Biochemical Genetics, Vol. 22, Nos. 7/8, 1984

Enzyme Markers in Inbred Rat Strains: Genetics of New Markers and Strain Profiles

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Received 2 Nov. 1983—Final 29 Feb. 1984

*Twenty-six inbred strains of the laboratory rat (*Rattus norvegicus*) were examined for electrophoretic variation at an estimated 97 genetic loci. In addition to previously documented markers, variation was observed for the enzymes aconitase, aldehyde dehydrogenase, and alkaline phosphatase. The genetic basis of these markers (*Acon-1, Ahd-2, *and* Akp-1*) was confirmed. Linkage analysis between 35 pairwise comparisons revealed that the markers* Fh-1 *and* Pep-3 *are linked. The strain profiles of the 25 inbred strains at 11 electrophoretic markers are given.*

KEY WORDS: enzyme markers; inbred strains; rat genetics; linkage; strain profiles.

INTRODUCTION

Electrophoretic markers have proved valuable in the laboratory mouse for genetic quality control, for the construction of gene maps, and as genetic markers (Festing, 1979a). The laboratory rat, however, suffers by comparison in the availability of electrophoretic markers (Womack and Cramer, 1980). The rat is in many respects a more suitable research animal than the mouse, and its full exploitation awaits a more comprehensive genetic characterization.

The literature relating to biochemical markers in *Rattus norvegicus* is limited and occasionally confusing. Festing (1979a) lists 10 electrophoretic

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0006-2928/84/0800-0611$03.50/0

markers, 2 of which (*Gl-1* and *Trf*) have since proved to be the same marker (Bender *et al.*, 1981). Since that compilation additional loci have been found to vary among inbred strains—*Cs* (Yamada *et al.*, 1981), *Pep-3* (Womack and Cramer, 1980), *Mdl-1* (Matsumoto *et al.*, 1982), *Es-6* (Bender *et al.*, 1982), *Es-7* (Lindena, 1981), *Lap-1, Mup-1, Svp-2* (Van Zutphen *et al.*, 1981), *Amy-1* (Mizuno and Susuki, 1978), *Upg-1* (Cramer, 1981), and *Glo-1* (Stolc *et al.*, 1980). Genetic variation has also been demonstrated for the proteins fumarase (Carleer and Ansay, 1976), a milk protein "L" (Brdicka, 1977), β-galactosidases (Douglas *et al.*, 1982), liver neuraminidase, and α-mannosidase (VandeBerg *et al.*, 1981). Additionally, a number of enzymes have been reported to be polymorphic in outbred (both wild and laboratory) populations but have not been used to characterize inbred strains, e.g., glucose-6-phosphate dehydrogenase and plasma alkaline phosphatase (Jiminez-Marin and Dessauer, 1973), phosphoglucomutase (Koga *et al.*, 1972), and aldehyde dehydrogenase (Truesdale-Mahoney *et al.*, 1981). Eriksson *et al.* (1976) also reported variation within "inred" strains of rats for glyceraldehyde-3-phosphate dehydrogenase, α-glycerophosphate dehydrogenase, and xanthine dehydrogenase (although see Discussion).

The present study was undertaken with a number of aims in mind:

(1) to screen inbred strains in an attempt to find additional markers and to establish the genetic bases of any such markers found;
(2) to test for linkage among a range of electrophoretic, morphological, and immunological markers;
(3) to determine the distribution within inbred strains of loci shown to be polymorphic within outbred *R. norvegicus;* and
(4) to determine the electrophoretic profiles of an extensive range of inbred strains of the laboratory rat.

MATERIALS AND METHODS

A total of 43 substrains of 26 inbred strains of the laboratory rat obtained from 12 institutions worldwide was used in the initial electrophoretic survey (Table I). Animals from Freiburg were kindly supplied by Dr. E. Günther from the Max Planck Institute for Immunology. Each substrain was represented by at least three individuals.

Mature animals were killed using halothane, and samples of liver, kidney, heart, skeletal muscle, brain, plasma, erythrocytes, and, where appropriate, testis were taken and frozen immediately at −70°C. Tissues and erythrocytes were homogenized by grinding in an equal volume of "lysing" solution (distilled water containing 1 μl of mercapetoethanol and 0.1 mg NADP per ml) at 4°C. Homogenates were then centrifuged at 10,000*g* for 15 min at 4°C and the supernatant was stored in separate aliquots of 5 μl at −20°C until use. Plasma samples were untreated.

