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Biochemical Polymorphism in the Rat: Genetics of Three Electrophoretic Variants and Characterization of Inbred Strains

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Nine inbred strains of the rat (Rattus norvegicus) *were screened for differences in electrophoretically detectable proteins. Interstrain variation was observed for 7 of 26 proteins. Three of these variants have not been described previously: leucine aminopeptidase* (Lap-1), *major urinary protein* (Mup-1), *and seminal vesicle protein* (Svp-2). *Genetic analysis revealed two autosomal alleles for each of these polymorphisms. The loci* Lap-1, Mup-1, *and* Svp-2 *are linked neither to one another nor to the previously described* Svp-1 *and* Es-4 *loci. Each of the nine strains can be identified now by a specific set of monogenic markers.*

KEY WORDS: genetics; electrophoresis; biochemical polymorphism; strain characterization; rat.

INTRODUCTION

The use of inbred strains in biomedical research requires the development of specific genetic monitoring procedures. The best guarantee for genetic constancy of inbred strains is obtained by a precise and accurate breeding system. It has been demonstrated however, that a system of regularly testing the authenticity and uniformity of an established inbred strain is necessary in

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order to prevent unintentional use of genetically contaminated strains (Festing, 1974; Hedrich *et al.*, 1975; Krog, 1976; Groen, 1977).

Genetic differences among inbred strains can be used for the identification of a strain. Each strain can be characterized by a specific set of monogenic markers. A practicable genetic monitoring system using monogenic markers, detectable with electrophoretic techniques, has been proposed by Moutier (1971) and has been developed further for the mouse by Krog (1976) and Groen (1977). The main advantage of using these techniques is that they are based on the visualization of qualitative differences in primary gene products.

A similar system based on biochemical polymorphic markers is currently being developed for the rat. Only a limited number of useful biochemical genetic markers have been reported in this species (Robinson, 1965; Bender and Günther, 1978; van Zutphen, 1981). Although this might be explained by the fact that the rat as a species is less polymorphic than the mouse, a more probable reason is that the mouse has been studied more extensively in this respect.

We have screened 26 proteins in nine inbred strains of the rat and found some variants which have not been reported before. These variants were studied genetically. Backcrosses were used to detect possible linkage groups. In combination with previously described markers, the new polymorphisms have been used for the characterization of the inbred strains.

MATERIALS AND METHODS

Animals

Interstrain variation was screened in five male rats of each of the following nine strains: BN/Cpb, CPB-B, CPB-G, CPB-WE, LEW/Cpb, SHR/Cpb, SD/Cpb, WAG/Cpb, and WKY/Cpb. All these strains are fully inbred (>20 generations b × s matings) and are maintained at the Central Institute for the Breeding of Laboratory Animals (CPB-TNO), Zeist.

Blood, Urine, and Organs

After ether anesthetization about 1.5 ml of blood was collected with heparinized pipets via the retroorbital sinus. Plasma and blood cells were separated by centrifuging (1500*g*) at 4 C for 15 min. Erythrocytes were washed three times with 0.9% saline and diluted with an equal volume of distilled water. Urine was collected with a pipet from the bottom of a clean cage.

Liver, kidney, intestine, and vesicle gland were collected after total

Table I. Compositions of the three gel buffers

Buffer	*p*H	Tris, ($\times 10^{-4}$ M)	Boric acid ($\times 10^{-4}$ M)	Citric acid ($\times 10^{-4}$ M)	1.0 M Lithium hydroxide (ml)
I	6.4	144	76	54	0.45
II	7.5	144	288	54	7.00
III	9.0	144	288	27	14.00

perfusion of anesthetized animals (60 mg/kg Nembutal). Liver and kidney supernatants were obtained by homogenizing these organs in distilled water (5 ml/g) and centrifuging for 15 min (1500*g*) at 4 C.

Seminal fluid was collected from the vesicle gland in 1 ml 0.1 M phosphate buffer (*p*H 7.5) and centrifuged for 15 min (1500*g*) at 4 C. The supernatant was used (Gasser, 1972).

All samples were stored at −20 C until used.

Electrophoresis

Horizontal starch gel electrophoresis was carried out with three different discontinuous buffer systems in order to estimate the optimal conditions for the separation of 26 proteins. The electrode buffer was the same for each of the systems and consisted of 0.38 M boric acid and 0.06 M lithium hydroxide (*p*H 7.8). The three gel buffers were composed as shown in Table I.

