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Biochemical and genetic characterization of esterase-27 (ES-27), the major plasma cholinesterase of the house mouse (*Mus musculus*)*

O. H. VON DEIMLING† AND A. GAA

Pathologisches Institut, Abteilung für Chemische Pathologie, Universtität Freiburg, D-7800 Freiburg i. Br., Bundesrepublik Deutschland

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Summary

Esterase-27A (ES-27A) was characterized in strain A/WySnA by a cascade of seven bands seen after disc electrophoresis of serum and subsequent staining for esterase. ES-27A catalyses the hydrolysis of thiocholine butyrate and is strongly inhibited by 100 μM tetraisopropyl pyrophosphamide (isoOMPA). Hence, the enzyme was concluded to be a cholinesterase EC 3.1.1.8. A heat-labile form termed ES-27B was represented by strain AKR/Han. From a three-point cross (AKR/Han, A/Wy) and a five-point cross (AKR/Han, SEG/1), the gene order on chromosome 3 was concluded to be centromere–*Car-2–Es-26–Es-27–Amy-1–Adh-1*.

1. Introduction

Heritable variation in a plasma cholinesterase of inbred mice was reported by Angel *et al.* (1967). The strains AKR and SJL were found to exhibit significantly less cholinesterase activity (measured quantitatively) than many other inbred strains. No other investigations of the genetics of plasma cholinesterase have since been reported. A variation in heat lability of an electrophoretically monomorphic mouse serum esterase, EST-A, was described by Bonhomme & Selander (1978): in strains AKR and SJL, EST-A was found to be inactivated by incubation of plasma at 50 °C for 20 min whereas 38 other inbred strains expressed a heat-stable form of EST-A. By examining various backcrosses it was concluded that this characteristic showed a codominant pattern of inheritance. The gene locus controlling the variation in heat-lability, however, was not identified.

Provisional data obtained in our laboratory a few years ago indicated that EST-A was a cholinesterase and that the EST-A locus was on chromosome 3 (Deimling, 1985). By typing the esterases of inbred laboratory strains kept in The Netherlands we found eight heat-sensitive forms of cholinesterase among 57 strains (Hilgers *et al.* 1988). Further studies revealed that the cholinesterase pattern in mouse serum was more complex than initially believed. Here we report the results of investigations on the biochemical nature and heredity of serum cholinesterase which indicate that at least seven electrophoretically separate enzymes (pseudoisoymes), including EST-A, are controlled by a single locus. According to the guidelines for gene nomenclature (International Committee for Standardized Genetic Nomenclature for Mice, 1985) we propose the systematic designation *Es-27* for this locus.

2. Material and methods

(i) *Animals and samples*

Strains A/WySnA, AKR/Han, BALB/cJ, CBA/J, C3H/He, C57BL/6J, C57BL/10Sn, DBA/2J, FTC/CpbU, IS/Cam, PUC/1Fre, PWD/Ph, PWK/Ph, SEG/1 (a partly inbred strain of *M. spretus*, F12) SK/Cam, and SJL/J were kept in the animal house of the Institute of Pathology in Freiburg. Mice of strain AKR/N were a gift of Dr H. Mossmann, Max Planck Institute of Immunology, Freiburg. MOLC/Rk, MOLD/Rk, and MOLE/Rk were obtained from Dr Roderick, Bar Harbour. Sera of MOLF/Ei and CAST/Ei were a gift of Dr E. Eicher, Bar Harbour.

Blood was obtained from the tail tip from mice which were in slight ether narcosis. Red blood cells were washed three times in isotonic saline and were lysed in 0·2% (w/v), aqueous Triton X-100 (red cells: 0·2% Triton X-100, 1:4 v/v). The following organs were examined: liver, kidney, lung, heart, tongue, and testis. Samples were prepared by homogenizing the organs in a threefold or sevenfold volume of 0·2%

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† Corresponding author.

Table 1. *Allele distribution at* Es-27 *and at four chromosome 3 loci* used for linkage studies*

	Car-2	*Es-26*	*Es-27*	*Amy-1*	*Adh-1*
A/WySnA	b	b	a	a	a
AKR/Han	a	c	b	a	a
SEG/1	r†	b	a	e†	b‡

* Assignment to chromosome 3 of *Car-2*, *Es-26*, *Amy-1*, and *Adh-1* had been established by Eicher *et al.* (1976), Deimling *et al.* (1984), and Bonhomme *et al.* (1979), respectively.
† Allelic designation according to Bonhomme *et al.* (1979).
‡ Allelic designation according to Britton & Thaler (1978).

aqueous Triton X-100 followed by centrifuging at 100000 *g* for 30 min. The supernatants were stored at −70 °C and recentrifuged at 25000 *g* for 10 min prior to use. In order to investigate heat stability samples were heated to 40 or 50 °C, for 15, 30, 60, or 120 min.

For linkage studies, two series of backcrosses were produced involving A/WySnA, AKR/Han, and SEG/1. The allelic segregation was studied at *Es-27* and at four marker loci (Table 1) covering a large segment of chromosome 3.

