzyme levels separately,‡ segregation of multiple alleles controlling hormonal levels and variations in the hormonal



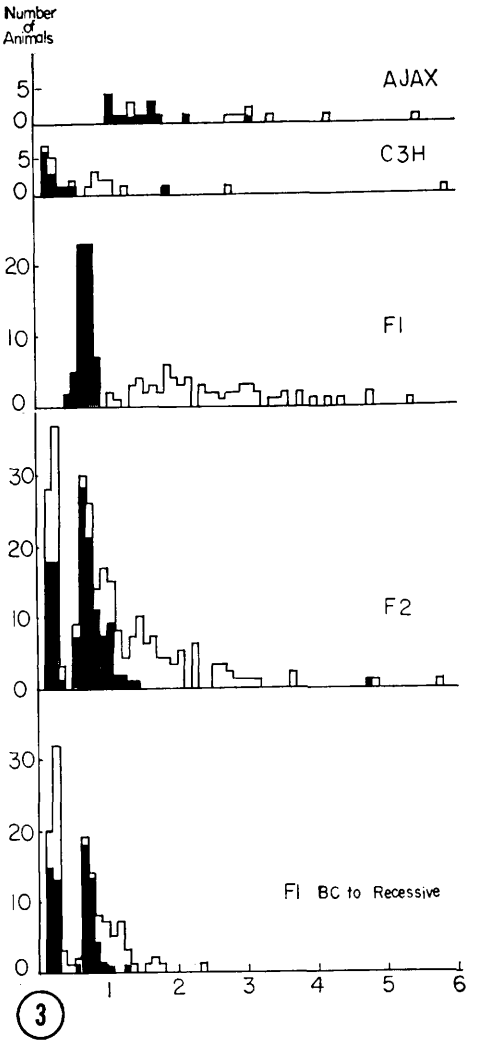
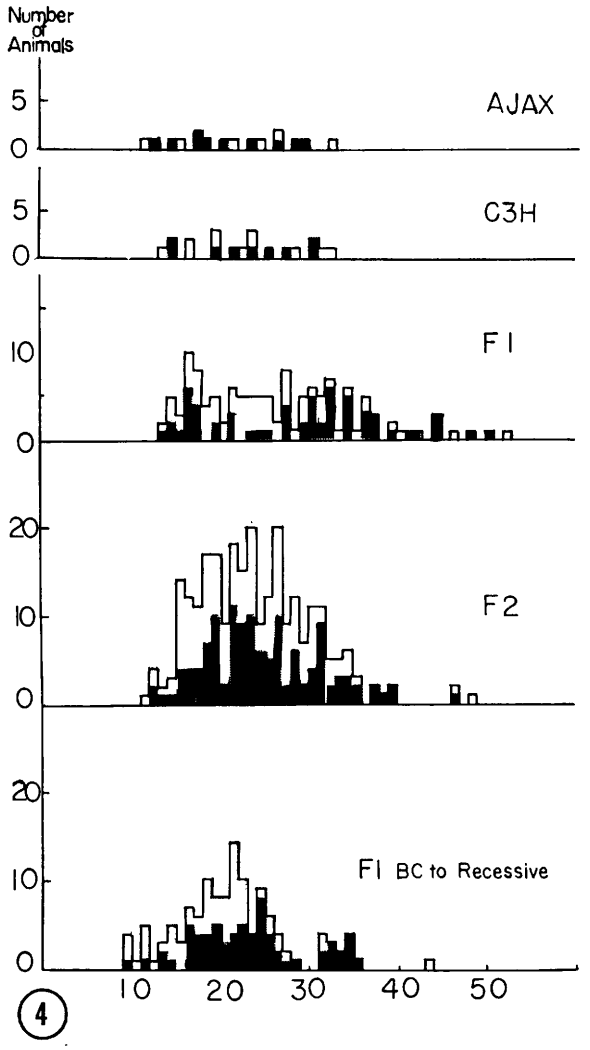


FIG. 2. Distribution of liver β-glucuronidase values in parents and their offspring. Abscissa is numbered in thousands of Fishman units per gram of fresh liver. Dark areas represent values in female mice, white areas, those of male mice.

FIG. 3. Distribution of kidney β-glucuronidase values in parents and their offspring. See legend to Fig. 2 for detail.



