Subcellular Expression of Liver Eaterase Isoenzymes in Mus musculus

by

Lloyd Eden

Biomedical Science Dept.

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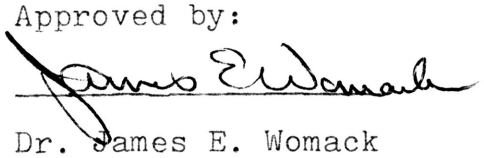
Approved by:



Dr. James E. Womack

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Abstract

The purpose of this study was to show the relationship of the the localization of esterase isoenzymes to the chromosomal position of their structural genes. Using a combination of subcellular fractionation, electrophoresis, and histochemical staining, I demonstrated that the products of the linked genes Es-2, Es-6, Es-9, and Es-11 were equally expressed in both the lysosomes and microsomes of mouse liver.

Key words: Mus musculus; Es-2, Es-6, Es-9, Es-11; Chromosome 8; Subcellular localization.

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INTRODUCTION

Here in is tested the hypothesis that esterase isoenzymes of closely linked genes are organelle specific. Esterases were chosen because Es-2, Es-6, Es-9, and Es-11 are all within a 12 centiMorgan span of each other. Es-3, Es-8, and Es-10 are all located on other chromosomes in the genome and are physiolgically distinctly different from those of chromosome 8. Lysosomes and microsomes were the organelles selected due to their quatities of esterases and the specificity of acid phosphatase to lysosomes, affording confirmation of fractional separation. Starch gel electrophoresis and histochmical staining provided determination of the mode of action of the individual esterase genes. The results obtained indicate that closely linked genes do not produce isoenzymes which are organelle specific.

MATERIALS AND METHODS

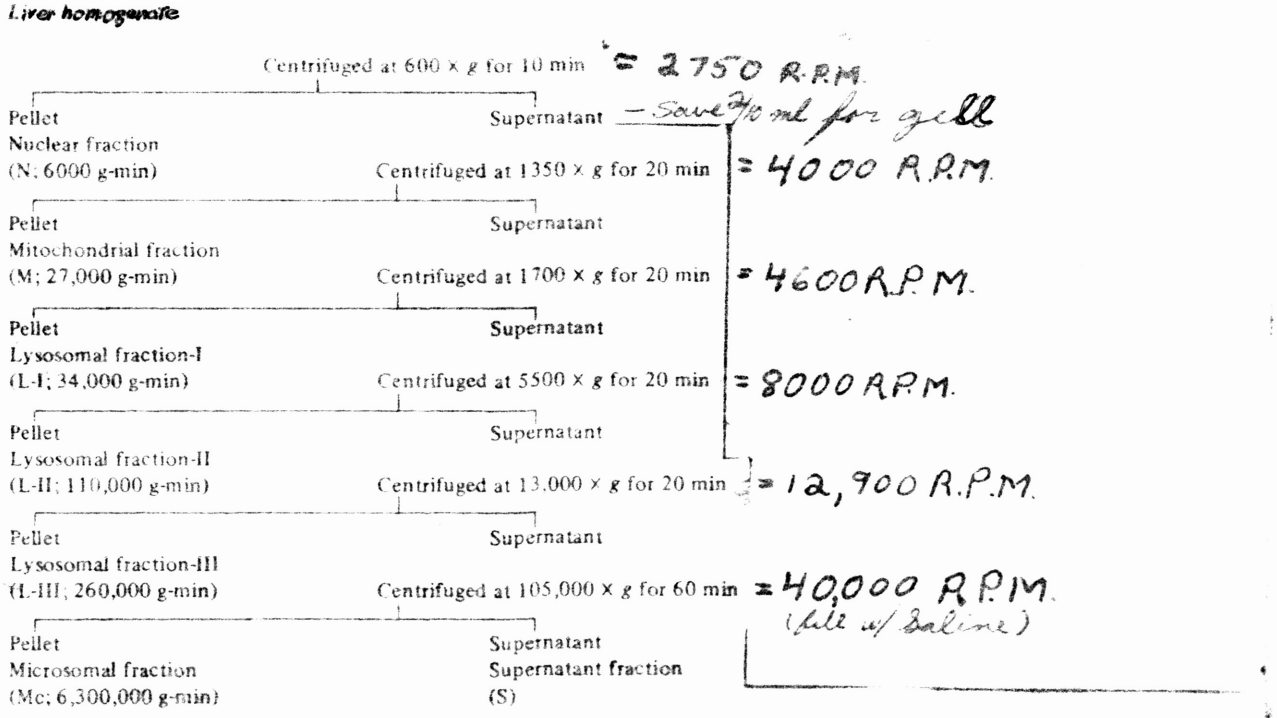
Source of Mice

All mice were from the colony of J.E. Womack and were inbred strains of the Jackson Laboratory, Bar Harbor, Maine.