Table I. Substrain Allelic Profiles at 11 Genetic Markers in the Laboratory Rat

Strain	Origin	Es-1	Es-10	Es-4	Gl-1	Fh-1	Acon-1	Akp-1	Pep-3	Ahd-2	Pgd	Igk-1
ACI	Univ. Melbourne	b	a	b	a	b	b	b	a		a	
AAW	Atomic Energy Comm., Melbourne	b	a	b	a	b	b	b	b	c	a	
AS	Freiburg	b	b	b	a	b	b	a	b	c	a	
	Univ. Otago	b	b	b	a	b	b	a	b	c	a	a
AS2	Freiburg	b	b	b	a	b	b	a	b	c	a	a
	Univ. Otago	b	b	b	a	b	b	a	b	c	a	
AUG	Univ. Otago	b	a	b	a	b	b	a	a	c	a	
	Monash Univ., Melbourne	b	a	b	a	b	b	a	a		a	
AVN	Freiburg	a	a	b	a	b	b	a	a	c	a	a
BD $\overline{V}$	Freiburg	b	a	b	a	b	b	b	a	c	a	b
BN	Freiburg	a	b	b	a	a	a	a	a	b	a	a
BS	Freiburg	b	a	b	a	b	b	a	a	c	a	a
	Univ. Otago	b	a	b	a	b	b	a	a	c	a	
BUF	Aust. Natl. Univ., Canberra	b	a	b	b	b	b	a	a		a	
	Univ. Melbourne	b	a	b	b	b	b	a	a		a	
DA	Aust. Natl. Univ., Canberra	b	a	b	a	b	b	b	b	b	a	
	Monash Univ., Melbourne	b	a	b	a	b	b	b	b	b	a	
	Walter Eliza Hall Inst., Melbourne	b	a	b	a	b	b	b	b	b	a	
	Inst. Med. Vet. Sci., Adelaide	b	a	b	a	b	b	b	b	b	a	b
	Freiburg	b	a	b	a	b	b	b	b	b	a	
	Univ. Otago	b	a	b	a	b	b	b	b	b	a	b
	Irvine	b	a	b	a	b	b	b	b	b	a	
F344	Aust. Natl. Univ., Canberra	a	a	b	a	b	b	a	b		a	
	Flinders Univ., Adelaide	a	a	b	a	b	b	a	b		a	
	Univ. Otago	a	a	b	a	b	b	a	b	c	a	
HS	Univ. Otago	b	a	b	a	b	b	a	a	c	a	
LEW	Walter Eliza Hall Inst., Melbourne	a	b	b	a	a	b	a	a	c	a	a
	Freiburg	a	b	b	a	a	b	a	a	c	a	
	Univ. Otago	a	b	b	a	a	b	a	a	c	a	
LEW.BN	Freiburg	a	b	b	a	a	b	a	a	c	a	a
JC	Aust. Natl. Univ., Canberra	a	b	b	a	a	b	a	a	c	a	
	Inst. Med. Vet. Sci., Adelaide	a	b	b	a	a	b	a	a	c	a	a
LOU/C	Aust. Natl. Univ., Canberra	a	a	b	a	b	b	a	b		a	
LOU/M	Aust. Natl. Univ., Canberra	a	a	b	a	b	b	a	b	b	a	
PVG	Aust. Natl. Univ., Canberra		a	b	a	a	b	a	a		a	
	Univ. Otago		a	b	a	a	b	a	a	c	a	
RHA/N	Inst. Med. Vet. Sci., Adelaide	b	b	b	a	b	b	b	a	c	a	
SHR	Flinders Univ., Adelaide	a	a	a	a	b	b	b	a	b	a	b
Wistar albino	Walter Eliza Hall Inst., Melbourne	b	a	b	a	b	b	b	a		a	
WAB	Monash Univ., Melbourne	b	a	b	a	b	b	a	a	c	a	
WAG	Univ. West. Aust.	b	a	b	a	b	b	a	a	c	a	
WF	Univ. West. Aust.	a	b	b	a	b	a	a	a	a	a	a
WKY/N	Flinders Univ., Adelaide	a	b	b	a	b	b	b	b	b	a	

Electrophoresis was conducted on Cellogel (Chemetron, Milan) according to techniques described elsewhere (Baverstock *et al.*, 1980). A total of 56 enzymes and other proteins was successfully stained for, yielding on a conservative estimate a minimum of 97 genetic loci screened. The proteins surveyed, the tissues examined, and an estimate of the minimum number of loci observable on cellogel are given in Table II. Detailed staining procedures

Table II. Details of Proteins Surveyed, Tissues Screened, and Estimated Number of Loci

Abbreviation	Common name	EC No.	Tissues screened	Minimum number of loci screened
ACON	Aconitase	4.2.1.3	K, L, H	2
ACP	Acid phosphatase	3.1.3.2	L, K, E	2
ADA	Adenosine deaminase	3.5.4.4	L	1
ADH	Alcohol dehydrogenase	1.1.1.1	L	2
AHD	Aldehyde dehydrogenase	1.2.1.5	L	2
AK	Adenylate kinase	2.7.4.3	K	2
ALB	Albumin	—	P	1
ALD	Aldolase	4.1.2.13	L, M, B	3
AKP	Alkaline phosphatase	3.1.3.1	L, K	3
AO	Aldehyde oxidase	1.2.3.1	L	1
ARG	Arginase	3.5.3.1	L	1
CA	Carbonic anhydrase	4.2.1.1	E, M	1
CK	Creatine kinase	2.7.3.2	M, B	1
DIA	Diaphorase	1.6.2.2	E, L, H, K	2
ENOL	Enolase	4.2.1.11	L, M, B	3
EST	Esterase	3.1.1.1	P, E, L, K, H, M, B, T	5
FDPASE	Fructosediphosphatase	3.1.3.11	L, K, M	2
FH	Fumarate hydratase	4.2.1.2	K	1
αGAL	α-Galactosidase	3.2.1.22	K	1
βGAL	β-Galactosidase	3.2.1.23	K	1
GA3PD	Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	L	1
GDH	Glutamate dehydrogenase	1.4.1.3	L	1
GLOI	Glyoxalase I	4.4.1.5	L	1
αGLU	α-Glucosidase	3.2.1.20	K, P	1
βGLU	β-Glucosidase	3.2.1.21	K	1
GOT	Glutamate-oxaloacetate transaminase	2.6.1.1	L	2
GP	General protein	—	L, K, H, M, P	4
G6PD	Glucose-6-phosphate dehydrogenase	1.1.1.49	L, K	1
αGPD	α-Glycerophosphate dehydrogenase	1.1.1.8	L, M	2
GPI	Glucose-phosphate isomerase	5.3.1.9	K	1
GPT	Glutamate-pyruvate transaminase	2.6.1.2	L	2
GSR	Glutathione reductase	1.6.4.2	L, E	1
GUK	Guanylate kinase	2.7.4.8	L, K, M	2
GUS	β-Glucuronidase	3.2.1.31	L	1
HB	Hemoglobin	—	E	2
HBDH	β-Hydroxybutyrate dehydrogenase	1.1.1.30	K	1
HEX	Hexosaminidase	?	K	1
HK	Hexokinase	2.7.1.1	L, H, M	2
IDH	Isocitrate dehydrogenase	1.1.1.42	L, H	2
LDH	Lactate dehydrogenase	1.1.1.27	K, T	3
MDH	Malate dehydrogenase	1.1.1.37	L	2

