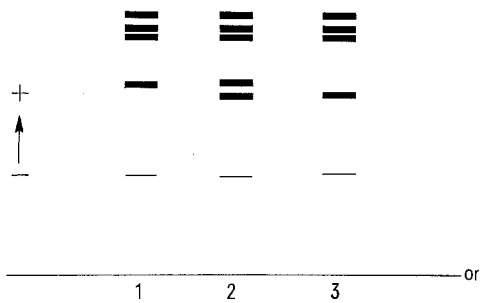
Genetic Control of an Esterase of Digestive Tract Tissues in *Rattus norvegicus*

This report identifies a pair of codominant autosomal alleles (a and b) at a single genetic locus (Es-3) which control the electrophoretic mobility of a soluble esterase in several digestive tract tissues of *Rattus norvegicus*. This esterase does not appear in serum or plasma as do two other rat esterases for which genetic loci have previously been identified[1,2].

Materials and methods. 164 adult rats of an outbred laboratory colony were killed by chloroform and 20 major organs and tissues excised immediately. All solid tissues were washed in saline and homogenized in an equal volume of water. Particulate material was removed by centrifugation at 30,000 × *g* for 30 min at 4 °C. The supernatant was stored at − 20 °C for up to 6 weeks until the following electrophoretic procedure could be performed on each sample.



Esterase zymogram phenotypes from rat small intestine extracts. Slot 1: zymograms pattern, A; proposed genotype, Es-3a/Es-3a. Slot 2: zymogram pattern AB; proposed genotype, Es-3a/Es-3b. Slot 3: zymogram pattern B; proposed genotype, Es-3b/Es-3b.

esterase activity. Most tissues also express slowly migrating esterase components just anodal to the origin. Liver, lung, kidney and most tissues of the digestive tract are also sources of esterases which migrate intermediate to these two extreme zones. Polymorphism for esterases of the intermediate zone was found in the large intestine, caecum, small intestine, cardiac stomach and esophagus. The small intestine zymogram is typical of intermediate zone esterases in these tissues and is diagrammed to illustrate the 3 patterns found (Figure). Polymorphism for esterases in serum, red cells, spleen, liver, lung, kidney, adrenal, thymus, pyloric stomach, heart muscle, skeletal muscle, brain, pancreas or gonads was not found in this colony. The variant enzyme appears to be specific to digestive tract tissues and is not present in serum or plasma.

Since killing of the animals was necessary in order to remove the tissues in which the Es-3 locus is expressed, the genetic data came from tissues of family groups rather than from prearranged matings. Seventeen pairs of animals and at least one litter of their offspring were examined. Animals with a single anodal esterase band in the intermediate zone were ascribed esterase pattern A, those with a single cathodal band pattern B, and those with two bands, pattern AB (Figure). No other patterns were observed in this colony. Family data were consistent with a hypothesis of 2 codominant alleles for a single autosomal locus. Matings of parents with identical single bands were assumed homozygous while animals with the AB pattern were assumed heterozygous. Both assumptions were affirmed by family data (Table). The allele responsible for the anodal of the 2 bands has been designated Es-3a and the control of the slower band has been attributed to an alternative allele, Es-3b. All 3 genotypes were found evenly distributed in both sexes. Heterozygotes express both bands without intermediate bands or other evidence of shared subunits[4].

Rat esterase patterns and proposed genotypes of parents and offspring of 17 matings

No. of families	Esterase pattern of parents	Proposed genotype of parents (Es-3)	No. of offspring	Esterase pattern of offspring	Proposed genotype of offspring (Es-3)
1	A × A	a/a × a/a	7	(7) A	(7) a/a
4	A × AB	a/a × a/b	31	(14 A, (17) AB	(14) a/a, (17) a/b
3	A × B	a/a × b/b	20	(20) AB	(20 a/b
1	B × B	b/b × b/b	8	(8) B	(8) b/b
3	B × AB	b/b × a/b	26	(15) AB, (11) B	(15) a/b, (11) b/b
5	AB × AB	a/b × a/b	38	(9) A, (18) AB, (11) BB	(9) a/a, (18) a/b, (11) b/b
17			130		

The buffer system described by SCHIFF and STORMONT[3] was used for horizontal starch gel electrophoresis. The electrolyle buffer of pH 8.2 contained lithium hydroxide and boric acid and the 13% starch gel was made in a lithium hydroxide, boric acid, *tris*, citric acid buffer of pH 7.3. Samples were inserted into the sliced gel on paper wicks which were removed after 30 min electrophoresis at 150 V. After removal of the wicks, electrophoresis was continued at 300 V for 4 h.

Horizontally sliced gel halves were then incubated for 30 min in a staining mixture of 2 ml 1% α-napthyl butyrate in acetone, 200 mg fast blue BB salt, 25 ml 0.4 *M tris*-HCl buffer and 175 ml H_2O. Electrophoretically distinct esterases were visualized as dark deposits of azo dye on the gel surface.

Results and discussion. With the exception of one pancreatic esterase, all enzymes identified by the above technique moved in an anodal direction. Most rat tissues express esterases which migrate in the serum albumin region and certain tissues contain a pre-albumin band of

Zusammenfassung. Bei *Rattus norvegicus* wird eine neue Mutation beschrieben, welche die elektrophoretische Wanderungsgeschwindigkeit eines Intestinalenzymes beeinflusst.

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[1] K. B. AUGUSTINSSON and B. HENRICSON, Biochim. biophys. Acta *123*, 323 (1966).
[2] J. E. WOMACK, J. Heredity *63*, 41 (1972).
[3] R. SCHIFF and C. STORMONT, Biochem. Genet. *4*, 11 (1970).
[4] This work supported by Brown-Hazen Fund of Research Corporation.