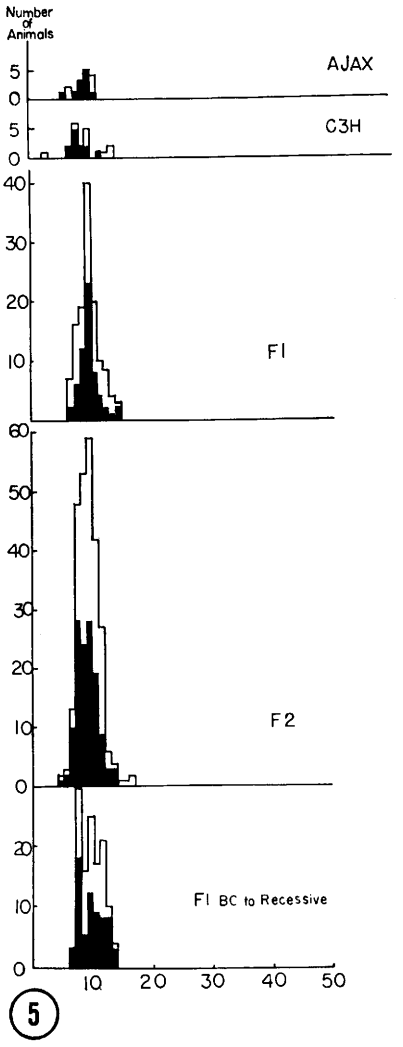


FIG. 4. Distribution of liver acid phosphatase values in parents and their offspring. Abscissa is numbered in thousands of acid phosphatase units (11) per gram of fresh tissue.

FIG. 5. Distribution of kidney acid phosphatase values in parents and their offspring. See legend to Fig. 4 for detail.

state of the animals due to environmental factors.

An examination of the breeding records and organ enzyme values has failed to support the first possible cause since disparate values appeared irregularly in offspring of parents in which liver and kidney were either both high or both low. Thus, an F1 male (both high) mated with C3H (both low) produced 4 offspring in the first litter in which the values were not disparate and in the second litter of 5, two in which kidney was "low" and liver "high." Altogether in this F1 backcross generation, only 2 out of 76 offspring of the first litter showed disparate values in contrast to 11 out of 51 in the second litter. From these facts, one can eliminate genetic mutation as a possible explanation and one

‡ Spontaneous mutations affecting C3H liver β-glucuronidase have been reported by Hamer(11) who observed that individual colonies of British C3H mice underwent a sudden transformation from low to high liver values. Observations were not made on the β-glucuronidase of the kidneys of mutant animals. Also, Paigen describes a C3H/st subline which is homozygous, GG.

TABLE II. List of Disparate Liver and Kidney β-Glucuronidase Values in F2 and Backcross of F1 to C3H/HeJ Generations.

Animal No.	Sex	β-Glucuronidase activity (units/g) Liver	Kidney
Second filial generation—F2			
355	♂	2010	226
358	♂	1760	147
499	♂	1775	129
511	♂	1125	227
293	♀	3260	187
Backcross of F1 to C3H/HeJ			
638	♂	1940	161
641	♀	3680	204
643	♂	2820	183
648	♀	3700	160
645	♀	2720	165
649	♀	4550	171
650	♂	2700	180
651	♂	3140	401
654	♂	3140	287
655	♂	2380	311
656	♂	2720	203

can suspect environmental factors.

In favor of segregation of multiple alleles is the fact that in Fig. 3 a definite reduction in the mean of "high" β-glucuronidase values occurred when the GG gene pair is transferred into a C3H background.

In retrospect, the environmental factor which is most suspect is that of "crowding" and of disturbing the society of some animals by introducing others in their midst. The crowding was unavoidable due to cage space limitations which became more acute as the numbers of animals were accumulating in the F2 and F1 backcross to recessive generations. Several days before autopsy the desired animals were labelled, taken from their own cages and collected into one pen in order to attempt to meet the cage shortage.

Marked changes in the hormonal status of mice have been induced in mice by crowding(9,10). The more aggressive mice produce adrenal exhaustion in their opponents as a consequence of fighting. Other effects noted include a suppression of seminal vesicles which is an indication of androgen deficit. Such an androgen deficiency might explain the low kidney values in the majority of the animals with disparate values. The liver would be unaffected as its activity is uninfluenced by androgen.

The extent of the renal β-glucuronidase response to androgenic hormones is known to be strain-determined(1). Also, offspring resulting from one of the matings performed as described here (backcross of the F2 to C3H) responded to testosterone(8) to a greater extent than was expected as compared to the parental C3H values. What was not known then were the resting levels of kidney β-glucuronidase in the offspring of the various generations. This is the baseline information which was particularly sought in the present experiments and has now been gathered.

It was not known also to what extent the response was related in general to acid hydrolases of lysosomes, the marker for which is now accepted to be acid phosphatase. The present data clearly show that the inheritance of kidney β-glucuronidase is independent from the renal acid phosphatase and presumably is unrelated to the economy of lysosomal hydrolases as presently understood.

Summary. In the course of a study of the inheritance of liver and kidney β-glucuronidase and acid phosphatase activities, animals aberrant with regard to β-glucuronidase were observed. Thus, 2.7% of the animals in the F2 and 7.1% of those in the F1-backcross to recessive generations exhibited liver β-glucuronidase levels characteristic of the "high" β-glucuronidase parental strain A/Jax while their kidney values were in the "low" category, typical of the C3H/Heston parents. On the other hand, the inheritance of acid phosphatase proceeded independently of that for β-glucuronidase. It is suggested that these aberrant animals may reflect the segregation of multiple alleles governing hormonal levels and probably also the alteration in hormonal status due to crowding.