Table II. (*Continued*)

Abbreviation	Common name	EC No.	Tissues screened	Minimum number of loci screened
ME	Malic enzyme	1.1.1.40	L	1
MPI	Mannose-phosphate isomerase	5.3.1.8	L	1
NP	Nucleoside phosphorylase	2.4.2.1	L	1
PEP	Peptidases	3.4.11 or 13	L, K	7
PGAM	Phosphoglycerate mutase	2.7.5.3	L, H	1
6PGD	6-Phosphogluconate dehydrogenase	1.1.1.44	L	1
PGK	Phosphoglycerate kinase	2.7.2.3	K, T	2
PGM	Phosphoglucomutase	2.7.5.1	K	2
PK	Pyruvate kinase	2.7.1.40	L, M, H	3
SOD	Superoxide dismutase	1.15.1.1	L, K, H	2
SORDH	Sorbitol dehydrogenase	1.1.1.14	L	1
TPI	Triose-phosphate isomerase	5.3.1.1	K, E	1
TRF	Transferrin	—	P	1
UMPK	Uridine monophosphate kinase	2.7.4.?	L	1
XO	Xanthine oxidase	1.2.3.2	L	1

[a]L, liver; K, kidney; H, heart; M, skeletal muscle; B, brain; T, testis; E, erythrocytes; P, plasma.

for so-called "new" loci are given in Table III (details of other procedures are available from the authors on request).

The two allelic forms of immunoglobulin kappa chains, controlled by the *Igk-1* locus (Gutman, 1981), were determined by a plate-binding radioimmunoassay as previously described (Gutman, 1982). Conventional anti-Igk-1a and Igk-1b antibodies were radiolabeled with ^{125}I and used for binding to LEWIS or DA IgG bound to microtiter wells. Sera or plasma for testing was added as inhibitor to the radiolabeled antibodies before addition to the wells. The recently described standard nomenclature for the rat κ-chain locus is used throughout (Gutman *et al.*, 1983).

RESULTS

1. New Loci

As a result of the initial screen, two enzymes previously known to be polymorphic in outbred or feral Norway rats, alkaline phosphatase and aldehyde dehydrogenase, were found to vary between inbred strains, while a new polymorphic system involving aconitase was revealed.

Table III. Electrophoretic Details of "New" Markers

Enzyme	Electrophoresis buffer	Electrophoresis conditions	Stain recipe	
ACON	0.02 M phosphates, *p*H 7.0	200 V, 2 hr	0.1 M Tris–HCl, *p*H 8.0	2 ml
	11.6 mM Na_2HPO4		*cis*-Aconitate (50 mg/ml, *p*H 8.0)	0.2 ml
	8.4 mM NaH_2PO4		NADP (20 mg/ml)	0.1 ml
			$MnCl_2$ (0.2 M)	0.1 ml
			MTT (6 mg/ml)	0.1 ml
			PMS (2 mg/ml)	0.1 ml
			Isocitrate dehydrogenase	0.5 IU
AHD	0.025 M Tris–glycine, *p*H 8.5	200 V, 2.5 hr	0.1 M Tris–HCl, *p*H 8.0	2 ml
	25 mM Tris		Acetaldehyde	0.1 ml
	192 mM glycine		NAD (25 mg/ml)	0.1 ml
			KCl (1 M)	0.2 ml
			Na pyruvate (50 mg/ml)	0.1 ml
			Pyrazole (50 mg/ml)	0.1 ml
			MTT (6 mg/ml)	0.1 ml
			PMS (2 mg/ml)	0.1 ml
AKP	0.015 M TEB–$MgCl_2$, *p*H7.8	200 V, 2 hr	0.1 M Tris–HCl, *p*H 8.6	2 ml
	15 mM Tris		β-Naphthyl acid phosphate	10 mg
	5 mM Na_2EDTA		Fast blue BB salt	8 mg
	5.5 mM boric acid		$MnCl_2$ (0.2 M)	0.1 ml
	10 mM $MgCl_2$		Mix strain well before application	

(a) Genetics

Aconitase (ACON). Following electrophoresis of kidney or liver homogenates, two zones of aconitase activity were observed after appropriate histochemical staining (ACON-A and ACON-B). The more anodal form under the conditions described (ACON-B) exhibited electrophoretic variation between inbred strains. Two phenotypes were observed corresponding to the common "fast" (F)-migrating form and a rarer "slow" (S)-migrating form. Crosses between strains possessing the alternative forms yielded male and female offspring, all of which were double banded (FS) for this zone of aconitase activity (Fig. 1). Such a finding is consistent with the observation that aconitase is a monomeric enzyme (Harris and Hopkinson, 1976).