(ii) *Separation of esterases*

Polyacrylamide disc electrophoresis was performed in slab gels (135 × 90 × 1 mm, 10 or 12 channels) containing 7·3 or 7·8% (w/v) acrylamide and was run using a Tris/HCl buffer system, pH 8·9 (Maurer, 1971). Sera, 12 μl, or tissue supernatants, 10–25 μl, were applied to each channel, and were subsequently separated at a field strength of about 16 V/cm for about 100 min.

(iii) *Staining of esterases*

Conventional procedures. Staining was performed in 150 mM phosphate buffer pH 6·5 at 20 or 37 °C with indigogenic (Holt, 1958) or azo dye (Gomori, 1952) methods. The substrates used were α-naphthyl acetate (αN-O-2: 32 mg/100 ml)/fast red TR, naphthol AS-D acetate (NASD-O-2: 16 mg/100 ml)/fast red TR, *N*-acetyl-L-methionine-α-naphthyl ester (αN-O-*met*: 20 mg/100 ml)/fast red TR and 5-bromoindoxyl acetate (5BrI-O-2: 35 mg/100 ml)/NBT.

Thiocholine procedures. The method of Karnovsky & Roots (1964) was modified as follows: (1) The gels were rinsed in 10 mM phosphate buffer pH 6·0 for 5 min followed by incubation in 10 mM phosphate buffer pH 6·0 containing 6 mM acetyl thiocholine iodide (Ch-S-2: Sigma A 5751 or Serva 10570) at 20 °C for 20 min. In some experiments the substrate was replaced by 6 mM butyryl thiocholine iodide (Ch-S-4: Sigma B 3253 or Serva 15510). In few cases, the pH of substrate solution was 7·3. (2) After another rinsing in 50 ml 150 mM acetate buffer pH 5·6 containing 300 mg sodium citrate ($C_6H_5O_7Na_3.2H_2O$) the gels were incubated in the precipitation solution at 40 °C for 40–60 min, until brown bands appeared indicating the cholinesterase activity.

Precipitation solution. 1450 mg sodium acetate ($C_2H_3O_2Na.3H_2O$), 120 mg sodium citrate ($C_6H_5O_7Na_3.2H_2O$) 60 mg cupric sulphate ($CuSO_4.7H_2O$), 27 mg potassium ferricyanide $K_3Fe(CN)_6$. Fill up with water to 80 ml and adjust with acetic acid to pH 5·6.

(iv) *Inhibitors of esterases*

Inhibition was performed at 20 °C for 30 min prior to staining by incubating the gels in 10 mM phosphate buffer pH 7·3 containing the inhibitor. The following inhibitors were applied: 100 μM eserine, 200 μM bis-*p*-nitrophenyl phosphate, BNPP (Heymann & Krisch, 1967) 200 μM di-isopropyl fluorophosphate (DFP), 100 μM tetraisopropyl pyrophosphamide (isoOMPA, Sigma T 1505) (Aldridge, 1953) or 50 mM 1,5-bis-(4-allyl dimethylammoniumphenyl)-pentane-3-one dibromide (BW 2284C51; Sigma A 9013) (Bisso, 1986).

(v) *Concanavalin A treatment*

For affinity experiments, stock concanavalin A-sepharose was prepared as recommended (Bog-Hansen *et al.* 1983). ConA-sepharose (Pharmacia, Freiburg) was washed with a fivefold volume of a 1:4 (v/v) dilution of spacer gel buffer and centrifuged. After removing the supernatant washing and centrifuging was repeated twice. Finally, the initial concentration of the ConA-sepharose was restored by adding the corresponding amount of the diluted buffer. This concentration served as stock. Inactive ConA-sepharose for controls was produced by repeated washing with 50 mM acetate buffer, pH 4·5, containing 5 mM-EDTA for removing the essential metal ions.

(vi) *Typing of CAR-2*

Isoelectric focusing was carried out on commercial gel plates, pH 3·5–9·5 (LKB, Bromma, Sweden). Seven μl of red-cell lysates were applied anodally (position 7). Voltage was limited to 1500 V, current to 40 mA, and

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power to 24 W. After isoelectric focusing for 90 min, the gels were incubated in trichloracetic acid:sulphosalicylic acid:redistilled water, 11·5:3·5:85·0 (w/w/v), for 15–20 h. Subsequently the gels were stained with Coomassie brilliant blue R 250. CAR-2A and CAR-2B focused as strong bands at about pH 6·3 and 6·4 respectively.

(vii) *Typing of ES-26*

By improving a method described previously (Deimling *et al.* 1984) isoelectric focusing was carried out using LKB gel plates, pH 3·5–9·5. Fresh liver supernatants (15–20 μl) were applied anodally (position 7), limiting the voltage to 1500 V, the current to 40 mA, and the power to 24 W. After isoelectric focusing for 90 min, the gels were incubated in 150 mM phosphate buffer pH 7·3 containing 10 mM-*p*-chloromercuriphenylsulphonate, at 20 °C for 30 min. By this procedure ES-26 was demasked from the cofocusing bands of ES-18. Thereafter, ES-26 was stained in 150 mM phosphate buffer pH 7·3 containing 2·5 mM indoxyl butyrate (I-O-4) as substrate and 0·6 mM nitrotetrazolium blue (NBT) as oxidizing reagent (McGadey, 1967).