Tissue Fractionation

Mortification was accomplished by CO_2 affixiation and livers were extracted within seconds. Livers were mechanically homogenized (using a Bodine Electric Co. fractional horsepower motor developing 1725 R.P.M.) in ice cold 15% w/v 0.25 M. sucrose solution containing 0.01 M. Tris-HCl buffer (pH 7.4). Separation of subcellular fractions was achived using the centrifugation scheme of Asano et al. (1979)[1] see Fig. 1. A second

FIGURE 1


Liver homogenate
Centrifuged at 600 × g for 10 min
= 2750 R.P.M.
Pellet
Nuclear fraction
(N; 6000 g-min)
Supernatant
– Save 3/4 ml for gell
Centrifuged at 1350 × g for 20 min
= 4000 R.P.M.
Pellet
Mitochondrial fraction
(M; 27,000 g-min)
Supernatant
Centrifuged at 1700 × g for 20 min
= 4600 R.P.M.
Pellet
Lysosomal fraction-I
(L-I; 34,000 g-min)
Supernatant
Centrifuged at 5500 × g for 20 min
= 8000 R.P.M.
Pellet
Lysosomal fraction-II
(L-II; 110,000 g-min)
Supernatant
Centrifuged at 13,000 × g for 20 min
= 12,900 R.P.M.
Pellet
Lysosomal fraction-III
(L-III; 260,000 g-min)
Supernatant
Centrifuged at 105,000 × g for 60 min
= 40,000 R.P.M.
(Dilute w/ Saline)
Pellet
Microsomal fraction
(Mc; 6,300,000 g-min)
Supernatant
Supernatant fraction
(S)

method attempted for subcellular separation was that used by Leighton et al. (1968)[2] see Fig.2. Several problems were encontered with this method. Triton WR-1339 (Tyloxapol or sodium alklaryl polyether sulfate) was injected interpertoneally 4 days prior to kill. Centrifugation was done in sucrose gradiants ranging from 45% to 14.3% w/w. The original scheme used 85 mgs. of Triton per 100 gs. of body weight (subjects were 200 g. rats). The viscosity of the Triton combined with the ten fold diffance in body weight (mice average 20 gs.) necessetated dilution. 25% ethanol and 0.9% sterile saline solvents were tried. The ethanol/Triton mixture proved fatal while the saline/triton afforded poor separation of lysosomes and microsomes ; see Fig. 3. Thus the method was abandoned for the straight centrifugation scheme mentioned above, (see Fig. 4 for results of this scheme). All fractions were resuspended in 0.25 M. sucrose solution and stored at -70°C until enzymatic and protein asseys were performed.

Enzyme Asseys

The acid phosphatase asseys were done as described by Womack et al. (1978)[3]; esterase as described by Gomeri (1952)[4]; and protein as described by Lowery et al. (1951)[5]. Both acid phosphatase and esterase level were expressed as per mg. of protein due to their membranous attachments.

Electropforesis and Histochemical Staining

The starch gels were prepared as described by Boyer et al. (1963)[6], using 35 gs. of hydrolyzed starch, 30 gs. of electro-

