COMPARATIVE AUTOSOMAL LINKAGE IN MAMMALS: GENETICS OF ESTERASES IN *MUS MUSCULUS* AND *RATTUS NORVEGICUS*

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ABSTRACT

Recombination between Esterase-4 and Esterase-2 in the rat was not observed in 278 backcross offspring. *Es-4* is thus included within the "esterase cluster" in Linkage group V. A new map of this region was constructed and the relationship of the four esterase loci was found to be: *Es-4*–(9.6 ± 1.6 cM)–*Es-2*, *Es-4*–(1.5 ± 0.7 cM)–*Es-3*. Homology of this region with a region of Linkage Group XVIII (Chromosome *8*) of the mouse was proposed on the basis of tissue distribution, subcellular localization and response of enzymes to inhibitors. Specifically, rat *Es-1* was suggested as the homolog of mouse *Es-2*, rat *Es-2* as the homolog of mouse *Es-1*, and rat *Es-4* as the homolog of mouse *Es-6*. An autosomal segment comprising at least 15 cM of the rat and mouse genomes appears to have remained relatively intact with respect to genetic content during rodent speciation. In addition, a new polymorphism for mouse esterase was described. The locus was designated Esterase-10 (*Es-10*) and proposed as the mouse homolog of human Esterase D. Linkage of *Es-10* with nucleoside phosphorylase-1 (*Np-1*) on Chromosome *14* was established.

AN understanding of the evolution of the mammalian genome is essential to the ultimate extrapolation of biological and biomedical data from laboratory animals to man. Progress toward such an understanding has been slow, although steady. Recently the amino acid sequencing of specific proteins from a variety of species has provided a wave of information regarding evolution at the cistron level. Comparative karyology using new banding techniques has provided evolutionary information at the chromosomal level. The study of comparative mammalian linkages comprises a discipline capable of giving genetic rather than cytological evidence in the relationships of chromosomal segments. Progress in this area, however, has been impeded by the lack of useful genetic markers and has had to rely largely on inter-specific homologies drawn from pigment genes or hereditary diseases (NACHTSHEIM 1958; SEARLE 1968). The zymogram technique for electrophoretic separation and histochemical staining of proteins (HUNTER and MARKERT 1957) has given new life to comparative mammalian genetics by greatly increasing the number of known genetic polymorphisms in several species.

Electrophoretic techniques have demonstrated genetic polymorphisms for at least seven esterase loci in *Mus musculus* (POPP and POPP 1962; RUDDLE and RODERICK 1966; PETRAS and BIDDLE 1967; PETRAS and SINCLAIR 1969; CHAPMAN, NICHOLS and RUDDLE 1974). The products of *Es-1*, *Es-2* and *Es-5* can be observed in serum, of *Es-1*, *Es-3*, *Es-7* and *Es-8* in erythrocytes and of *Es-1*, *Es-2*, *Es-3* and *Es-6* in kidney homogenates. A locus originally designated *Es-4* was shown to be identical to *Es-1* (RUDDLE and RODERICK 1968); therefore, the gene symbol *Es-4* is undesignated and probably should remain so. *Es-1*, *Es-2*, *Es-5*, *Es-6* and *Es-7* are within a 15 cM segment on Chromosome *8* (CHAPMAN, NICHOLS and RUDDLE 1974), *Es-3* is in Chromosome *11* (RODERICK, HUTTON and RUDDLE 1970), and *Es-8* is in Chromosome *7* (CHAPMAN, NICHOLS and RUDDLE 1974). Polymorphism for alleles of *Es-1*, *Es-2* and *Es-3* exists between inbred strains (RODERICK *et al.* 1971); variant alleles for *Es-7* and *Es-8* have been found in laboratory stocks of the Asian house mouse, *Mus musculus castaneus* (CHAPMAN, NICHOLS and RUDDLE 1974); and variants for *Es-5* have been observed only in feral populations of *Mus musculus* (PETRAS and BIDDLE 1967). A variant allele at *Es-6* was originally reported in a wild population (PETRAS and SINCLAIR 1969). Variants at this hormone-influenced locus have since been observed in laboratory stocks of *M. m. molossinus* (WOMACK 1975) and in the inbred strain SK/Cam (WOMACK, unpublished data). Polyacrylamide gels have recently been employed to differentiate alleles at a locus designated *Es-9* (SCHOLLEN, BENDER and VON DIEMLING 1975). Tissue distribution, response to testosterone, and distribution of alleles among strains lead us to suspect that this locus does not differ from *Es-6*. Nevertheless, the gene symbol *Es-9* should be reserved for this locus, should it be confirmed as distinct from *Es-6*. We, therefore, will use *Es-10* to designate a new mouse esterase locus described in this report.