(viii) *Typing of AMY-1*

Kidney supernatants (10 μl) were applied anodally (position 7) to a LKB gel plate, pH 3·5–9·5, and isoelectric focusing was carried out as for ES-26. The gels were stained with iodine using an overlayer of starch-agarose-gel, as described by Kaplan *et al.* (1973).

(ix) *Typing of ADH-1*

ADH-1 was typed after isoelectric focusing on LKB gel plates, pH 3·5–9·5, of liver supernatants (1:4 w/v). Fifty μl each was applied to the gel at positions 6 and 7. Voltage, current and power was limited to 1500 V, 40 mA and 24 W, respectively. After isoelectric focusing, the gels were rinsed in 100 mM Tris/HCl buffer pH 8·0 for 15 min. Staining was performed using ethanol as the substrate and nitrotetrazolium blue (NBT) as tetrazolium salt as described previously (Holmes, 1978). The ADH-1 bands focused near the cathode.

3. Results

(i) *Staining pattern of serum esterases*

Table 2 summarizes the electrophoretic bands stainable with αN-O-2 or Ch-S-2. These bands can be subdivided into four groups:

(1) Three bands or zones assigned to carboxylesterase isozymes which have been defined previously by their gene locus, namely ES-1 (Popp & Popp, 1962), ES-2 (Petras, 1963) and ES-5 (Petras & Biddle, 1967) (Figs. 1, 2).

(2) Four bands assigned to genetically non-defined carboxylesterases which do not stain with Ch-S-2 or Ch-S-4 as the substrate, and provisionally designated the non-standardized term SE-I (serum esterase-I) SE-II, SE-III (Ronai *et al.* 1985), and SE-V (Figs. 1*a*; 2*a*).

(3) Four bands capable of hydrolyzing Ch-S-2 but not Ch-S-4 and strongly inhibited by BW2284C51, termed SE-VI (Fig. 2*b*, *c*), SE-VII, SE-VIII, and SE-

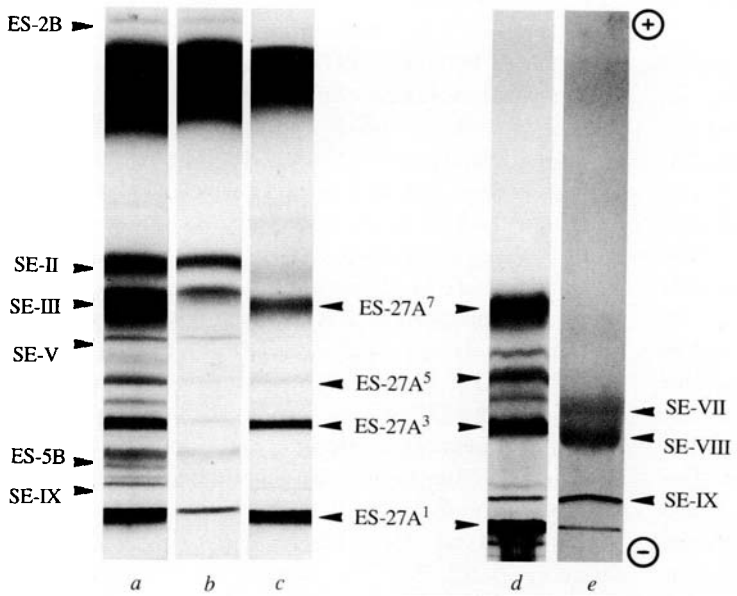

Fig. 1. Serum of an A/WySnA female following disc electrophoresis (7·3% acrylamide, pH 8·9) and subsequent staining for esterase, using αN-O-2 (*a–c*) or Ch-S-2 (*d*, *e*) as the substrate. The gel load was 10 μl. (*a*) Untreated serum. (*b*) Serum incubated with the fourfold volume of ConA-sepharose for 30 min prior to electrophoresis. (*c*) Untreated serum, inhibition of the gel by 1 mM-BNPP for 30 min. (*d*) Untreated serum. (*e*) Untreated serum, inhibition of the gel by 250 μM-isoOMPA for 30 min.