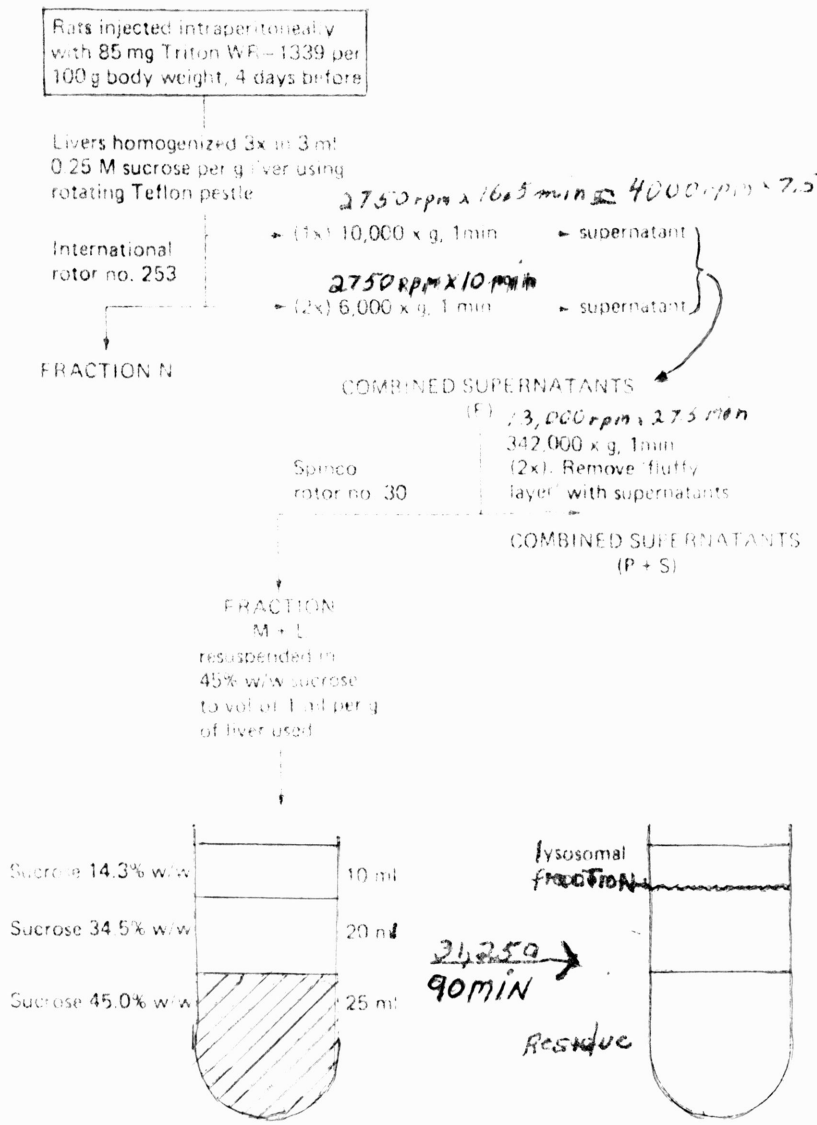


Fig. 1. Isolation of lysosomes containing Triton WR-1339 (after Leighton et al., 1968).

Sucrose 46.8% w
(ρ_m = 1.22)
Sucrose 57.4% w
(ρ_m = 1.28)
Sucrose 67.3% w
(ρ_m = 1.34)
Sucrose 62% w/
(ρ_m = 1.38)