At least four esterase loci are known in *Rattus norvegicus* (WOMACK 1973). Linkage of *Es-1*, *Es-2* and *Es-3* has been established (WOMACK 1973). In this report, we will provide evidence that *Es-4* is linked to the other three loci on Linkage Group V.

It is important to know if the evolution of chromosomal banding patterns necessarily corresponds to the evolution of genetic content. Mouse and rat chromosomes have been examined for similarity of banding patterns (NESBITT 1974). The esterase loci provide a unique opportunity to examine the conservation of genetic information over a 10 to 15 centiMorgan segment of the genomes of these rodents.

MATERIALS AND METHODS

Mapping of rat Es-4

Various alleles of Esterase-1 (*Es-1*), Esterase-2 (*Es-2*), Esterase-3 (*Es-3*), and Esterase-4 (*Es-4*) were identified by electrophoretic separation and histochemical staining as described previously (WOMACK 1973). Alleles at *Es-1* and *Es-2* carried by each rat were determined from sera, alleles at *Es-3* from intestines and alleles at *Es-4* from kidneys of chloroform-asphyxiated rats.

Animals designated ACC are from a stock maintained at Abilene Christian College. These animals, although in only the fifth generation of full-sib inbreeding at the time of this study,

were homozygous for the alleles *Es-1*^{b}, *Es-2*^{b}, *Es-3*^{b}, and *Es-4*^{a}. Strains Lewis and BN, kindly provided by DR. DAVID L. GASSER, have been described elsewhere (GASSER 1972). Both these strains carry the alleles *Es-1*^{a}, *Es-3*^{a}, and *Es-4*^{b}. The Lewis strain carries *Es-2*^{c} and BN carries *Es-2*^{d} (GASSER *et al.* 1973). *Es-2*^{b}/*Es-2*^{b} animals are easily distinguished from either *Es-2*^{b}/*Es-2*^{c} or *Es-2*^{b}/*Es-2*^{d} animals by sera zymograms.

Two backcrosses were established: (ACC × Lewis)F^1 females backcrossed to ACC males, and (ACC × BN)F^1 females backcrossed to ACC males. In both backcrosses segregation data for all four esterase loci were obtained.

Criteria for homology

Attempts to establish homology or non-homology of products of rat and mouse esterase loci were made on the basis of tissue distribution, subcellular localization and response to inhibitors of the genetically determined esterases in both species. We examined only those loci for which we could obtain two allelic forms to serve as genetic controls in the following tests.

Serum, erythrocytes, kidneys, liver, and intestine from strains known to differ at specific loci were prepared and subjected to electrophoresis as previously described (WOMACK 1973; WOMACK 1975). Since strain by strain and tissue by tissue comparisons were made on the same gels, the multi-tissue expression of a gene could be established on the bases of both electrophoretic and allelic identity.

Subcellular fractions of mouse and rat kidneys were prepared by the differential centrifugation technique of RAGAB *et al.* (1967) and inserted into electrophoretic slots as described above. Crude kidney homogenates from strains differing at esterase loci served as controls on the same gels with the subcellular fractions. Subcellular expression of a locus was determined when the same allelic differences observed in crude homogenates could be observed on gels containing subcellular preparations from the same genetically different strains.