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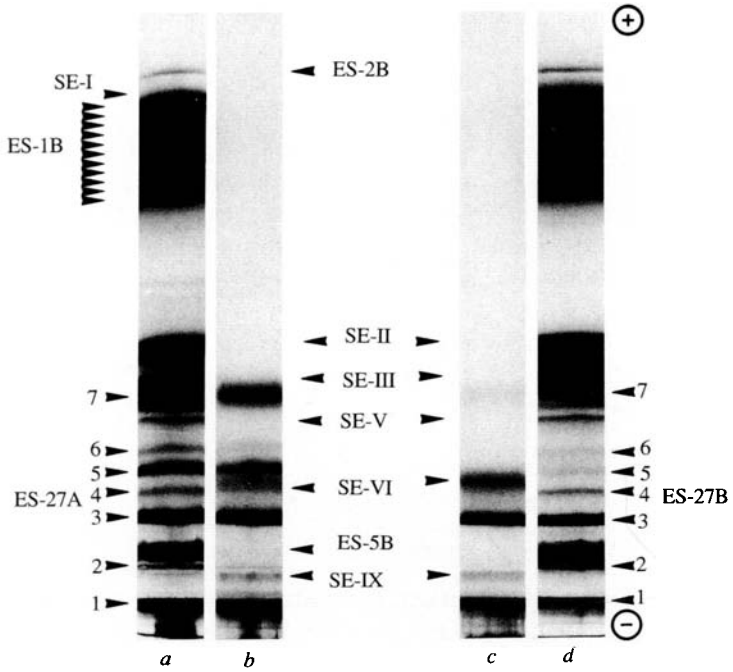

Fig. 2. Serum of A/WySnA (*a*, *b*; high cholinesterase activity strain) and AKR/Han (*c*, *d*; low cholinesterase activity strain) females following disc electrophoresis (7·8% acrylamide, Tris-glycine buffer pH 8·9) and subsequent staining for esterase. The gel load was 12 μl per channel. The gels were stained for 30 and 20 min using αN-O-2 (*a*, *d*) and Ch-S-2 (*b*, *c*) as substrates.

IX (Fig. 1*e*) and possibly representing acetylcholine esterases EC 3.1.1.7.

(4) Seven bands hydrolysing Ch-S-4, Ch-S-2 and αN-O-2 (Fig. 2*a*, *b*).

The seven bands of group 4 shared several properties and may represent a group of pseudoisozymes (Lebherz, 1983) under the control of a single structural locus, *Es-27* (see below). The phenotype present in A/WySnA was termed ES-27A and the pseudoisozymes were labelled by indices 1–7 as shown in Table 2 and Fig. 2*a*.

The most sensitive substrate for demonstrating the seven pseudoisozymes of ES-27 was αN-O-2 (Fig. 2). However other enzymes such as carboxylesterases were strongly stained with this substrate and ES-27A^7 was partly masked by SE-III (Fig. 1*a*, *c*, *d*). Although the latter problem could be overcome by inhibiting SE-III with BNPP (because SE-III is much more sensitive to this compound than ES-27A^7, Fig. 1*c*) the staining method of choice for demonstrating the various ES-27A pseudoisozymes was the thiocholine method at pH 6·0. Ch-S-2 was the most suitable substrate. When incubated with this substrate, the pseudoisozymes appeared in the temporal sequence ES-27A^1, A^3, A^7, A^5, A^4, A^6, A^2, according to their decreasing activity. They were more active in females than in males (Fig. 3) and the activity in females increased during pregnancy except for ES-27A^1 (Fig. 3*c*). They disappeared after treating the gels with 200 μM-DFP or 100 μM-isoOMPA (Fig. 1*e*); but remained unchanged when 50 μM-BW 2284C51 was used as inhibitor (not shown). Hence, we conclude that ES-27 is a cholinesterase, EC 3.1.1.8.

Substrate incubation at pH 7·3 gave similar results regarding ES-27. However, other bands such as SE-VI and SE-IX were more prominent at this pH than at pH 6·0 (cf. Figs. 3*i*, 4*f*).

(ii) *Allozymes of ES-27 and strain distribution*

The allelic nomenclature of ES-27 is not uniform in the literature. When only two forms of the cholinesterase were distinguished the designations S (heat-sensitive) and R (heat-resistant) were used (Bonhomme & Selander, 1978). These designations were maintained even after the enzyme designation EST-A was replaced by ES-27 (Hilgers *et al.* 1988). By anticipating more detailed results (see below) at least one heat-sensitive and three heat-resistant forms are distinguished by now. Therefore, the designations R and S were changed to a, c, d (heat-resistant) and to b (heat sensitive) respectively.

The phenotype ES-27A was observed in many other laboratory strains: A/J, CAST/Ei, IST/Ola, MOLC/Rk, MOLD/Rk, MOLE/Rk, MOLF/Ei, PUC/1Fre, SEG/1, and SK/Cam. Since the activity of the separate pseudoisozymes depends on the hormonal status (see Discussion), the genetic background of the various inbred strains may influence some details of the phenotype. Particularly, the expression of the weaker band ES-27A^6 may vary from strain to strain.