Fig. 2. Isolation

Triton/Saline-Microsome/Lysosome Separation And Esterase Content

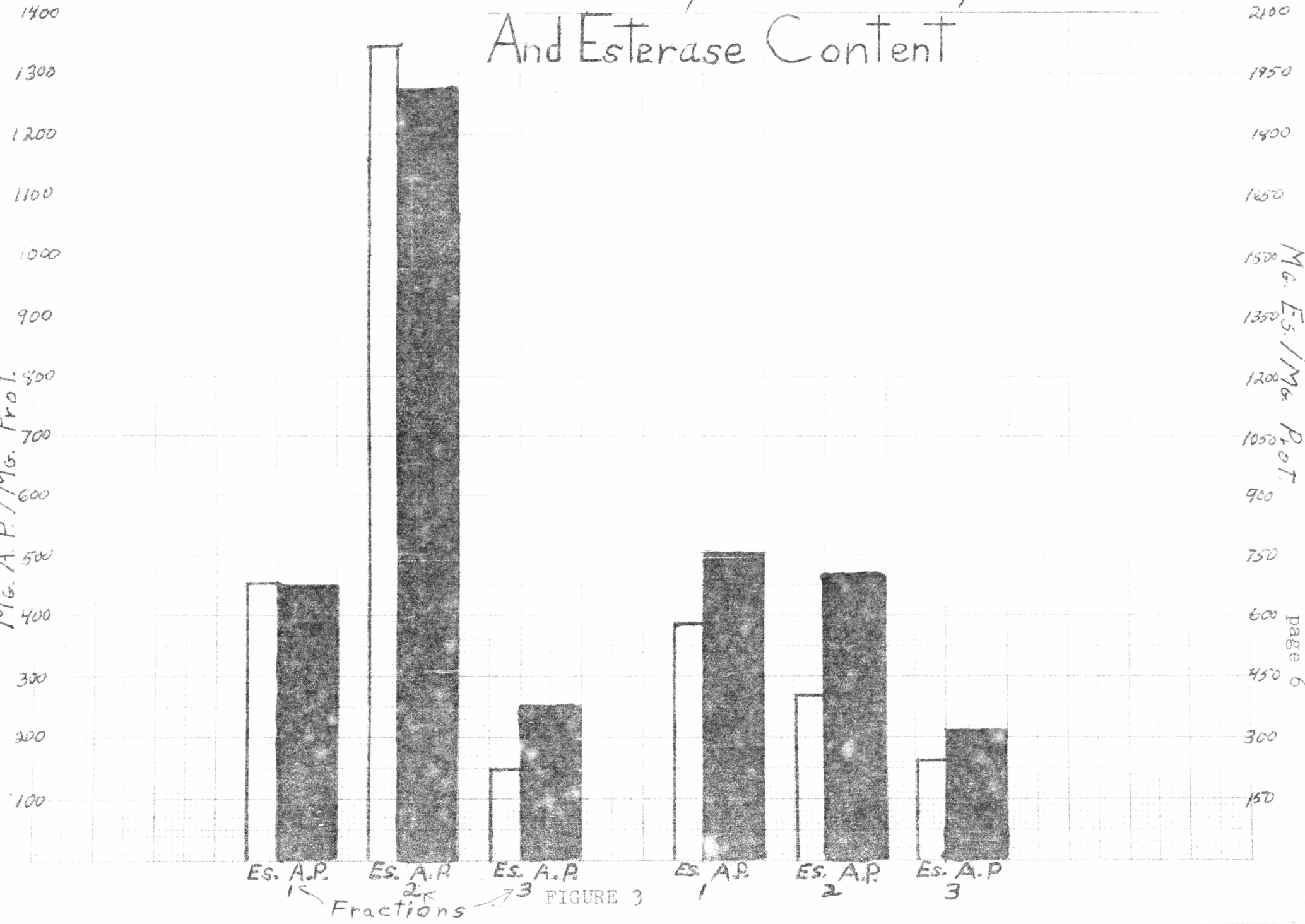


FIGURE 3

Microsome / Lysosome Separation And Esterase Content-Straight Centrifugation

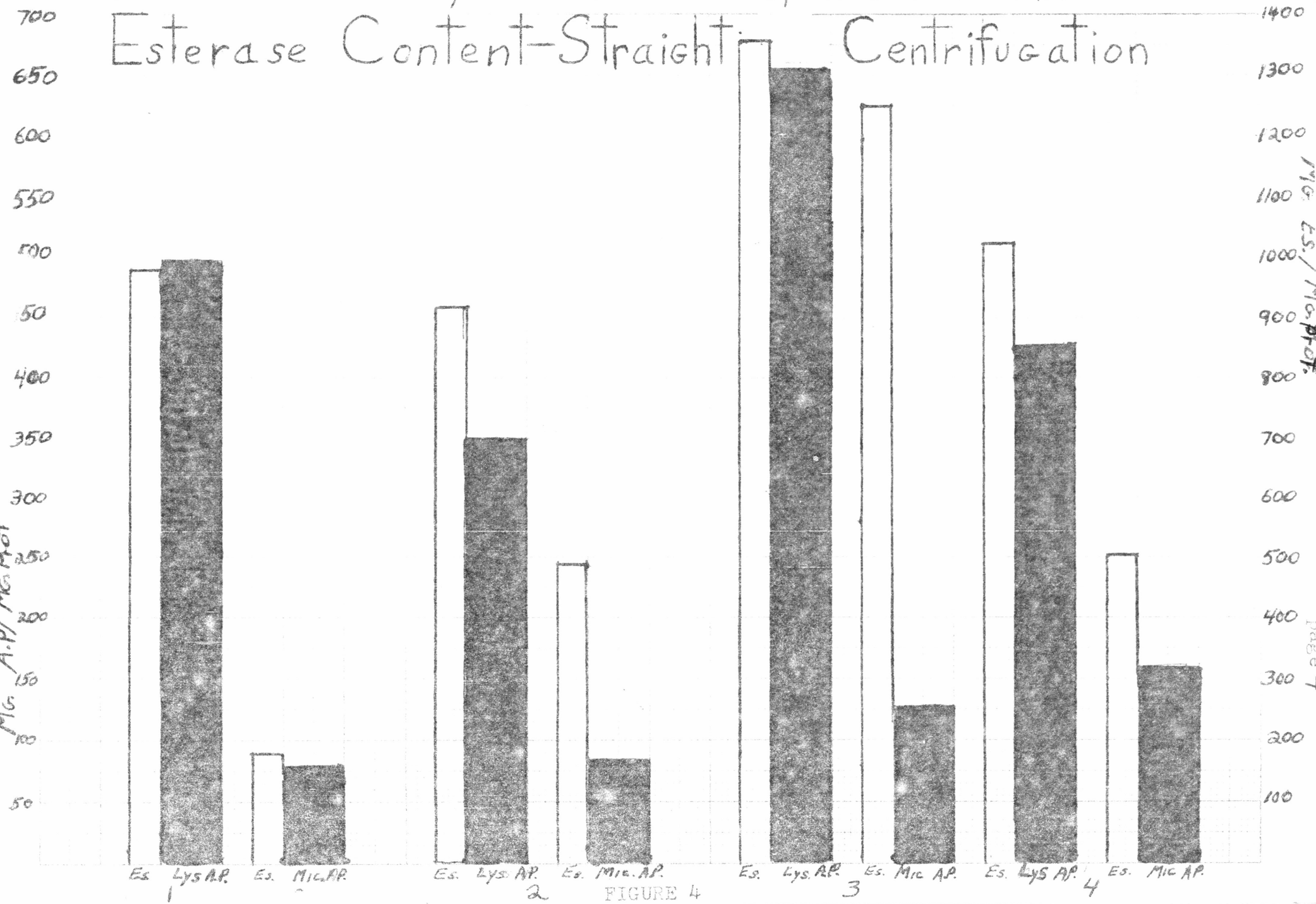


FIGURE 4

starch, and 25:475 mls. E.B.T. buffer (0.02 M. E.D.T.A., 0.05 M. boric acid, and 0.9 M. Tris HCl):water. The gels were run for 16 hours at 200 volts. Histochemical staining was performed as described by Boyer et al.(1963)[7]. 100 mls. of stock pH 7.0 buffer + 1 ml. alpha naphthaline acetate + 1 g. alpha naphthaline in 6 mls. of acetone (1 ml. used) +100 mgs. of fast blue B.B. salt. The stain is light sensative and therefor was developed for 30 minutes to 3 hours (depending on the sample strength) in complete darkness. The gels were then examined for esterase activity and photographed to preserve their information see Fig. 5.

RESULTS

A strain matrix of mice with known allelic differances for esterase genes 2, 6, 9, and 11 was surveyed. PL/J, $C57/Bl_6$, M-73, and a combination mouse were asseyed for overall esterase content and electrophoretically examined for specific isoenzymes. Fig. 6 shows a clear separation of lysosomal and microsomal fractions while their overall esterase content remained relativly constant. Examination of the starch gels revealed that all gene products were expressed as expected see Fig. 7; (the combination had all alleles, PL/J is a varient on Es-2, $C-57/Bl_6$ is a varienton Es-1, M-73 is a varient on Es-11, Es-3,and Es-9, and is null for Es-2. The results indicate that the products of closely linked esterase genes are not organelle specific.