Staining Procedures

Variation of proteins in plasma, erythrocytes, urine, and vesicle fluid was investigated by staining the gel according to Brewer (1970).

α-Amylase (plasma) was stained according to Mazumber and Spooner (1970). Acid phosphatase (plasma), alkaline phosphatase (plasma), alcohol dehydrogenase (kidney), glycerol-3-phosphate dehydrogenase (kidney), glucose phosphate isomerase (erythrocytes), glutamate oxaloacetate transaminase (liver), isocitrate dehydrogenase (kidney), lactate dehydrogenase (erythrocytes, kidney, liver), leucine aminopeptidase (serum, kidney, liver), peptidase (kidney, liver), phosphoglucomutase (erythrocytes, kidney), and 6-phosphogluconate dehydrogenase (erythrocytes) were stained according to Shaw and Prasad (1970).

Esterases (plasma, intestine, kidney) were stained according to van

Zutphen (1974). Gene symbols as proposed by Womack (1972, 1973) were used for intestine and kidney esterases (*Es-3* and *Es-4*).

Indophenol oxidase (erythrocytes) and mannose phosphate isomerase (kidney) were stained according to Nichols and Ruddle (1973).

β-Galactosidase (kidney) was stained according to Seyedyazdani *et al.* (1975) and glutamate pyruvate transaminase (liver) was stained according to Eicher and Womack (1977).

Genetic Analysis

Crosses were made between LEW/Cpb (♀) and SHR/Cpb (♂). Three F_1 females of the crosses were backcrossed to their male parent. The male progeny of this backcross were examined at 12 weeks of age for electrophoretic variants of leucine aminopeptidase, major urinary protein, seminal vesicle proteins, and esterase (*Es-4*).

RESULTS

Screening for Polymorphisms

In order to detect interstrain variation, electrophoresis of body fluids and tissue homogenates of nine inbred strains was carried out in different gel buffer systems. A total of 26 enzymes and other proteins was tested for variation. With these methods no variation between the nine strains was found for 19 of these proteins (Table II). Seven proteins were found to be polymorphic (Table III). Polymorphism for *Es-1, Es-3, Es-4,* and *Svp-1* has been described previously, whereas polymorphism for *Lap-1, Mup-1,* and *Svp-2* has not been reported before. The zymotypic variation of the latter three proteins is demonstrated in Figs. 1–3.

For leucine aminopeptidase, LEW/Cpb is the only inbred strain with zymotype bb; all other inbred strains tested have zymotype aa (Fig. 1). The aa zymotype for major urinary protein was found in CPB-G, CPB-WE, SHR/Cpb, and WKY/Cpb, whereas the other inbred strains have the bb zymotype for this protein (Fig. 2).

The newly detected variation for seminal vesicle protein was found in the region between the $Svp\text{-}1^a$ and $Svp\text{-}1^b$ zones. Most strains tested possess the aa zymotype in which a distinct zone is visible just anodal to the $Svp\text{-}1^a$ zone. Three inbred strains (CPB-WE, LEW/Cpb, and WKY/Cpb) have the bb zymotype for this protein (Fig. 3).

Table II. Proteins for which no interstrain variation was found in the nine inbred rat strains tested

Name	EC No.
Alcohol dehydrogenase	1.1.1.1
Glycerol-3-phosphate dehydrogenase (NAD^+)	1.1.1.8
Lactate dehydrogenase	1.1.1.27
Isocitrate dehydrogenase ($NADP^+$)[a]	1.1.1.42
6-Phosphogluconate dehydrogenase[a]	1.1.1.43
Indophenol oxidase	1.9.3.1
Glutamate-oxaloacetate transaminase	2.6.1.1
Glutamate-pyruvate transaminase	2.6.1.2
Phosphoglucomutase[a]	2.7.5.1
Carboxyl esterase (*Es-2*)[a]	3.1.1.1
Alkaline phosphatase	3.1.3.1
Acid phosphatase	3.1.3.2
α-Amylase	3.2.1.1
β-Galactosidase	3.2.1.23
Peptidase	3.4.13.1
Mannose phosphate isomerase	5.3.1.8
Glucose phosphate isomerase	5.3.1.9
Hemoglobin[a]	
Transferrin[a]	

[a] Polymorphism for these proteins has been described in the rat.

Genetic Analysis

Crosses between SHR/Cpb (♀) and LEW/Cpb (♂) were made in order to study the genetic basis of the three new polymorphic systems. F_1 hybrid zymotypes for each of the three polymorphic systems are demonstrated in Figs. 1–3, together with the zymotypes of the two parental strains.