Another phenotype consisting of seven bands of similar electrophoretic mobility but of considerably reduced activity was found in AKR/N and AKR/Han. This phenotype was designated ES-27B with the

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Table 2. *Serum esterase bands of A/WySnA in order of decreasing R_m values. Characteristics and provisional classification*

Designation		T_m value† (%)	Staining and Inhibition‡						Endocrine status		Genetic variation in inbred strains	Provisional classification EC number
			Ch-S-2				αN-O-2					
Provisional	Genetic*		Control	isoOMPA	BW2284C51	Ch-S-4	Control	BNPP	Non-pregnant	Day 18 of pregnancy		
	ES-2B	0·905	−	−	−	−	+	−	♀ = ♂		Yes	3.1.1.1
SE-I		0·860	−	−	−	−	+	−	♀ = ♂		Yes	3.1.1.1
	ES-1B	0·706	−	−	−	−	+	(+)	♀ > ♂		Yes	3.1.1.1
SE-II		0·480	−	−	−	−	+	−	♀ < ♂		Yes	3.1.1.1
SE-III		0·400	−	−	−	−	+	−	?		Yes	3.1.1.1
	ES-27A[7]	0·379	+	−	+	+	+	+	♀ > ♂	♀ ≫ ♂	Yes	3.1.1.8
SE-V		0·352	−	−	−	−	+	−	?		?	3.1.1.1
	ES-27A[6]	0·330	+	−	+	+	+	+	♀ > ♂	♀ ≫ ♂	Yes	3.1.1.8
	ES-27A[5]	0·291	+	−	+	+	+	+	♀ > ♂	♀ ≫ ♂	Yes	3.1.1.8
SE-VI		0·281	+	+	−	−	−	−	♀ = ♂		No	3.1.1.7
SE-VII		0·275	+	+	−	−	+	−	♀ = ♂		No	3.1.1.7
	ES-27A[1]	0·252	+	−	+	+	+	+	♀ > ♂	♀ ≫ ♂	Yes	3.1.1.8
	ES-27A[3]	0·202	+	−	+	+	+	+	♀ > ♂	♀ ≫ ♂	Yes	3.1.1.8
SE-VIII		0·194	+	+	−	−	+	?	♀ = ♂		No	3.1.1.7
	ES-5B	0·160	−	−	−	−	+	−	♀ = ♂		Yes	3.1.1.1
	ES-27A[2]	0·123	+	−	+	+	+	+	♀ > ♂	♀ ≫ ♂	Yes	3.1.1.8
SE-IX		0·110	+	+	−	−	+	+	♀ = ♂		No	3.1.1.7
	ES-27[1]	0·057	+	−	+	+	+	+	♀ > ♂		Yes	3.1.1.8

* Genetic designation of ES-27 as proposed in this study.
† 7·3% acrylamide concentration, Tris/HCl buffer system, pH 8·9.
‡ +, Bands clearly stained; inhibitory effect weak or negligable; (+), inhibitory effect clearly visible; −, bands not or very weakly stained; inhibitory effect strong.

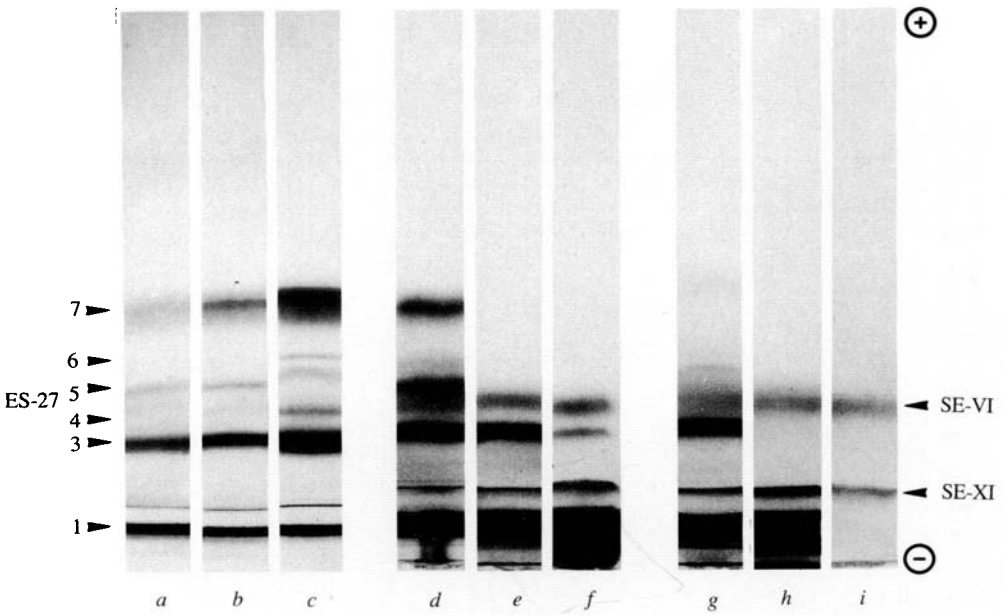

Fig. 3. Serum of A/WySnA (*a–f*) and AKR/Han mice (*g–i*) following disc electrophoresis [7·3% acrylamide (*a–c*); 7·8% acrylamide (*d–i*)] and subsequent staining for cholinesterase, using Ch-S-2 as the substrate. The gel load was 8 μl (*a–c*) or 12 μl (*d–i*), the incubation period was 25 min (*a–c*) or 20 min (*d–i*) at 37 °C. Lanes *a–c* show the influence of sex and of pregnancy on ES-27A bands: (*a*) male; (*b*) female; (*c*) pregnant female, day 18. Lanes *d–i* show the influence of heat treatment on the various pseudoisozymes of ES-27A (*d–f*) and ES-27B (*g–i*). (*d, g*) Untreated controls; (*e, h*) sera heated at 50 °C for 15 min; (*f, i*) sera heated at 50 °C for 120 min.