FIGURE 5
PHOTOGRAPHS OF STAINED STARCH GELS

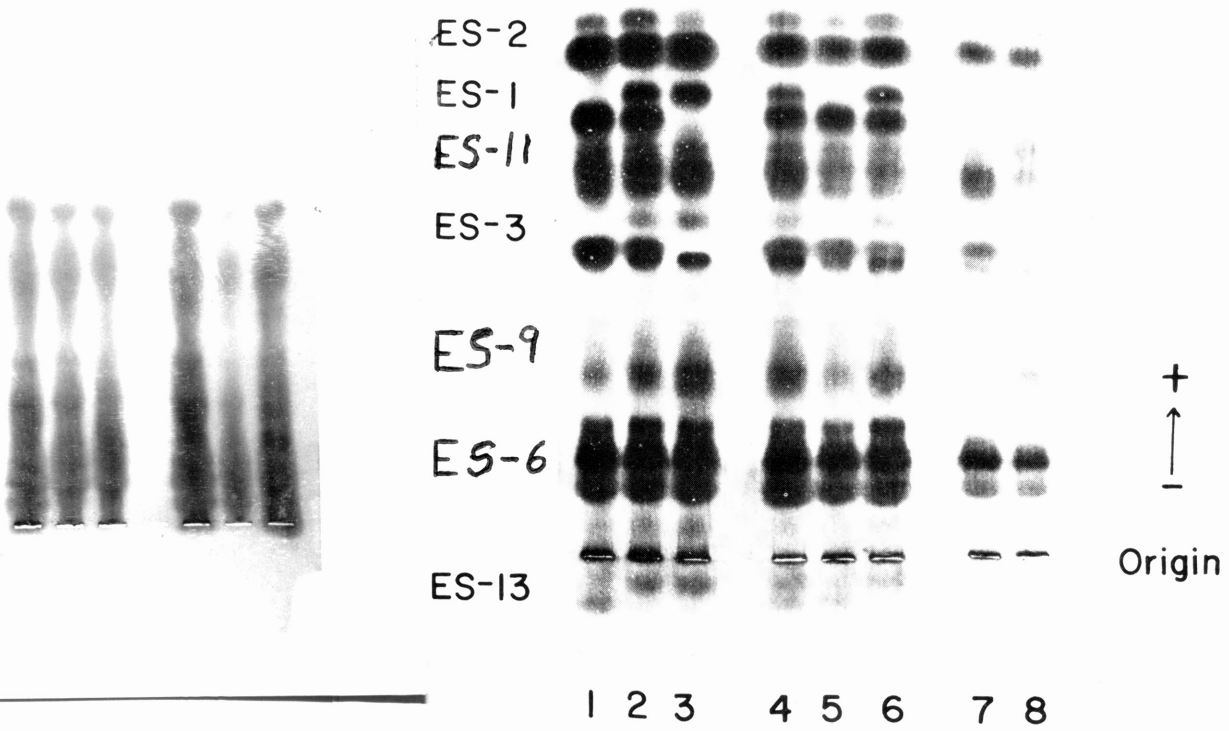


Fig. 1. Starch gel zymogram of kidney esterases stained with α-naphthylbutyrate as substrate. Slots: 1, SJL/J; 2, SJL/J × C57BL/6J F_1; 3, C57BL/6J; 4, SJL/J plus C57BL/6J incubated mixture; 5, SJL/J diluted 1:1 with normal saline; 6, SJL/J × C57BL/6J F_1; 7, SJL/J incubated with neuraminidase; 8, C57BL/6J incubated with neuraminidase.

*Womack, J.E., Benjamin, A.T.,and Barton,J.E. (1978). Esterase 13, a New Mouse Locus with Recessive Expression and Its Genetic Locatio on Chromosome 9. Biochem. Genetics, Vol.16 Nos. 11/12