After backcrossing the F_1 females to their male parents, a total of 32

Table III. Seven biochemical markers found to be polymorphic in the nine inbred rat strains tested

Marker	Gene symbol	Sample for optimal separation	Gel buffer for optimal separation
Esterase 1	*Es-1*	Plasma	II
Esterase 3	*Es-3*	Intestine	II
Esterase 4	*Es-4*	Kidney	II
Leucine aminopeptidase 1	*Lap-1*	Kidney	III
Major urinary protein 1	*Mup-1*	Urine	III
Seminal vesicle protein 1	*Svp-1*	Seminal fluid	III
Seminal vesicle protein 2	*Svp-2*	Seminal fluid	III

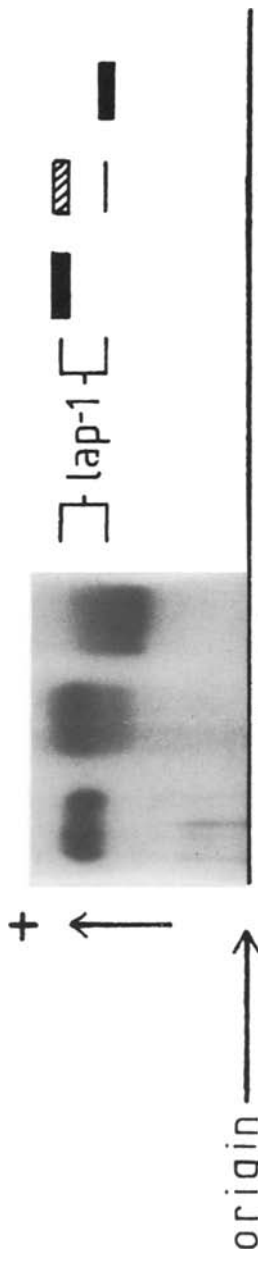

Fig. 1. Leucine aminopeptidase patterns in kidneys of SHR/Cpb (aa), LEW/Cpb (bb), and F_1 hybrid (ab) after starch gel electrophoresis and staining with L-leucyl-2-naphthylamide as a substrate.

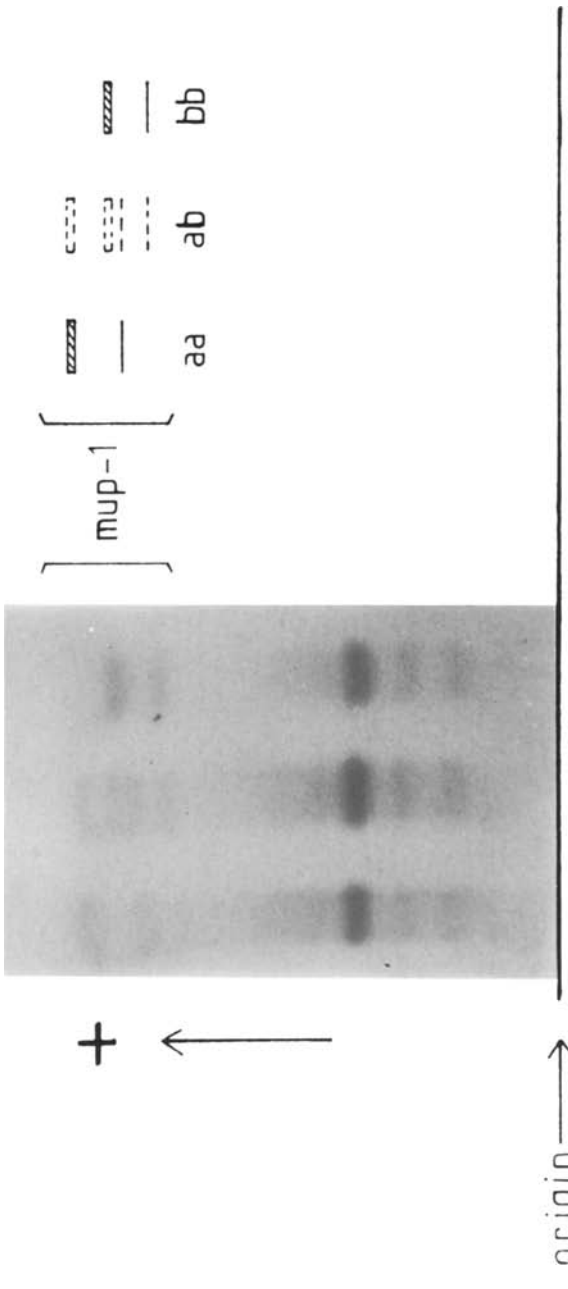

Fig. 2. Major urinary protein patterns of SHR/Cpb (aa), LEW/Cpb (bb), and F_1 hybrid (ab) after starch gel electrophoresis.