corresponding indices 1–7 (Fig. 2*d*). The order of activity of the 7 pseudoisozymes was about the same as described for ES-27A. Because of the generally low activity of ES-27B, only the two strongest bands, ES-27B[1] and ES-27B[3], could be clearly demonstrated using CH-S-2 as the substrate (Fig. 2*c*). ES-27B[7] appeared as a faint shadow (Fig. 2*c*). However, the weak pseudoisozymes ES27B[2], ES-27B[4], ES-27B[5] and ES-27B[6] could be clearly seen if αN-O-2 was used as the substrate (Fig. 2*d*).

A third phenotype was observed in IS/Cam (not shown). This phenotype, also seven-banded, was characterized by a high sensitivity towards BNPP. As backcrosses have not been performed with IS/Cam, it is not yet clear whether this variation is allelic at *Es-27*.

Another seven-banded phenotype of serum cholinesterase occurs in PWD/Ph and in PWK/Ph (not shown). Originally, this phenotype was thought to be identical with that of BALB/c and, therefore, was termed ES-27A (Deimling *et al.* 1988). However, the most anodal band of this phenotype shows a stronger activity than that of ES-27A[7] and its electrophoretic mobility was slightly reduced. Also, sexual dimorphism of this phenotype was less clear than in the case of ES-27A and ES-27B. As for the IS/Cam phenotype, it remains unclear whether this phenotype is allelic at ES-27. Provisionally the genetic designations ES-27C and ES-27D may be reserved for the serum cholinesterases of IS/Cam and PWD/Ph, PWK/Ph respectively.

(iii) *Heat treatment*

Sera of A/WySnA and AKR/Han females were incubated at 40 or 50 °C for 0–120 min. The pseudoisozymes of ES-27B were inactivated at the lower temperature and, at 50 °C more rapidly than those of ES-27A (Fig. 3*d–i*; Table 3). Thus, the heat inactivation was a useful tool for distinguishing the ES-27B from the ES-27AB phenotypes of backcrosses (see below).

(iv) *Binding to Concavalin A*

Each 20 μl serum of A/WySnA female were mixed with 80 μl of washed stock ConA-sepharose, either undiluted or diluted 1:4 (v/v) or 1:16 (v/v) using spacer gel buffer: aqua dest 1:4 (v/v). After incubation for 20 min, the mixtures were centrifuged and 40 μl of the supernatants were subjected to disc electrophoresis. ConA-sepharose did not change the banding pattern at the 1:16 dilution. The 1:14 dilution led to a slight decrease of the intensity of ES-27A bands. However in the channel containing the undiluted ConA-sepharose, the various ES-27 activities were considerably weakened whereas the other major esterase bands were still present (Fig. 1*b*). Inactivated ConA-sepharose did not weaken the activities of ES-27 bands. It would thus appear that ES-27 was bound to active ConA-sepharose, indicating that it is a glycoprotein. Variation in the carbohydrate moiety presumably underlies the different response of ES-

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Table 3. *Effect of heat on ES-27A and ES-27B (four most active pseudoisozymes each). The substrate was Ch-S-2. Electrophoretic conditions as in Fig. 2*

Heating period	Temperature (°C)	Relative activity*							
		ES-27A^{7}	ES-27A^{5}	ES-27A^{3}	ES-27A^{1}	ES-27B^{7}	ES-27B^{5}	ES-27B^{3}	ES-27B^{1}
0′	40	++	+	+++	++++	+	(+)	++	++++
	50	++	+	+++	++++	+	(+)	++	++++
15′	40	++	+	+++	++++	−	−	++	+++
	50	−	−	++	++++	−	−	−	+++
60′	40	+	+	+++	++++	−	−	++	++++
	50	−	−	++	++++	−	−	−	−
120′	40	(+)	(+)	+++	++++	−	−	+	++++
	50	−	−	+	++	−	−	−	−

* − not visible; (+) very weak; + weak; ++ moderate; +++ strong; ++++ very strong.