Microsome/Lysosome Separation And Their Respective Esterase Content

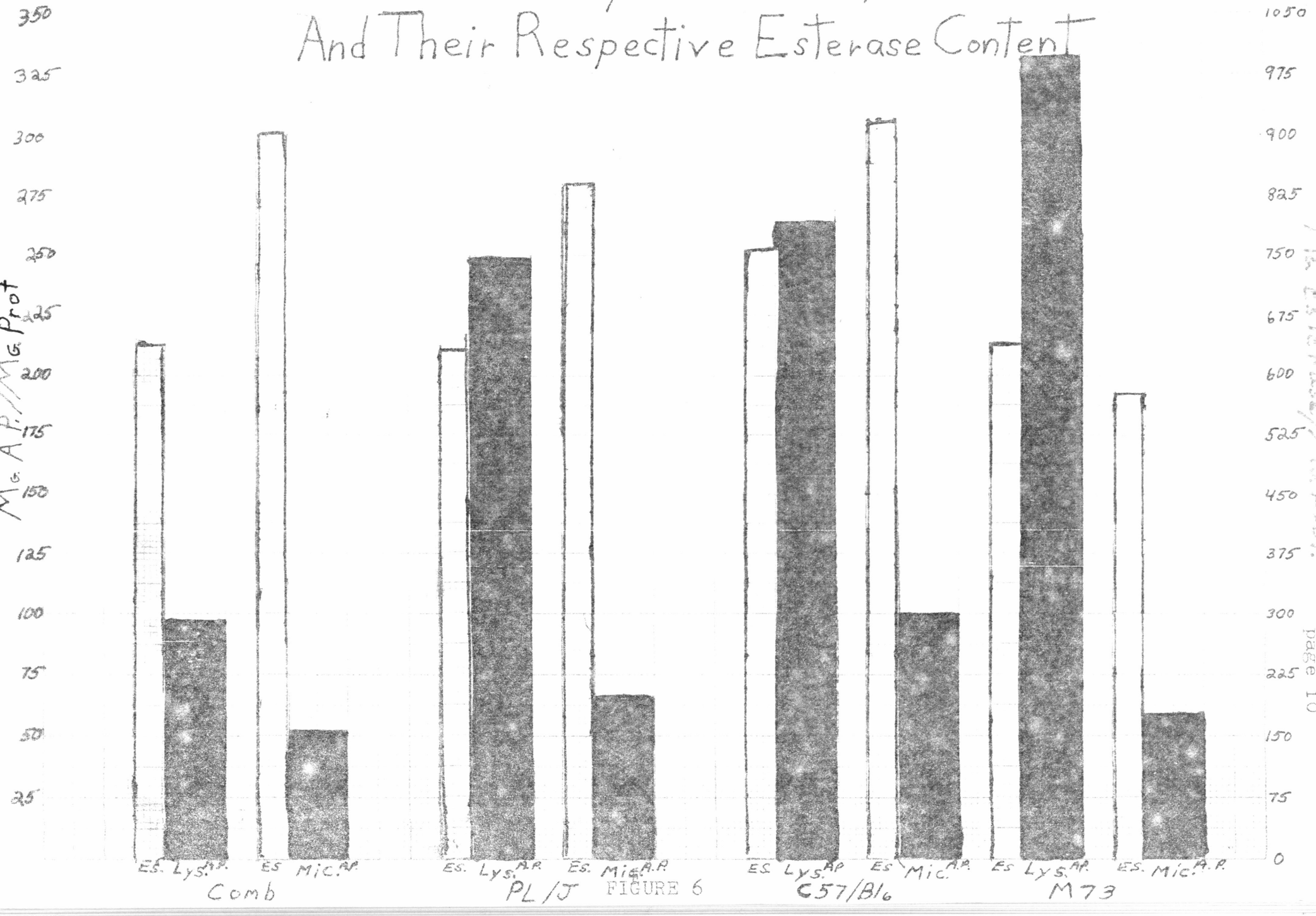
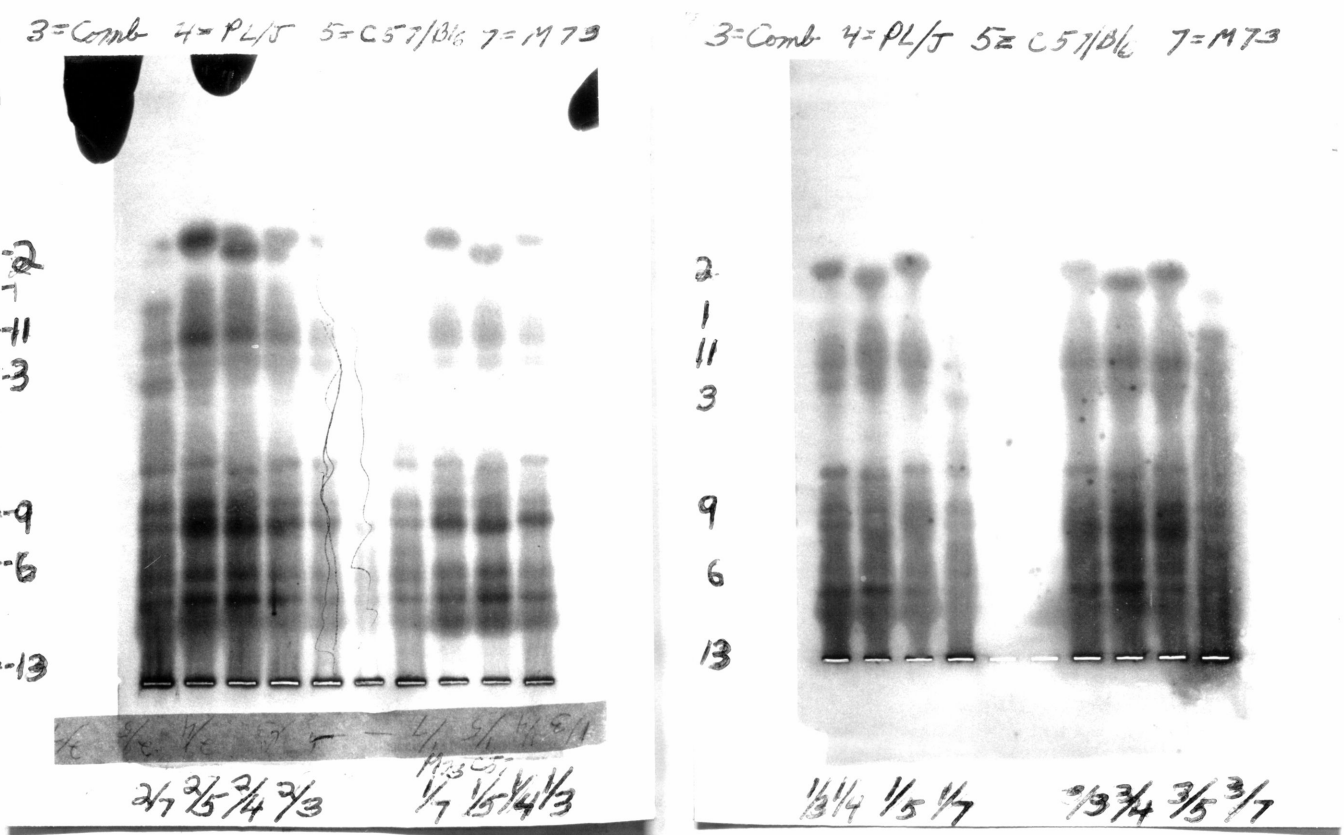