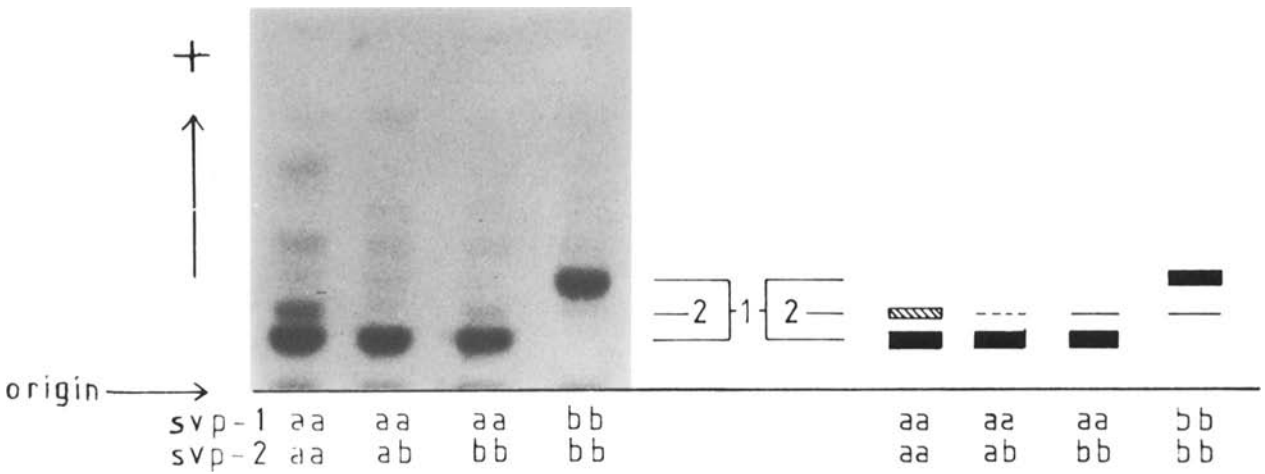

Fig. 3. From left to right: seminal vesicle protein patterns of CPB-G, (CPB-G × CPB-WE) F_1, CPB-WE, and LEW/Cpb after starch gel electrophoresis.

Table IV. Distribution of zymotypes in the progeny of backcross (SHR/CPB × LEW/Cpb) F_1 × LEW/Cpb

	ab	bb	χ^2	P(df 1)
Leucine aminopeptidase (*Lap-1*)	15	17	0, 13	>0.50
Major urinary protein (*Mup-1*)	16	16	0	1.00
Seminal vesicle protein (*Svp-2*)	19	13	1, 13	>0.10

male backcross progeny was available for the study of the genetic basis of each of the proteins and for testing possible linkage relationships.

The distribution of the zymotypes of the three systems as found in the progeny of the backcross is given in Table IV. The data presented in this table corroborate the hypothesis that two alleles (*a* and *b*) are responsible for the variation of each of the proteins leucine aminopeptidase, major urinary protein, and seminal vesicle protein. We therefore propose the allelic designations *Lap-1*^{a} and *Lap-1*^{b}, *Mup-1*^{a} and *Mup-1*^{b}, and *Svp-2*^{a} and *Svp-2*^{b}. Both the *Lap-1* alleles and the *Mup-1* alleles are codominant. Although the alleles at the *Svp-2* locus may also be indicated as codominant, it should be noted that the codominance for this locus is not always clearly recognizable. *Svp-2*^{a} codes for a protein which is stained rather intensively, whereas the zone of the *Svp-2*^{b} allele, having the same mobility, is less distinct. In the heterozygotes, one would expect a zone with intermediate staining intensity; however, generally a very faint zone, one less distinct than the *Svp-2*^{b} zone, is visible.