The genetic basis of electrophoretic variation in ACON-B was studied in crosses of WF and SHR. The results (Table IV) are consistent with a Mendelian pattern of inheritance for two codominant alleles at an autosomal locus. Considerations of relative mobility, comparative appearance of isozymes, and differential activity between kidney and liver on the one hand and

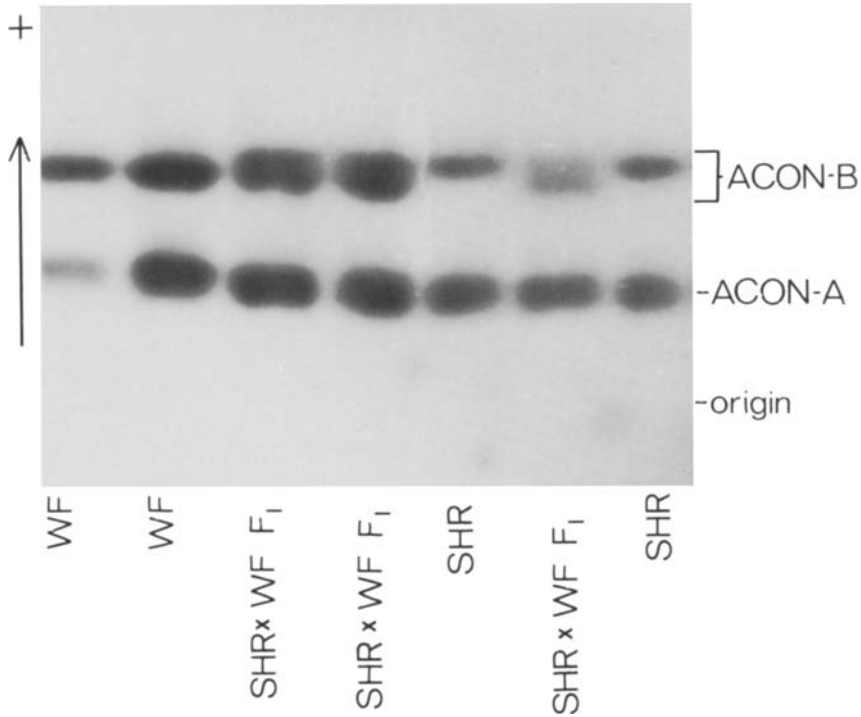

Fig. 1. Aconitase patterns of WF (ACON-B slow), SHR (ACON-B fast), and F_1 hybrids after cellulose acetate electrophoresis of kidney homogenates.

Table IV. Inheritance Pattern of ACON-B Phenotypes in WF (S) × SHR (F) Crosses

Cross	Phenotypic distribution of offspring			
	F	FS	S	
FS × S				
Obs	0	35	43	$\chi_1^2 = 0.82$
Exp	0	39	39	$P > 30\%$
FS × F				
Obs	40	41	0	$\chi_1^2 = 0.01$
Exp	40.5	40.5	0	$P > 90\%$
FS × FS				
Obs	13	21	10	$\chi_2^2 = 0.50$
Exp	11	22	11	$P > 70\%$

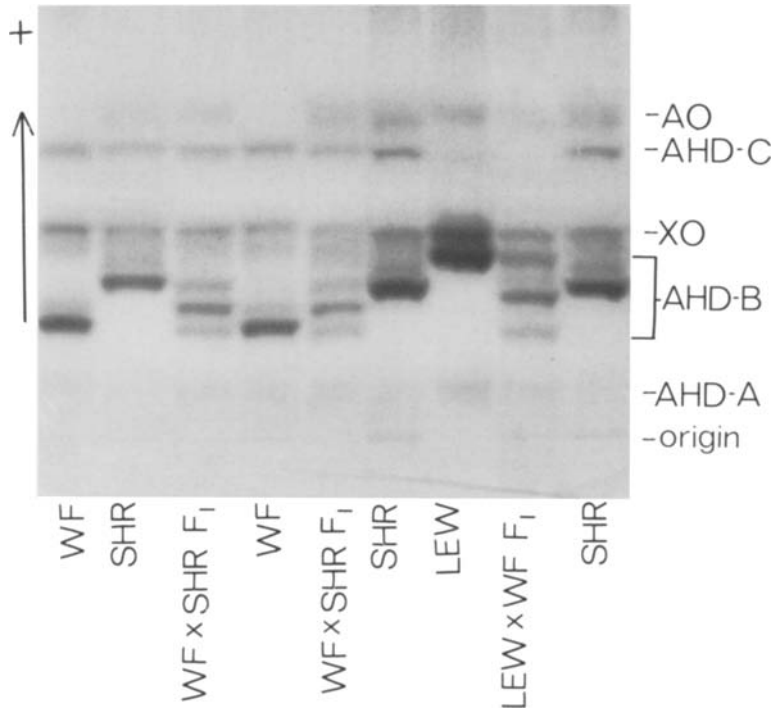

Fig. 2. Cellulose acetate zymograms of rat liver stained for aldehyde dehydrogenase, indicating that three areas of AHD activity are present, AHD-A, AHD-B, and AHD-C. The other isozymes indicated are xanthine oxidase (XO) and aldehyde oxidase (AO). Variant patterns are shown for AHD-B in WF (slow AHD-B), SHR (intermediate AHD-B), and LEW (fast AHD-B) and in F_1 hybrids of WF × SHR and WF × LEW.

heart on the other lead us to suggest that the variant enzyme is the cytoplasmic form of aconitase. We propose to designate the locus *Acon-1,* with two alleles, *Acon-1*[a] and *Acon-1*[b], coding for the slow- and fast-migrating forms, respectively.