Solutions of 10^{-3} M eserine sulfate, 300 mg/ml protamine sulfate, 10^{-4} M mercuric nitrate, 10^{-3} M sodium fluoride, 10^{-4} M E600 (diethyl p-nitrophenyl phosphate), and 10^{-4} M DFP (diisopropyl fluorophosphate) were prepared in 0.025 M phosphate buffer, pH 7.0. One-half of a gel was pre-incubated in an inhibitor solution without substrate and dye coupler, then immersed in 200 ml of inhibitor solution containing 1 ml 10% α-naphthyl butyrate in acetone and 200 mg Fast Blue BB salt. The other half was stained identically without the inhibitor. The relative inhibition of the products of esterase loci was subjectively appraised by a visual comparison of inhibited and non-inhibited gels.

Mouse Esterase-10

Electrophoretic separation of tissue homogenates followed by staining with 4-methylumbelliferyl acetate was employed in an effort to find additional esterase variants in the mouse. Tissue preparation and electrophoresis were by the method described previously (WOMACK 1975). The gels were stained by incubation for 15 min in 50 ml 0.025 M phosphate buffer, pH 7.0, containing 10 mg of 4-methylumbelliferyl acetate (Sigma). Visualization under UV light revealed fluorescent bands of esterase activity. Linkage analysis involved backcrossing C57BL/6J × *M. m. molossinus* hybrids to C57BL/6J.

RESULTS

Linkage of rat Es-4

Backcross data are presented in Table 1. Recombination between *Es-2* and *Es-4* was not observed in either cross. Recombination frequencies in (Lewis × ACC)F_1 females are not significantly different ($p > 0.5$) from those of (BN × ACC)F_1 females for either the *Es-1* to [*Es-2*, *Es-4*] region or the [*Es-2*, *Es-4*] to *Es-3* region. Consequently, data were pooled to obtain map distances. In 278 backcross offspring examined, 27 were found to be recombinants between *Es-1* and [*Es-2*, *Es-4*]. Five recombinants between [*Es-2*, *Es-4*] and *Es-3* were observed in the

TABLE 1

Esterase genotypes of offspring from (Lewis × ACC)F_1 females backcrossed to ACC males and from (BN × ACC)F_1 females backcrossed to ACC males

Region of recombination	Genotype				No. offspring	Total
	Es-1	*Es-2*	*Es-4*	*Es-3*		
	(Lewis × ACC)F_1 × ACC					
None	*b/b*	*b/b*	*a/a*	*b/b*	68	115
	a/b	*c/b*	*b/a*	*a/b*	47	
Es-1–[*Es-2,Es-4*]	*b/b*	*c/b*	*b/a*	*a/b*	8	11
	a/b	*b/b*	*a/a*	*a/b*	3	
[*Es-2,Es-4*]–*Es-3*	*b/b*	*b/b*	*a/a*	*a/b*	3	3
	a/b	*c/b*	*b/a*	*b/b*	0	
	(BN × ACC)F_1 × ACC					
None	*b/b*	*b/b*	*a/a*	*b/b*	68	131
	a/b	*d/b*	*b/a*	*a/b*	63	
Es-1–[*Es-2,Es-4*]	*b/b*	*d/b*	*b/a*	*a/b*	8	16
	a/b	*b/b*	*a/a*	*b/b*	8	
[*Es-2,Es-4*]–*Es-3*	*b/b*	*b/b*	*a/a*	*a/b*	2	2
	a/b	*d/b*	*b/a*	*b/b*	0	

same population. Previously (WOMACK 1973), 5/55 recombinants between *Es-1* and *Es-2* and 0/55 recombinants between *Es-2* and *Es-3* had been reported. Pooling the data from the two experiments produced the following genetic map (distances in centiMorgans): *Es-1* (9.6 ± 1.6) *Est-2*, *Es-4* (1.5 ± 0.7) *Es-3*.

Tissue expression and subcellular localization

The relative expressions of four mouse esterase loci and four rat esterase loci in different tissues and in subcellular fractions of kidney homogenates are given in Figure 1. It should be mentioned that blood was probably not totally removed from kidneys and livers by perfusion and some of the activity observed in these tissues may be attributed to erythrocyte or serum components.