Test for Linkage

In addition to the three loci *Lap-1, Mup-1,* and *Svp-2,* differences between the parental strains SHR/Cpb and LEW/Cpb were also observed for *Svp-1* and *Es-4*. SHR/Cpb is *Svp-1*^{a}–*Es-4*^{a} and LEW/Cpb is *Svp-1*^{b}–*Es-4*^{b}. Thus, the backcross progeny can be used for testing linkage among five loci. Backcross (SHR/Cpb × LEW/Cpb) F_1 × LEW/Cpb can be written as follows:

$$\frac{Es\text{-}4^a}{Es\text{-}4^b} = \frac{Lap\text{-}1^a}{Lap\text{-}1^b} = \frac{Mup\text{-}1^a}{Mup\text{-}1^b} = \frac{Svp\text{-}1^a}{Svp\text{-}1^b} = \frac{Svp\text{-}2^a}{Svp\text{-}2^b}$$

$$\times \frac{Es\text{-}4^b}{Es\text{-}4^b} = \frac{Lap\text{-}1^b}{Lap\text{-}1^b} = \frac{Mup\text{-}1^b}{Mup\text{-}1^b} = \frac{Svp\text{-}1^b}{Svp\text{-}1^b} = \frac{Svp\text{-}2^b}{Svp\text{-}2^b}.$$

Although the total number of progeny from this backcross is limited, the data

Table V. Segregation analysis of backcross (SHR/Cpb × LEW/Cpb) F_1 × LEW/Cpb for testing linkage between *Es-4*, *Lap-1*, *Mup-1*, *Svp-1*, and *Svp-2*

Compared markers	Genotype of backcross progeny		% Recombination	χ^2 P(df 1)
	Parental	Recombinant		
Es-4–Lap-1	11	21	66	3.13 > 0.05
Es-4–Mup-1	16	16	50	0.00 1.00
Es-4–Svp-1	18	14	44	0.15 > 0.10
Es-4–Svp-2	15	17	53	0.13 > 0.50
Lap-1–Mup-1	18	14	44	0.50 > 0.10
Lap-1–Svp-1	14	18	56	0.50 > 0.10
Lap-1–Svp-2	18	14	44	0.50 > 0.10
Mup-1–Svp-1	14	18	56	0.50 > 0.10
Mup-1–Svp-2	19	13	40	1.13 > 0.10
Svp-1–Svp-2	15	17	53	0.13 > 0.50

summarized in Table V indicate that none of the five loci is linked to one of the others.

Genetic Characterization of Nine Inbred Strains

The three markers *Lap-1*, *Mup-1*, and *Svp-2* can be used for the characterization of the inbred strains in combination with the previously described biochemical markers *Es-1*, *Es-3*, *Es-4*, and *Svp-1* and the coat color marker *albino*. The allelic distribution of these polymorphic markers as found by testing five animals of each inbred strain is given in Table VI. With the

Table VI. Distribution of alleles in nine inbred strains of the rat

Strain	Loci							
	Es-1	*Es-3*[a]	*Es-4*[a]	*Lap-1*	*Mup-1*	*Svp-1*	*Svp-2*	*Albino*
BN/Cpb	a	b	b	a	b	b	a	C
CPB-B	a	a	b	a	b	b	a	C
CPB-G	a	b	b	a	a	a	a	c
CPB-WE	a	a	b	a	a	a	b	C
LEW/Cpb	a	b	b	b	b	b	b	c
SHR/Cpb	a	b	a	a	a	a	a	c
SD/Cpb	b	a, b	b	a	b	a	a	c
WAG/Cpb	a	a, b	b	a	b	b	a	c
WKY/Cpb	a	b	b	a	a	a	b	c

[a] *Es-3* and *Es-4* according to Womack (1973). The *Es-3*^{b} zymotype of SHR/Cpb differs from the other *Es-3*^{b} zymotypes. This might indicate a third allele at the *Es-3* locus.

exception of *Es-3*, no intrastrain variation was found for any of these markers. As can be seen from the table, each of the nine strains has a specific set of alleles and can be identified by these monogenic markers.

DISCUSSION

Three markers which have not been described previously in the rat were analyzed genetically. The variation of leucine aminopeptidase, major urinary protein, and seminal vesicle protein, as found in this study, appeared to be under the control of three independent autosomal loci, each with two alleles.

Although variation in leucine aminopeptidase was demonstrated in the kidney, it can also be found in homogenates of liver and in the serum. The electrophoretic mobility was greater when tested in liver than in kidney homogenates, whereas the mobility of the serum enzyme was intermediate. Monis (1964) also found that rat kidney leucine aminopeptidase is moving more slowly than the enzyme in the serum. He postulated that the two enzymes are of different origin. We, however, observed a simultaneous pattern change in the kidney, liver, and serum when animals of different genotypes were compared. This indicates that the enzyme in kidney, liver, and serum is controlled by a common gene. Variation in leucine aminopeptidase has also been found in other species (Tanabe, 1974; Womack *et al.*, 1975). In the mouse Womack *et al.* (1975) found variation in intestinal but not in kidney homogenates. One locus with two alleles was localized on chromosome 9.