Aldehyde Dehydrogenase (AHD). Gels stained for aldehyde dehydrogenase activity in vertebrates typically exhibit complicated banding patterns resulting from the added presence of xanthine oxidase, aldehyde oxidase, and occasionally alcohol dehydrogenase and "phenazine oxidase" (Holmes, 1978). The unequivocal identification of AHD requires the concurrent use of control gels stained for these other enzymes. For example, AO, XO, and "phenazine oxidase" isozymes will appear in the absence of added NAD. Using this approach, three major areas of activity of aldehyde dehydrogenase were observed following electrophoresis of rat liver homogenates. These areas are designated in Fig. 2 AHD-A, AHD-B, and AHD-C, in order of increasing mobilities. Liver was found to be the only tissue suitable for the screening of rat aldehyde dehydrogenases, in contrast to the mouse, where both liver and kidney exhibit high activities (Holmes, 1978). Variation was observed in the

Table V. Inheritance Pattern of AHD-B Phenotypes in Backcrosses of WF(S) × SHR(I), LEW(F) × DA(I), AS(F) × DA(I), and C(F) × DA(I)[a]

	Phenotype distribution of offspring			
Cross	S	SI	I	
SI × I				
Obs	0	35	47	$X_1^2 = 1.76$
Exp	0	41	41	$P > 10\%$
SI × S				
Obs	31	39	0	$X_1^2 = 0.91$
Exp	35	35	0	$P > 30\%$
SI × SI				
Obs	13	27	6	$X_2^2 = 3.53$
Exp	11.5	23	11.5	$P > 10\%$
	I	FI	F	
FI × I				
Obs	106	143	0	$X_1^2 = 5.5$
Exp	124.5	124.5	0	$P > 2\%$
FI × F				
Obs	0	102	104	$X_1^2 = 0.02$
Exp	0	103	103	$P > 90\%$

[a]Data for LEW × DA, AS × DA and C × DA pooled.

strong AHD-B isozyme, with three distinct electrophoretic patterns, slow (S), "intermediate" (I), and fast (F), being found among inbred strains (see Fig. 2). Male and female offspring of S × I, S × F, and I × F matings all exhibited three major bands of AHD-B activity, consistent with them being heterozygous at an autosomal locus encoding a dimeric enzyme (Fig. 2). Table V details the results obtained from the various backcrosses set up to test the genetics of this variation. These results are consistent with the existence of an autosomal locus possessing three alleles responsible for the three mobility classes observed among inbred rats. We propose a locus *Ahd-2,* with alleles *a*, *b*, and *c* coding for the S, I, and F forms of the enzyme, respectively (see Discussion for a more detailed rationale).

Alkaline Phosphatase (AKP). A representative zymogram obtained for the alkaline phosphatases of rat kidney is shown in Fig. 3. Four major zones of activity are evident, designated A, B, C, and D in order of increasing mobility. Variation was observed between inbred strains for the presence or absence of a

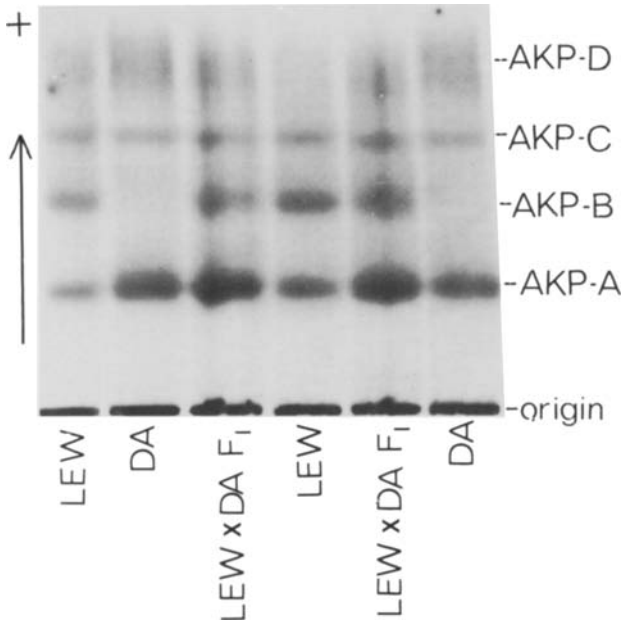

Fig. 3. Cellulose acetate zymograms of rat kidney stained for alkaline phosphatase. Four zones of activity are revealed in kidney, AKP-A, AKP-B, AKP-C, and AKP-D. AKP-B shows interstrain variation of a presence/absence type, being present in LEW (*Akp-l*^{aa}), absent in DA (*Akp-l*^{bb}), and present in the F_1's (*Akp-l*^{ab}).

Table VI. Inheritance Pattern of AKP-B Phenotypes in Backcrosses of LEW(+) × DA(N), C(+) × DA(N), and AS(+) × DA(N)

Cross	Phenotype distribution of offspring		
	+	N	
+(+ × N hybrid) × N			
Obs	134	133	$X_1^2 = 0.004$
Exp	133.5	133.5	$P > 95\%$
+(+ × N hybrid) X +			
Obs	156	0	
Exp	156	0	

band at AKP-B. Confirmation of the genetic basis of the apparent "null" allele at this locus was obtained from a number of crosses and backcrosses involving the DA strain (null phenotype—N) to various other strains possessing AKP-B(+). Thus F_1's of such crosses were all + (Fig. 3), backcrosses to the + parent were all +, and backcrosses to the DA parent produced approximately equal numbers of the two phenotypic classes (Table VI). We designate the locus *Akp-1,* with two alleles, *Akp-1*[a], responsible for the + phenotype, and *Akp-1*[b], responsible for the N phenotype. We were not able to demonstrate the expression of *Akp-1* in plasma or in any tissue other than kidney under the electrophoretic conditions used.