Effect of inhibitors

The relative effects of esterase inhibitors on specific gene products are presented in Figure 2. Mouse *Es-3* is extremely sensitive to eserine sulfate, E600, and DFP. Mouse *Es-6* and rat *Es-4* share a common resistance to the inhibitory effects of E600 and DFP. Mouse *Es-1* and rat *Es-2* are resistant to E600, whereas mouse *Es-2* and rat *Es-1* are resistant to protamine sulfate in the concentration used in this study.

Mouse Esterase-10

Mouse *Es-2*, *Es-3* and *Es-6* catalyze the substrate 4-methylumbelliferyl acetate. In addition, this substrate is catalyzed by a slow-migrating (− to +) component

		Tissue expression					Subcellular localization (kidney)		
		Serum	RBC	Kidney	Liver	Intestine	Cell Sap	Microsomal	Lysosomal
MOUSE	ES-1	+++	+	+	+	−	+	−	−
	ES-2	+	−	++	++	+++	+	+++	+++
	ES-3	−	+	++	++	−	++	−	−
	ES-6	−	−	+++	++	−	+	+++	+++
RAT	ES-1	+	−	+	+	+++	+	+	+
	ES-2	+++	+	+	+	−	+	−	−
	ES-3	−	−	−	−	+++	−	−	−
	ES-4	−	−	+++	++	−	+	+++	+++

FIGURE 1.—Tissue expression and subcellular localization (in kidney) of mouse and rat esterases. Range of relative activity on stained gel indicated by — (no apparent stain) to +++ (most intense stain).

		Eserine Sulfate 10^{-3}M	Protamine Sulfate 300 mg/ml	Mercuric Nitrate 10^{-4} M	Sodium Fluoride 10^{-3} M	E600 10^{-4} M	DFP 10^{-4}m
MOUSE	ES-1	0	2	1	2	0	1
	ES-2	1	0	0	2	1	1
	ES-3	3	0	0	0	3	3
	ES-6	0	0	0	2	0	0
RAT	ES-1	1	0	0	2	1	1
	ES-2	0	2	1	2	0	1
	ES-3	1	0	0	0	3	3
	ES-4	0	0	0	2	0	0

FIGURE 2.—Effect of inhibitors on staining intensity of mouse and rat esterases. Range of relative inhibition indicated by 0 (no apparent inhibitory effect) to 3 (total inhibition of staining reaction).

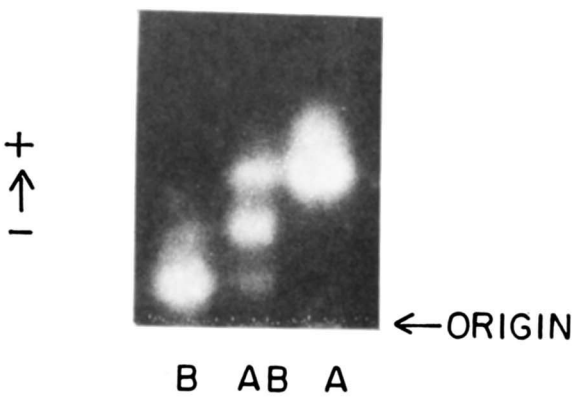

FIGURE 3.—Zymogram pattern of *Es-10* in erythrocytes. B = *M. m. molossinus*, AB = C57BL/6J × *M. m. molossinus* F_1, A = C57BL/6J.