FIGURE 6

FIGURE 7

PHOTOGRAPHS OF STRAIN MATRIX STARCH GEL



Fraction 2 All Mice

Fraction 1 All Mice

Fraction 1 All Mice

Fraction 3 All Mice

DISCUSSION

The "enormous difficulties involved in the isolation of pure lysosomes" (Dingle 1972)[8] were experianced by this author. In the Materials and Methods section I discussed the two methods I tried for the isolation and purification of not only lysosomes but for microsomes as well. The difficulties stem from the fragility and scarceness of the lysosome "and from the wide dispersion of physical properties within populations of these particles"(Dingle 1972)[9]. The biochemical data presented here was the advantage of leading to unbiased qualitative evaluation of the purity achieved. Such criteria rests upon the two postulates put foward by de Duve and Berthet (1954) and by de Duve et al. (1955) as a basis for interpreting patterns of enzyme distributions in tissue fractionation experiments. The first states that ' a given enzyme belongs to a single tintercellar components in the living cell'. The second states that 'granules of a given population are enzymically homogeneous or at least cannot be separated by centrifugation into subgroups differing in enzymic content' (Dingle 1972)[10].

The first assumtion proved to be true for certain enzymes like acid phosphatase but not true for others like the esterases. Thus the measurement of acid phosphatase is a valid test of the relative purity of lysosomes and microsomes; and electrophoresis and staining employment afforded identification of the individuals of the 'subgroups'isoenzymes. That the four closely linked esterase genes are all expressed in both the microsomal and lysosomal fractions is, however, consistant

with the multiple gene theory;'a series of genes results from tandem duplications of a single gene' and **therefor** may share some of the same physiological functions.

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