(b) Strain Distribution of New Loci

Table I shows the strain distribution of alleles for *Acon-1, Ahd-2,* and *Akp-1.*

Note that there are several "missing values," especially for *Ahd-2.* Aldehyde dehydrogenase isozymes are difficult to type on tissues that are not fresh, and by the time that the original electrophoretic screen and subsequent genetic crosses were completed, the original tissues were not suitable for typing the strains. The missing values refer to cases where fresh material could not be obtained to confirm the original typing.

For *Acon-1* all strains carried the *b* allele, except BN and WF. Of the three alleles at *Ahd-2,* allele *a* occurred in only 1 strain, allele *b* in 5 strains, and allele *c* in 16 strains. For *Akp-1,* 8 strains possessed allele *b,* while 18 possessed allele *a.*

2. Linkage Testing

Crosses used for linkage testing are shown in Table VII along with the differentiating loci involved. The numbers of recombinants and parentals for each pair of loci are given in Table VIII.

Table VII. Crosses Used for Linkage Testing and the Differentiating Loci[a]

	Differentiating locus											
	LGI	LGIV		LGVI	LGIX	Others						
Cross	*c*	*a*	*Svp-1*	*h*	*RT-1*	*Cu-2*	*Ahd-2*	*Acon-1*	*Akp-1*	*Fh-1*	*Pep-3*	*Igk-1*
SHR × WF			x				x	x				x
JC × DA	x			x			x		x	x	x	x
AS × DA	x	x		x	x		x		x			x
LEW × DA	x	x	x	x			x		x	x	x	x
C[b] × DA						x	x		x	x		x

[a]Completely recessive loci could be scored in backcrosses only one way, e.g., *a, c, Akp-1*. H is incompletely dominant to h so that Hh animals are distinguishable from HH (Robinson, 1965).
[b]Strain C from NIH is randombred but the C grandparent was homozygous for *Cu-2, Ahd-2, Akp-1, Fh-1,* and *Igk-1*.

Convincing evidence for linkage is evident in two cases. One confirms a previously reported tight linkage relationship between *a* and *Svp*. The loci *Fh-1* and *Pep-3* are loosely linked ($\chi_1^2 = 57.13$, $P < 0.001$), with an estimated recombination distance of 0.32 (SE = 0.02). There is a possibility of very loose linkage between *Fh-1* and *Igk-1* ($\chi_1^2 = 5.23$, $0.01 < P < 0.05$).

3. Strain Characterization for Loci Polymorphic Within Outbred *R. norvegicus*

The enzymes, AHD, AKP, GA3PD, αGPD, G6PD, 6PGD, PGM, and xanthine dehydrogenase (XDH) have all been reported as exhibiting electrophoretically detectable variation in outbred populations of the Norway rat (see Introduction for references). In the case of AHD, plasma AKP, and 6PGD, the genetic bases of the observed variation have been demonstrated (Truesdale-Mahoney *et al.,* 1981; Jiminez-Martin and Dessauer, 1973; Krog, 1977). The variation at the 6PGD locus is noteworthy in that the original "S" and "F" terminology has yet to be changed, despite the fact that a number of inbred strains have already been characterized (Bender *et al.,* 1978; Festing 1979a).

In this survey, no variation was detected in the enzymes GA3PD, αGPD, G6PD, 6PGD, and PGM. The fast form of 6PGD was present in several outbred strains examined concurrently and thus we are confident that the typing is valid for the inbred strains indicated. Our inability to detect variants for the other enzymes (and indeed all monomorphic proteins) does not necessarily mean that genetic variation does not actually exist, rather that any present was not detectable with our electrophoretic methods.

The results for the enzyme XDH warrant special mention. We were not

Table VIII. Summary of Linkage Testing[a]

Locus	Locus										
	a	*c*	*h*	*Cu₂*	*Acon-1*	*Ahd-2*	*Akp-1*	*Fh-1*	*Pep-3*	*Svp-1*	*Igk-1*
a		ns	ns	–	–	ns	–	ns	ns	—**	ns
c	+		ns	–	–	ns	–	ns	ns	ns	ns
h	+	+		–	–	ns	ns	ns	ns	ns	ns
Cu₂	–	–	–		–	ns	ns	ns	–	–	ns
Acon-1	–	–	–	–		ns	–	–	–	ns	ns
Ahd-2	20/32	106/119	163/132	34/34	80/60		ns	ns	ns	ns	ns
Akp-1	–	–	89/80	39/34	–	111/119		ns	ns	ns	ns
Fh-1	34/34	132/121	163/171	40/33	–	239/248	132/111		—**	ns	—*
Pep-3	26/35	128/106	85/78	–	–	184/196	84/77	291/135		ns	ns
Svp	17/1	15/16	37/38	–	29/42	61/68	13/17	37/40	36/29		ns
Igk-1	+	+	+	39/37	82/78	278/281	121/109	142/106	87/84	35/41	

[a]Number of parentals/number of recombinants given in the lower left-hand matrix; significance levels given in the upper right-hand matrix. +, tests for linkage are well documented; –, linkage not testable in any of the crosses set up; ns, not significant ($P > 0.05$).
*$0.01 < P < 0.05$.
**$P < 0.001$.

able to demonstrate any enzyme exhibiting a specific xanthine dehydrogenase activity on gels stained for XDH. The major isozyme present was referable to a xanthine oxidase, an enzyme capable of using hypoxanthine but without the requirement for NAD. No electrophoretic variation was detected for this xanthine oxidase.