TABLE 2

Genotypes of gametes transmitted by F_1 parents in the backcross, (C57BL/6J × M. m. molossinus)F_1 × C57BL/6J

	Number	
Es-10^a Np-1^a	20	38
Es-10^b Np-1^b	18	
Es-10^a Np-1^b	2	5
Es-10^b Np-1^a	3	
Total		43

of kidney, liver, and red cell lysates. The electrophoretic mobility of the additional band is identical in all inbred strains examined to date. *M. m. molossinus*, however, produces an even slower band (Figure 3), and F_1 hybrids produce a triple-banded zymogram indicative of a dimeric enzyme. Backcrosses of the F_1 to C57BL/6J produced 22 offspring with the single fast band and 21 offspring with the triple-banded pattern. We have designated this new autosomal locus as *Es-10* and the common and *M. m. molossinus* alleles as *Es-10^a* and *Es-10^b*, respectively. The backcross of F_1 hybrids to C57BL/6J produced evidence for linkage of *Es-10* and *Np-1* (Table 2). Polymorphism for a rat homolog of *Es-10* was not found among the strains examined (WOMACK 1973).

DISCUSSION

The rat linkage data in this report, along with the linkage data for *Ag-C* and *Es-2* reported by GASSER *et al.* (1973), suggest the map of rat Linkage Group V presented in Figure 4. Other linkage groups shown in the map were compiled from ROBINSON (1972), FRENCH, ROBERTS and SEARLE (1971), GASSER (1972), and MOUTIER, TOYAMA and CHARRIER (1973). As suggested by ROBINSON (1972), the assigned order of loci that have not been tested together should be regarded as tentative.

The "esterase cluster" provides an approach to the study of mammalian evolution. Those poorly defined but apparently related enzymes are coded by a 10 to 12 centiMorgan segment of both the rat and mouse genomes (Figure 5) that may have arisen by repeated tandem duplication of an ancestral gene. Precise genetic mapping within this segment coupled with biochemical characterization of the products of homologous genes can provide evolutionary information with regard to both genes and genomes. The value of such information depends upon the establishment of gene homology. Several approaches may be applied to problems of esterase homology. Tissue distribution, subcellular localization, and response to inhibitors are among the most useful criteria. Plasma from most mammals contains an arylesterase closely connected with the plasma albumin under most electrophoretic conditions (AUGUSTINSSON 1968). A predominant plasma esterase has been shown to be under the control of *Es-1* in the mouse and *Es-2* in the rat (POPP and POPP 1962; RUDDLE and RODERICK 1966; WOMACK

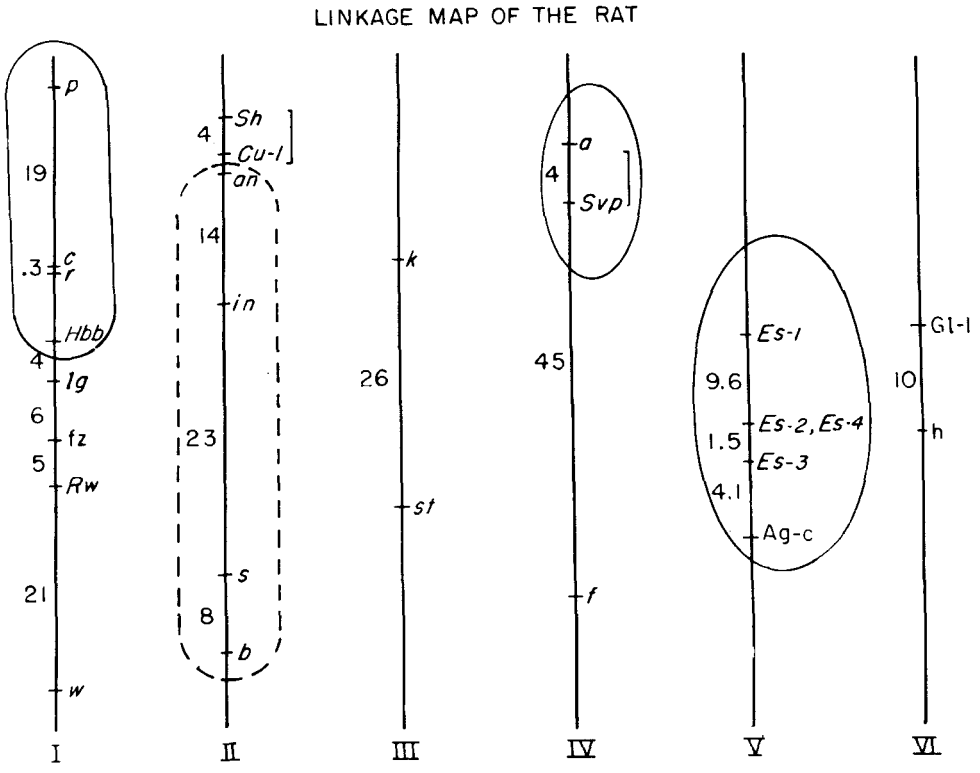

FIGURE 4.—Current linkage map of the rat. Linkages enclosed by circles are presumed homologous to portions of the mouse linkage map.

