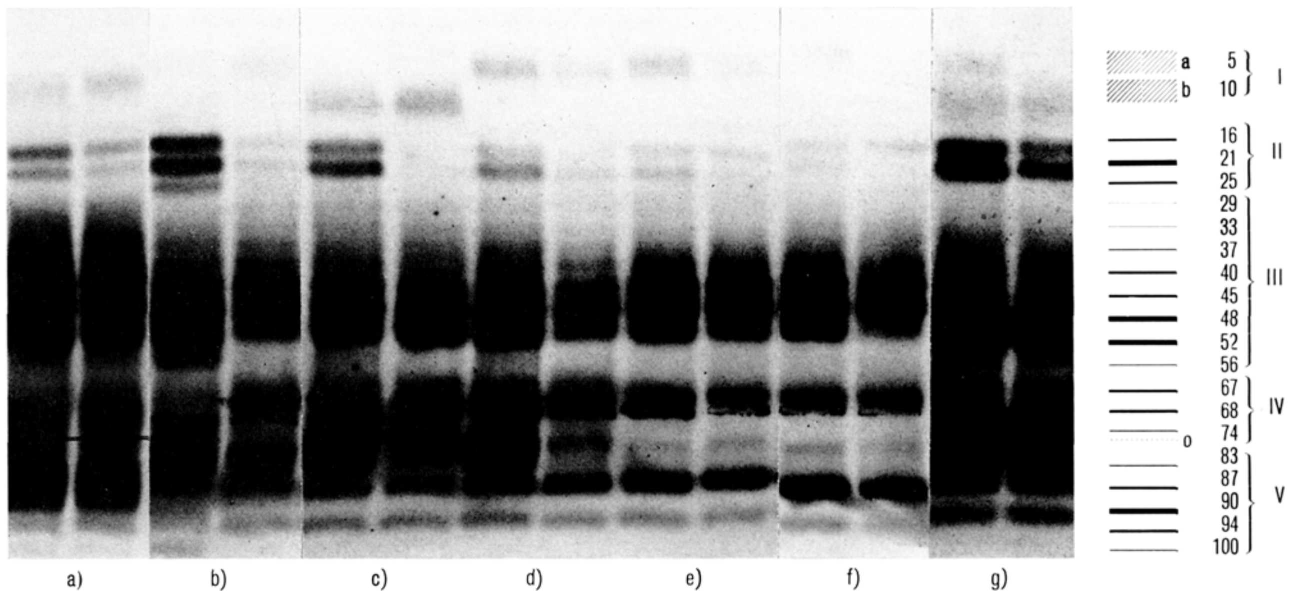

Esterase zymograms of liver extracts from 7 different neurological mutants of the mouse employing β-naphthyl acetate as substrate. a) Teetering; b) jolting; c) shambling; d) ducky; e) myelin synthesis deficiency; f) reeler; g) tottering. The mutant is on the right and the normal control on the left of each channel.

may function in protein metabolism since at least some hepatic aliesterases of the rat are capable of splitting amides and amino acid esters[15]. Indeed, bands of zone-II, including II-21 are ali-esterases. Bands III-29, III-52, and III-56 represent cholinesterases and are inhibited by eserine. The absence of either or both III-52 and III-56 in all but jolting, shambling and tottering mice, if true as well for the brain, may relate to the convulsive behavior and reduced lifespan of these mutants.

The sensitivity of bands III-52 and III-56 to inhibition by eserine sulfate and their relative electrophoretic mobilities suggest that they may correspond to esterase-3 bands controlled by *Es*-3, a locus whose linkage is not as yet known[16]. Our uncertainty stems from the fact that previous analyses of esterase isozymes were made with starch rather than thin-layer agar electrophoresis. Although *Es*-3 is not yet located, 4 other known esterase loci are mapped and belong to linkage group XVIII[17]; it seems unlikely that the deletions in zone III represent allelic differences at the *Es*-3 locus in these mutants.

Bands IV-74 and V-83, lacking singly or together in 3 of the mutants, represent lipolytic active esterases because of their ability to hydrolyse α-naphthyl stearate. Thus, they are important in lipid or lipoprotein metabolism.

The severe esterase isozyme deletions as well as deficiencies in total esterase activity occurring in livers of so-called neurological mutations may be considered as due either to a pleiotrophic gene effect or adaptive responses to the primary gene effect. Thus, in either case, hereditary neurological diseases represent primary or secondary systemic disorders[18,19].

Zusammenfassung. Alle von uns in Mäusen untersuchten vererblichen neurologischen Erkrankungen stellen entweder primäre oder sekundäre gesamtkörperliche Prozesse dar. Verschiedene allelische Substitutionen produzieren spezifische Leber-Esterase-Isozym-Defekte.

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The Jackson Laboratory, Bar Harbor (Maine 04609, USA), 15 September 1970.

[15] D. K. MYERS, J. W. TOL and M. H. T. DE JONGE, Biochem. J. *65*, 223 (1957).

[16] F. H. RUDDLE and T. H. RODERICK, Genetics *51*, 445 (1965).

[17] M. C. GREEN, *Biology of the Laboratory Mouse*, 2nd edn (McGraw-Hill, New York 1966), chap. 8, p. 87. – M. L. PETRAS and P. SINCLAIR, Can. J. Genet. Cytol. *11*, 97 (1969).