Table 4. *Segregation of alleles at* Car-2, Es-26, *and* Es-27 *in the backcross (A/WySnA × AKR/Han) F_1 × AKR/Han*

	Genetic locus			Number		
	Car-2	*Es-26*	*Es-27*	♂	♀	Σ
No recombination	a (alleles carried by AKR/Han)	c	b	10	11	21
	b (alleles carried by A/WySnA)	b	a	9	2	11
Recombination *Car-2–Es-26*	a	b	a	3	3	6
	b	c	b	0	8	8
Total				22	24	46

Percentage recombination *Car-2–Es-26* 14/46 = 30·4 ± 6·8
Gene order: *Car-2* (*Es-26–Es-27*)

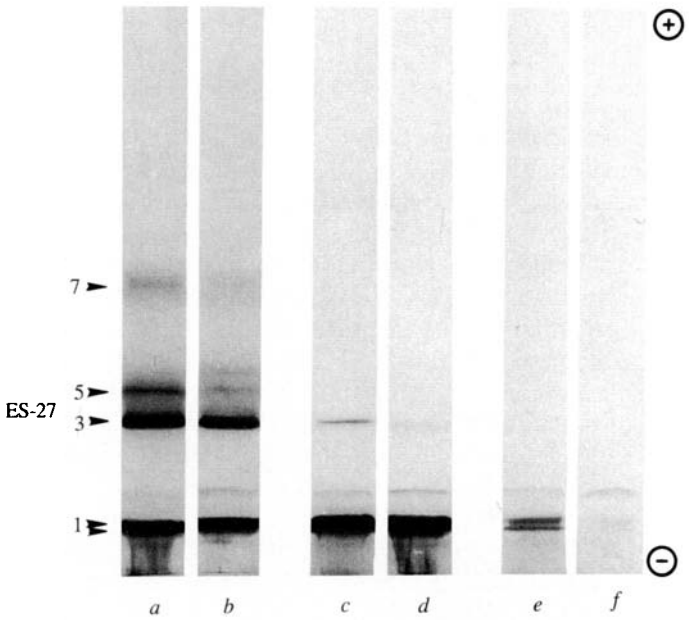

Fig. 4. Sera of two different backcross progeny (SEG/1 × AKR/Han) F_1 × AKR/Han following disc electrophoresis (7·3% polyacrylamide, pH 8·9) and subsequent staining for esterase at pH 6·0 using Ch-S-2 as the substrate. Gel load was 10 μl per channel. The ES-27AB (channels *a*, *c*, *e*) and ES-27B (channels *b*, *d*, *f*) phenotypes are demonstrated at the levels of the four strongest bands (arrows) of the ES-27 cascades. Channels *a* and *b*, bands R_m 0·291 and 0·379 after heating the sera to 40 °C for 15 min. Channels *c* and *d*, bands R_m 0·202 after heating the sera to 50 °C for 15 min. Channels *e* and *f*, bands R_m 0·057 as seen after preheating the sera to 50 °C for 60 min.

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Table 5. *Segregation of alleles at* Car-2, Es-26, Es-27, Amy-1, *and* Adh-1 *in the backcross* (*AKR/Han* × *SEG/1*) F_1 × *AKR/Han*

	Genetic locus					Number		
	Car-2	*Es-26*	*Es-27*	*Amy-1*	*Adh-1*	♂	♀	Σ
No recombination	a	c	b	a	a	11	14	25
		(alleles carried by AKR/Han)						
	r	b	a	e	b	8	12	20
		(alleles carried by SEG/1)						
Single recombination	a	b	a	e	b	5	2	7
	r	c	b	a	a	7	6	13
	a	c	a	e	b	1	0	1
	r	b	b	a	a	0	0	0
	a	c	b	e	b	3	3	6
	r	b	a	a	a	6	5	11
	a	c	b	a	b	2	1	3
	r	b	a	e	a	3	1	4
Double recombination	a	b	a	e	a	4	3	7
	r	c	b	a	b	1	1	2
	a	b	a	a	a	0	0	0
	r	c	b	e	b	0	1	1
Total						51	49	100
Percentage recombination								
Car-2–Es-26	30/100 = 30 ± 4·6							
Es-26–Es-27	1/100 = 1 ± 1							
Es-27–Amy-1	18/100 = 18 ± 3·8							
Amy-1–Adh-1	16/100 = 16 ± 3·7							

27A[5] to ConA-sepharose treatment; indeed, such variations may be responsible for the occurrence of all the ES-27 electrophoretic bands.

(v) *Inheritance and linkage*

Females of the cross (AKR/Han × A/WySnA) were backcrossed to AKR/Han males. The segregation of ES-27 in the backcross progeny was examined after heating the sera prior to electrophoresis basing on the different temperature sensitivity of ES-27A and ES-27B outlined in Table 3. Pseudoisozyme R_m 0·057 was typed after heat treatment at 50 °C for 60 min. Pseudoisozyme R_m 0·202 was scored after heat treatment at 50 °C for 15 min. Pseudoisozymes R_m 0·291 and R_m 0·379 were scored after keeping the sera at 40 °C for 15 min. The four pseudoisozymes showed concordant segregation throughout indicating that they are different forms of one enzyme, ES-27. Two classes of progeny were distinguished, ES-27B and ES-27AB, at a ratio of 29:17 (Table 4). It was concluded that ES-27A and ES-27B are inherited as allelic products at a single autosomal locus designated *Es-27*, with the alles *ES-27*^{a} (originally termed *Es-27*^{r}, heat resistant; Hilgers *et al.* 1988) in A/WySnA and *Es-27*^{b} (originally termed *Es-27*^{s}, heat sensitive) in AKR/Han.