1973; GASSER *et al.* 1973). Our data suggest that these two loci are indeed homologous.

Similar electrophoretic mobility, tissue distribution and preference to butyryl substrates suggest homology between the products of rat *Es-1* and mouse *Es-2* (RUDDLE and RODERICK 1965; WOMACK 1973). The presence of the products of these loci in lysosomal and microsomal fractions and the similarity of their response to inhibitors strengthens the support for this proposed homology.

Mouse *Es-6* is a predominant slow-migrating (− to +) kidney esterase that is hormone-influenced (PETRAS and SINCLAIR 1969; WOMACK 1975). Rat *Es-4* has similar electrophoretic mobility and tissue distribution. MANDA and OKI (1967) report hormone-influenced expression in what appears to be *Es-4* in rat kidney. The products of both loci are found in lysosomes and microsomes (Figure 1) and are inhibited by sodium fluoride (Figure 2). We propose that there are homologous loci in the two species.

Rat ES-3 is an enzyme of digestive tract tissue. Genetic variation for an analogous mouse enzyme has not been reported. Likewise, mouse loci *Es-3*, *Es-5*, *Es-7*, and *Es-8* appear as yet to have no rat homologs. MOUTIER, TOYAMA and CHARRIER (1973) reported genetic variation for four rat loci using different

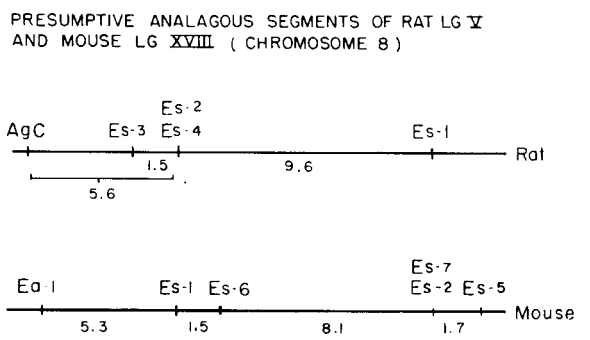

FIGURE 5.—Genetic map of rat Linkage Group V and a partial genetic map of mouse Chromosome *8* illustrating comparative linkage relationships of presumptive homologous loci.

electrophoretic media and a variety of tissue sources. Some of their loci probably correspond to ours; others may be homologous to one or more of the unmatched mouse loci. Additional testing on a common electrophoretic system must be performed.

The new mouse locus, *Es-10*, also appears non-homologous to rat loci. It is unique among mouse esterases, however, in that heterozygotes express a triple-banded pattern indicative of a multimeric enzyme. Esterase-D in humans (HOPKINSON *et al.* 1973) hydrolyzes the same substrate, has the same tissue distribution, and expresses a similar pattern in heterozygotes. On the basis of these similarities we propose that mouse *Es-10* and human *Es-D* are homologous loci. In a series of backcrosses involving Robertsonian translocations we have obtained evidence that *Np-1* is located in mouse Chromosome *14* (WOMACK *et al.*, manuscript in preparation). The linkage of *Es-10* and *Np-1* allows the assignment of mouse *Es-10* to that chromosome as well.

The suggested homology of erythrocyte antigen loci *Ag-C* in the rat and *Ea-1* in the house (GASSER *et al.* 1973) provides additional support for the conservation of a relatively large autosomal segment in the respective genomes. Linkage of two loci controlling rabbit erythrocyte esterases (SCHIFF and STORMONT 1970) and two loci controlling plasma esterases in Microtus (SEMENOFF 1972) probably represents the identity of this conserved segment in other species.