4. Strain Profiles

Table I lists the allelic profiles at 11 genetic markers for the inbred strains surveyed on a substrain by substrain basis. There are no detected cases of subline differentiation for the 10 strains involved in multiple sampling. Attempts to score hemoglobin variation and variation at the *Es-2, Es-3,* and *Es-9* esterase loci were only partially successful and the incomplete typings obtained have been omitted from the table. Note that in the table and text we have followed the esterase nomenclature suggested by the Committee on Genetic Nomenclature of the Rat (Van Zutphen, 1983).

DISCUSSION

1. New Markers

This paper reports the results of a systematic search for new electrophoretic markers in the laboratory rat. As a consequence of a large survey involving many enzymes and proteins and many different strains, we report the

discovery of a hitherto undiscovered marker, *Acon-1,* and the formal characterization of two others, *Ahd-2* and *Akp-1.*

The variation at the *Ahd-2* locus has been previously documented (Truesdale-Mahoney *et al.,* 1981), although the authors referred to only two alleles (presumably the *b* and *c* alleles). They also demonstrated that the enzyme involved was located in the cytosolic fraction of rat liver. Aldehyde dehydrogenase is known to exist as a number of different isozymes that are differentially localized in subcellular fractions of mammalian tissues (Timms and Holmes, 1981a). Three separate loci have been characterized thus far in the mouse (Holmes, 1978; Timms and Holmes, 1981a, b): *Ahd–1,* encoding a mitochondrial form; *Ahd-2,* encoding a form located in the cytoplasm; and a regulatory locus, *Ahd-3,* controlling the expression of a microsomal aldehyde dehydrogenase. On this basis, the variant locus in the rat is homologous to mouse *Ahd-2.* However, there remains a conflict between the expected tetrameric nature of the enzyme and the observation of only three isozymes in heterozygotes. Perhaps, as Truesdale-Mahoney *et al.* (1981) suggest, the three bands correspond to the A_4, A_2B_2, and B_4 tetramers only, and the absence of A_3B and AB_3 isozymes is due to the fact that tetramer formation takes place only between the A_2 and the B_2 homodimers. An alternative possibility is that the enzyme is dimeric in some mammalian lineages.

The relationship between *Akp-1* and previously observed genetic variation in plasma alkaline phosphatases (Jiminez-Marin, 1974) remains equivocal. We have characterized $Akp\text{-}1^b$ as a null activity variant at a locus expressed only in kidney homogenates using cellogel as the electrophoretic support medium. However, under different conditions it may be possible to characterize the allele as a mobility variant perhaps with reduced activity. The situation regarding variation at *Es-1* (Womack, 1973) indicates that the so-called null $Es\text{-}1^b$ allele is actually a mobility variant which in some instances is obscured by the product of the *Es-2* locus. This may be true of $Akp\text{-}1^b$ and the AKP-C or AKP-A areas as shown in Fig. 3. Further work needs to be done to determine whether the "fast"/"slow" plasma alkaline phosphatase variation reported by Jiminez-Marin (1974) is encoded by the *Akp-1* locus or represents an additional marker.

The variation documented at *Ahd-2* and *Akp-1* will be especially useful in the area of genetic quality control. The rarer $Ahd\text{-}2^b$ and $Akp\text{-}1^b$ alleles are sufficiently common to be of considerable use when taken in conjunction with other markers in uniquely defining several strains. The $Acon\text{-}1^a$ and $Ahd\text{-}2^a$ alleles are both possessed by WF, with BN possessing $Acon\text{-}1^a$ and $Ahd\text{-}2^b$. These markers serve to identify these strains uniquely.

The genetics of the electrophoretic variation observed in 6PGD has yet to be formalized. This variation has been shown to be genetic (Krog, 1977) and a number of inbred strains have been characterized (Bender and Günther, 1978;

Krog, 1977). We propose that the locus be called *Pgd* in line with the *Mus* nomenclature and that the slow and fast phenotypes refer to products of alleles *a* and *b*, respectively. Table I uses this terminology and indicates that all inbred strains tested possessed the *a* allele.

Similarly, the situation regarding the marker fumarate hydratase is somewhat confused in the literature. Carleer and Ansay (1976) described electrophoretic variation for this enzyme and confirmed the genetic basis of the two mobility forms. They simply stated that their results ". . . support the assumption that the 3 phenotypes are under the genetic control of 2 co-dominant alleles, *FHa* and *FHb* at a single autosomal locus. . . ." Elsewhere (Nezlin *et al.*, 1979) the marker has been characterized by the symbol *Fum.* We suggest that the locus be designated *Fh-1* in line with the reserved gene symbol for the mouse, with alleles $Fh\text{-}1^a$ and $Fh\text{-}1^b$ encoding the fast and slow forms of the enzyme, respectively.

2. Linkage Relationships

Altogether we have tested the linkage relationships between 35 pairwise combinations of markers. The one conclusive finding is the linkage of *Fh-1* and *Pep-3,* with an estimated map distance of 32 recombination units. This results in the construction of a new linkage group, $\overline{X}$ (Cramer, 1982), containing *Fh-1* and *Pep-3.* Our results also allude to a possible linkage between *Fh-1* and *Igk-1.* If this were true , *Igk-1* would then have to be on the opposite end of linkage group $\overline{X}$ to *Pep-3* since there is no evidence of linkage between *Pep-3* and *Igk-1.* The comparative linkage relationships in *Mus musculus* are of no direct help in this matter, since although *Pep-3* has been mapped to chromosome 1, *Fh-1* and *Igk-1* have both yet to be mapped in the mouse.