Rodents, particularly male mice and rats, have long been known to excrete unusually large amounts of proteins in the urine. The variation in separation patterns has been studied extensively in the mouse (Finlayson *et al.*, 1968; 1969; Groen and Lagerwerf, 1979). The variation in mouse major urinary proteins is controlled by at least four alleles. The two codominant alleles found in the rat control the variation of proteins detectable in the urine of male animals. This variation which is visible in the most anodal region of the gel could not be visualized in the urine of females. Additional variation can be found when urine of male animals is separated with isoelectrofocusing techniques. Thus, as in the mouse, more than two alleles or more than one locus may also be found in the rat if the isoelectrofocusing patterns are analyzed in more detail.

Moutier *et al.* (1971) described a polymorphism of seminal vesicle proteins (*Svp-1*) in the rat that resembles the polymorphism controlled by the *Svp-1* locus in the mouse. We propose the *Svp-2* symbol for the new seminal vesicle protein variant in the rat. Rats homozygous for $Svp\text{-}2^a$ have a distinct zone between the zones of *Svp-1*, whereas this zone is faint in the homozygous $Svp\text{-}2^b$ animals. This might indicate that *Svp-2* is a regulatory rather than a

structural gene. In heterozygotes the zone is usually stained less intensively than in either of the homozygotes. The *Svp-2* patterns are fairly well evident after electrophoresis on starch gels, but preliminary studies have indicated that the distinction can be improved by isoelectrofocusing on polyacrylamide gels.

In the nine inbred strains tested, we also found variation for some previously described esterase variants (*Es-1*, *Es-3*, and *Es-4*). Only two alleles have been described for *Es-3* by Womack (1972, 1973), but we have found intestinal esterase patterns which might indicate that a third allele occurs at this locus. In addition, we also observed esterase variation in seminal vesicle homogenates, which might be identical to the recently described *Es-6* esterase variant found in testis, ovary, brain, and lymph nodes by Bender *et al.* (1979). These and other patterns are now being studied in relation to the previously described esterase loci. As in most mammalian species which have been studied, the genetic basis for the esterase isozymes of the rat seems to be rather complex. A cluster of esterase loci has been localized on linkage group V (Womack and Sharp, 1976). These clustered esterase loci have been indicated as *Es-1* through *Es-4*. However, the nomenclature of esterase loci in the rat is rather confusing and a revision of this nomenclature is badly needed. In this paper we have adopted the *Es-3* and *Es-4* symbols as proposed by Womack (1972, 1973), but Moutier *et al.* (1973*a*, *b*) have also been using the *Es-3* and *Es-4* symbols for esterase polymorphisms which are different from the ones described by Womack (1972, 1973).

Each strain used in this study is characterized by a specific set of genetic markers (Table V). These markers are all autosomal. With the exception of the esterase loci, the biochemical markers mentioned in Table VI segregate independently.

Independently segregating markers are of particular value for genetic monitoring programs in which the genetic contamination of inbred strains is regularly tested (Bender *et al.*, 1979).

Biochemical markers may also be used for testing differences between substrains. For example, two substrains of WAG (WAG/Orl and WAG/Frei) have both been characterized as $Svp\text{-}1^a$ by Moutier (1971) and Bender and Günther (1978), respectively, while in our study substrain WAG/Cpb appeared to possess $Svp\text{-}1^b$. In addition, WAG/Cpb was characterized as $Es\text{-}1^a$, whereas Bender and Günther (1978) found the $Es\text{-}1^b$ allele fixed in the WAG/Frei strain. The fact that more differences among WAG substrains have been found (Festing, 1979) may indicate that in the past genetic contamination occurred in the WAG strain or in one or more of the sublines of this strain. The finding of intrastrain polymorphism for *Es-3* in WAG/Cpb is surprising since this strain has been inbred for more than 115 generations at

the same institute. Strain SD/Cpb, which also showed intrastrain variation for *Es-3*, has been inbred for more than 30 generations at different institutes. Intrastrain variation within rat inbred strains has also been observed by Eriksson *et al.* (1976).

A more systematic search for intrastrain variation seems needed in order to establish whether rat inbred strains are less uniform than mouse inbred strains.

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