NESBITT (1974) has analyzed trypsin-banded karyotypes of rat and mouse chromosomes for banding similarities and found identical banding patterns covering about 40% of the genome of each species. She could not find a segment of rat chromosome corresponding in banding pattern to the segment of mouse Chromosome *7* thought to carry *p,c,Hbb* group. She also noted that rat and mouse *X* chromosomes differ with respect to banding patterns, although the genetic content of most mammalian *X* chromosomes is probably similar (OHNO 1969). Our data indicate that a 15 cM segment of mouse Chromosome *8* and rat linkage Group V are genetically homologous. Since NESBITT (1974) has found banding similarities between rat Chromosome *10* and mouse Chromosome *8*, it would be tempting to assign rat linkage Group V to Chromosome *10* if banding patterns in the two species were more nearly identical.

TABLE 3

Presumptive homologous linkages in the rat and mouse genome

	Genes and map distance	Linkage group
rat	*p* (19) *c* (9) *Hbb*	L.G. I
mouse	*p* (15) *c* (5) *Hbb*	L.G. I (Chromosome *7*)
rat	*an* (45) *b*	L.G. II
mouse	*an* (7) *b*	L.G. VIII (Chromosome *4*)
rat	*a* (4) *Svp*	L.G. IV
mouse	*a* (9) *Svp*	L.G. V (Chromosome *2*)
rat	*Ag-C* (5.6) *Es-2* (0) *Es-4* (9.6) *Es-1*	L.G. V
mouse	*Ea-1* (5.3) *Es-1* (1.5) *Es-6* (8.1) *Es-2*	L.G. XVIII (Chromosome *8*)

Data from ROBINSON (1972), GASSER *et al.* (1973), and WOMACK (1975).

Regions of homology between rat and mouse genomes appear to be extensive. The rat linkages enclosed by circle in Figure 4 have apparent homologies in the mouse genome (GASSER *et al.* 1973). These homologies are presented in Table 3. Similar recombination frequencies in the rat and the mouse have been observed in both the *p,c,Hbb* and the *a,Svp* linkages (GASSER 1972). Chiasma frequencies in the two species appear to be roughly equivalent (HUSKINS and HEARNE 1936; BRYDEN 1932; ROBINSON 1972); thus mapping units in the two species are assumed equivalent in the absence of either supporting or non-supporting evidence. Of the six known linkage groups in the rat, four appear to have homologs in the more extensive mouse map. The two without recognized homologs are pairs of linked loci. It appears that the genetic homology between the species may exceed the 40% banding homology and that the conservation of genetic content has exceeded the conservation of banding similarities.

The linkage of genes for brown hair and anemia is much tighter in the mouse than in the rat (CASTLE and KING 1941). Coat color and disease homologies are difficult to establish and these two loci may not be homologous in the two species. On the other hand, an internal rearrangement such as an inversion, a deletion, or an insertion may have accompanied the evolution of the two species. NESBITT (1974) found no evidence of inversions in her study of comparative banding patterns.

A reasonable explanation for the conservation of the *X* chromosome in mammalian evolution has been presented (OHNO 1969). The case for obligatory linkage is more difficult to argue in autosomal conservation, especially when 10 to 12 cM segments are involved. Autosomal conservation of shorter segments, perhaps contiguous tandemly duplicated loci, are not uncommon. For example, two carbonic anhydrase loci are linked in the guinea pig (CARTER 1972), the pig-tailed macaque (DESIMONE, LINDE and TASHIAN 1973), and the mouse (EICHER *et al.*, 1976). Two amylase loci are linked in humans (MERRITT, RIVAS and WARD 1972) and the mouse (KAPLAN, CHAPMAN and RUDDLE 1973), as are immunoglobin heavy chain loci (HERZENBERG 1964; KUNKEL *et al.* 1969). Conservation of autosomal linkage is therefore real, but its extent and relationship to chromosome banding is yet unknown.

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