Similar results were obtained from a second backcross, (AKR/Han × SEG/1) F_1 × AKR/Han (Table 5). The different appearance of four components (bands R_m 0·057, 0·202, 0·291, 0·379) of ES-27A and ES-27AB following heat treatment is documented in Fig. 4. Linkage of *Es-27* to loci on chromosome 3 was established as indicated in Tables 4 and 5. We suggest the gene order *Car-2*, *Es-26*, *ES-27*, *Amy-1*, *Adh-1* as the most probable, since it gives the fewest double recombinations.

4. Discussion

Biochemical properties and genetic assignment to chromosome 3 of ES-27 are described in the present report. ES-27 is characterized by a cascade of seven bands seen after disc electrophoresis of serum and subsequent staining for esterase. Four phenotypes were distinguished: ES-27A occurs in A/WySnA and in 57 other laboratory strains or substrains including those reported previously (Hilgers *et al.* 1988). ES-27B occurs in AKR/Han and in eight other laboratory strains reported previously (Hilgers *et al.* 1988). ES-27C is reserved for the phenotype occurring in IS/Cam. ES-27D was provisionally assigned to PWD/Ph and PWK/Ph.

Certain components of the ES-27 cascade have already been mentioned in the literature. Increased activities of distinct mouse serum esterases in females, particularly during pregnancy, have been described by Allen & Moore (1966), by Pantelouris & Arnason (1966), by Oki *et al.* (1967), and by Tyndall & Daniel (1975). The probable synonyms are compiled in Table 6. ES-27A[1] may be identical with the EST-A band described by Bonhomme & Selander (1978). ES-27A[3]

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Table 6. *Pseudoisozymes of ES-27A and probable synonymous esterase bands of mouse serum described previously*

	Esterase band						References
ES-27	A^1	A^3	A^4	A^5	A^6	A^7	Present work
	17	13		←10–18→		7	Allen & Moore (1966)
		11		←10→		5	Pantelouris & Arnason (1966)
	C4	C3				C2	Oki *et al.* (1967)
	12	8		←7,	6→	5	Tyndall & Daniel (1975)
	3	6	7	8	9	11	Davidson *et al.* (1978)
	Est-A						Bonhomme & Selander (1978)
	1	3				5	Bisso (1986)

Table 7. *Cumulative percentages of recombination between four loci on chromosome 3 based on data from three backcross series*

Backcross series*	Loci	Recombination ratio (RR)	Cumulative RR	Cumulative RR (%)
1	*Car-2–Es-26*	22/81		
2		14/46	66/227	29·1 ± 3·0
3		30/100		
2	*Es-26–Es-27*	0/46	1/146	0·68 ± 0·68
3		1/100		
1	*Es-26–Amy-1*	11/81	30/181	16·6 ± 2·8
3		19/100		

* Backcross series:
(1) (A/WySnA × *M.m. mol*) F_1 × A/WySnA (Deimling *et al.* 1984).
(2) (A/WySnA × AKR/Han) F_1 × AKR/Han (this report).
(3) (AKR/Han × SEG/1) × AKR/Han (this report).

through ES-27A^7 may correspond to those serum esterases which showed a highly increased activity upon treatment of the mice with diethyl nitrosamine (Davidson *et al.* 1978). ES-27 as a whole may correspond to the plasma cholinesterase activity measured by Angel *et al.* (1967) which was characterized by significantly higher activities in females than in males. ES-27 may be identical with the trichloroethylene-inducible plasma cholinesterase described by Kjellstrand *et al.* (1983) which also showed higher activity in females than in males.

The multiplicity of electrophoretic bands of ES-27 may, in part, be brought about by the presence of monomeric, dimeric, and tetrameric forms of cholinesterase, as established in rat serum (Massoulie & Bon, 1982). Some multiple forms may differ in their sialic acid content. Rat cholinesterases are capable of associating with non-catalytic serum components which may also give rise to additional electrophoretic forms (Massoulie & Bon, 1982). The activity levels of mouse serum cholinesterase have been considered to be regulated by both estradiol and testosterone (Oki *et al.* 1967). Thus, the hormonal status of the animals and the ability to form associates may both contribute to the minor variations in the ES-27 banding pattern observed in mice with different genetic backgrounds.

Our results indicate that the seven pseudoisozymes of ES-27 are controlled by a single autosomal locus designated *Es-27*. We conclude that *Es-27* is a structural locus. From our linkage data, locus *Es-27* was mapped on chromosome 3 close to *Es-26*. The distance between *Es-26* and *Es-27* appears to be smaller than originally thought (Deimling, 1986). The cumulative distances between the loci involved in this study are given in Table 7.

There are at least five loci on chromosome 3 which control enzymes exhibiting esterase activity: *Car-1*, *Car-2*, *Es-16*, *Es-26*, and *Es-27*. Biochemically, ES-27 appears to be more closely related to ES-26 than to the other three enzymes: ES-26 and ES-27 are outstanding in their sensitivity to eserine and ability to hydrolyse Ch-S-2. We suggest that the biochemical similarity reflects evolutionary origin of the two loci from a common ancestral gene.

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