Our sincere thanks go to Sidney Green for his help and his patient cooperation. The authors are indebted to Dr. Earle Green, the Jackson Laboratories, and Dr. F. H. Bronson, Hamilton Biological Station, for their aid in arriving at the present interpretation of the data. To Dr. W. P. Anslow, Jr. we owe sincere thanks for his critique of the manusript before its submission for publication.

1. Fishman, W. H., Farmelant, M., Endocrinology, 1953, v52, 536.

2. Fishman, W. H., Artenstein, M., Green, E., En-

docrinology, 1955, v57, 646.

3. Fishman, W. H., in Mechanism of Action of Steroid Hormones, C. A. Villee, L. L. Engel, eds., Oxford, Pergamon Press, 1961, 157.

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

5. Fishman, W. H., in Methods of Hormone Research, Chap. 10, Academic Press, Inc., New York, 1964.

6. Stolbach, L. L., Nisselbaum, J. S., Fishman, W. H., Am. J. Clin. Path., 1958, v29, 37.

7. Paigen, K., Exp. Cell Research, 1961, v25, 286.

8. Fishman, W. H., in Advances in Enzymology, Interscience Pub., New York, 1955, v16, p361.

9. Bronson, F. H., Eleftherion, B. E., Physiol. Zoology, 1963, v36, 161.

10. ———, Science, 1965, v147, 627.

11. Hamer, D., Brit. J. Cancer, 1958, v12, 661.

Received June 6, 1965. P.S.E.B.M., 1965, v120.

Effects of Oophorectomy and Various Doses of Estradiol-17β on Corticosterone Production by Rat Adrenal Slices.* (30483)

JULIAN I. KITAY

Departments of Medicine and Physiology, University of Virginia School of Medicine, Charlottesville

Production of corticosterone by rat adrenal slices *in vitro* was decreased by prepubertal oophorectomy and increased after replacement with a single injection of a depot preparation of estradiol-17β(1). These observations were made at only one time interval after replacement therapy (2 weeks) and only one large dose of estradiol (2 mg/100 g body weight) was used. The present study was undertaken to obtain additional data concerning adrenal production of corticosterone in female rats and to evaluate the effects of oophorectomy and estrogen administration in greater detail.

Materials and methods. Female rats of the Sherman strain obtained from Camm Research Institute, Wayne, N. J., were maintained under standardized conditions of light (14 hr/24 hr) and temperature (75 ± 1°F) on a diet consisting of Purina Laboratory Chow and water *ad lib.* Unless otherwise specified, oophorectomy was performed at 30 days of age. Six weeks later, estrogenic hormone replacement was administered as a single, subcutaneous injection of polyestradiol phosphate (Estradurin, Ayerst). Control rats were uninjected, because preliminary experiments revealed no effects attributable to a single injection of the vehicle, distilled water, alone. Animals were rapidly decapitated, and adrenal slices were prepared and incubated according to a technique previously described(2). ACTH was added *in vitro* in doses (mU per 100 mg adrenal weight) indicated for each experiment. Corticosterone was measured by a fluorometric technique(3).

TABLE I. Corticosterone Production by Adrenal Slices from Intact, Untreated Female Rats of Selected Body Weights (μg/100 mg Adrenal Wt/2 Hr).

Body wt (g)	Adrenal wt (mg)	Corticosterone produced	
		5 mU ACTH	200 mU ACTH
116 ± 1	30.6 ± 1.1	9.6 ± .3	26.4 ± 1.7
133 ± 1	33.9 ± 1.6	9.2 ±1.1	.24.8 ± 3.7
174 ± 4	53.8 ± 2.6	6.3 ± .4	15.8 ± 1.2*
203 ± 2	64.6 ± 5.9	8.1 ± .2	21.4 ± .2
230 ± 2	67.2 ± 4.7	11.6 ± .7	24.1 ± 2.1

Entries represent means ± S.E. of 4 observations.
* Value differs from all others in column at $P < .01$.

Results. Untreated female rats were divided into groups with homogeneous body weights (Table I). Adrenal glands from animals with a mean body weight of 174 g (168-181 g) produced significantly less corticosterone *in vitro* than did adrenal tissue from rats of any other body weight tested. This difference was most clearly manifested when 200 mU of ACTH per 100 mg adrenal weight was added to the incubation medium. Significant differences with the 5 mU dose of ACTH were obtained only upon comparison with steroid yields from rats of the lowest

* Supported in part by U.S.P.H.S. Research Grant AM-03370 and Research Career Development Award AM-K3-13960.