3. Strain Profiles

The strain profiles shown in Table I represent the largest single compilation of such information yet published. Furthermore, the screening for and lack of variation found in the enzymes GA3PD, αGPD, G6PD, and PGM add to the dimensions of the biochemical characterization obtained. There are however, several notable omissions regarding the choice of markers. The esterase loci *Es-2, Es-3, Es-6, Es-7,* and *Es-9* are not indicated. We were not aware of the existence of *Es-6* and *Es-7* until after the completion of our work. Attempts to score *Es-3* and *Es-9* on Cellogel were not successful; it would seem that other support media may be more suitable for scoring multiple locus systems much as esterases. We were able to score some but not all of the allelic types of *Es-2;* thus it was deemed safer to omit the marker completely. The esterases are an important group of markers for inbred strains of the Norway rat. They are

unfortunately quite difficult to work with electrophoretically, thus limiting their usefulness for routine genetic typing. This is probably true of many of the markers available for the rat, including the three described in this paper. Furthermore, the close linkage of all esterase loci except *Es-6* (Bender *et al.*, 1982) limits the usefulness of these markers in extending the gene map of *R. norvegicus*.

The strain profiles listed do not by themselves uniquely characterize each individual inbred strain. While most stains (ignoring coat color genes) have unique strain profiles, a few pairs (e.g., AS and AS2, WAB and AUG) are indistinguishable biochemically. The addition of a few commonly variable markers such as *Hb, Svp-1, Es-2, Es-3,* etc., should result in the unique characterization of every inbred strain of the laboratory rat. The aim of a genetic quality control program ought to be to have a minimum of two biochemical markers differentiating between any two inbred strains held within an institution. Rat genetics is rapidly heading toward this goal; it is unfortunate, however, that many of the markers available are "difficult" to work with and, in several cases, are such that heterozygotes are difficult or impossible to distinguish from one or another homozygote.

It seems appropriate here to refer to the work of Eriksson *et al.* (1976) on enzyme variation in inbred and outbred rats. The authors screened two inbred strains F344 and ACI, several outbred strains, and two wild populations for a number of liver enzymes. They concluded that ". . . of the laboratory strains the inbred and outbred strains are equally polymorphic" and interpreted their results regarding the inbred strains as being due to heterosis. As Festing (1979b) points out, a more likely explanation is genetic contamination of the two inbred strains involved. There are also a number of intrinsic anomalies in their results. The three main enzymes which contributed to the observed heterozygosity of F344 and ACI were GA3PD, αGPD, and XDH, three NAD-dehydrogenases all detected using the same basic enzyme straining mechanism. The staining regimes of these three enzymes are noted for their tendency to allow the simultaneous staining of the common "phantom" dehydrogenases, alcohol dehydrogenase and lactate dehydrogenase, unless special precautions are taken in the stain ingredients (Harris and Hopkinson, 1976). Furthermore, the very existence of a "xanthine dehydrogenase" is open to some doubt. We have never been able to demonstrate anything other than xanthine oxidase, alcohol dehydrogenase, lactate dehydrogenase, or "phenazine oxidase" (see Holmes, 1978) on gels stained for xanthine dehydrogenase in this laboratory's experience with a wide range of vertebrates and invertebrates. The data presented for the GA3PD polymorphism in F344 indicate that there were 28 homozygous $Ga3pd^{100}$, 5 homozygous $Ga3pd^{110}$, and only 1 heterozygote! The authors blame the problems of overlap of superoxide dismutase (SOD) as a contributor to scoring uncertainties. Overall we feel

that the paper is open to criticism on various counts and that, in the absence of any specific supportative genetic data, the enzymes GA3PD, αGPD, and XDH should be dropped from the laboratory rat genetics literature.

Ten strains in this survey were represented by more than one substrain. We have found no evidence for substrain differentiation, although the literature does refer to a case of subline differentiation in WAG (Festing, 1978; Van Zutphen *et al.,* 1981). A comparison of the results in Table I with those of the published literature indicates one other such case. The BN rats tested by us possessed the *a* allele at *Pep-3,* whereas those (BN/Pitt) tested by Womack and Cramer (1980) possessed the *b* allele. We have also observed the *b* allele in a BN sub-line held within Australia (unpublished data).

Finally, a comment on the comparative degree of polymorphism observed within the laboratory rat compared to the laboratory mouse is warranted. A number of workers (Womack and Cramer, 1980; Bender and Günther, 1978; Van Zutphen *et al.,* 1981) have commented on the relative scarcity of biochemical markers in the laboratory rat, and in general, most have attributed this phenomenon to the bias in screening intensity devoted to the laboratory mouse. The results reported herein suggest that the laboratory rat is in fact genetically less variable than the laboratory mouse. Indeed there is strong evidence (Sage, 1981) that the laboratory mouse arose from at least two species of *Mus* (*M. domesticus* and *M. molossinus*), a factor which would enhance the extent of genetic variation between inbred strains. This conclusion, if correct, does not augur well for the future expansion of laboratory rat genetics using inbred strains. It may be that more reliance will have to be placed on discovering variants in outbred and wild populations of the Norway rat if we are to progress significantly in the area of gene mapping and genetic homology comparisons.

ACKNOWLEDGMENTS

We are grateful for the technical assistance provided by S. Palmer-Wood and J. Birrell.

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