[18] This work was supported in part by NIH research grant No. NB 06448 from the National Institute of Neurological Diseases and Stroke, a grant from the National Foundation for Neuromuscular Diseases, Inc., a grant from the Health Research Fund, United Fund of Schenectedy County, Inc., and an allocation from NIH General Research Support Grant No. RR 05545 from the Division of Research Resources to The Jackson Laboratory.

[19] The principles of laboratory animal care as promulgated by the National Society for Medical Research are observed in this Laboratory.

Preparation of a Plasma Membrane Fraction from the Brown Adipose Tissue

The plasma membranes of brown and white adipose tissues are of great interest as the site of numerous metabolic controls. As a useful approach to the biochemical properties of the plasma membranes, it seemed of interest to isolate this fraction free of contaminants.

Preparation and characterization of plasma membranes from liver and ascites cells have been the subject of several investigations[1-9]. Two different methods have been described: in the first, the plasma membranes were separated from heavy fractions (nuclei, mitochondria),

Mg^{2+}-Na^+-K^+-ATPase, 5'-nucleotidase and NADH-diaphorase activities of plasma membrane (PM) and microsomal (Mc) fractions of brown and white adipose tissues of the rat

	Brown adipose tissue			White adipose tissue		
	PM	Mc	PM/Mc	PM	Mc	PM/Mc
Mg^{2+}-Na^+-K^+-ATPase	840 (5)[a]	140 (5)	6.0 ± 1.6	1100 (5)	260 (5)	4.2 ± 0.9
5'-nucleotidase	316 (5)[a]	62 (5)	5.1 ± 1.8	962 (5)	183 (5)	5.3 ± 1.3
NADH-diaphorase						
Non sonicated	268 (4)[b]	507 (4)	0.53 ± 0.11	638 (4)	1180 (4)	0.54 ± 0.12
Sonicated	238 (4)[b]	600 (4)	0.40 ± 0.04	613 (4)	1370 (4)	0.45 ± 0.04

The results are expressed as: [a] nmoles of Pi/mg protein per min. [b] nmoles NADH oxidized/mg protein per min. Numbers in parenthesis represent the number of experiments. Values represent means ± standard error of the means.

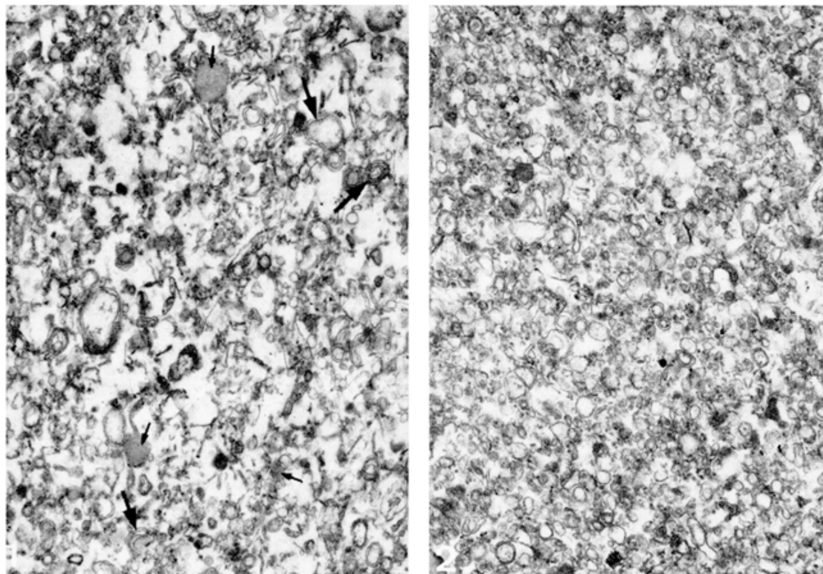

Fig. 1 and 2. Membrane fractions of brown adipose tissue isolated according to a modification of the procedure of KAMAT and WALLACH[8].
Fig. 1. Plasma membrane fraction. The fraction contains membrane profiles of varying diameters, some of which appear multilayered (thick arrows). A few lipid droplets can be seen (thin arrows). ×18,500.
Fig. 2. Microsomal fraction. The fraction is composed mostly of small single-walled vesicles. ×18,500.

after gentle homogenization[1-4,6]. In the second method, which was first described by KAMAT and WALLACH[8] with Ehrlich ascites cells, and subsequently by GRAHAM et al.[5] with liver cells, the plasma membranes were separated from the light fraction (microsomes) after vigorous homogenization. Recently, McKEEL and JARETT[10] succeeded in preparing plasma membranes of isolated white fat cells, starting from the mitochondrial fraction. In brown adipose tissue, because of the high amount of mitochondria, this procedure would present a great risk of contamination. It therefore seemed interesting to use the procedure of KAMAT and WALLACH[8] for isolating the plasma membranes of brown adipose tissue, and to extend this study to white adipose tissue.

Methods. Groups of 3–4 Sprague-Dawley male rats weighing 450–500 g and fed ad libitum on Nafag chow (St. Gall, Switzerland) were used for each fractionation experiment; after sacrifice by decapitation, brown and white adipose tissues were excised and rapidly placed in an homogenization medium at 4 °C, containing $CaCl_2$ (0.5 mM), HCO^-_3 (1 mM) and $MgSO_4$ (0.2 mM), pH 7.5. Such a medium, described by RAY[7] for the liver, should increase the yield and the enzymatic activities of the plasma membranes. The method described by KAMAT and WALLACH[8] was used for the separation of the plasma membranes from brown and white adipose tissues with the following modifications: 1. As described by GRAHAM et al.[5] for liver cells, we used a Potter-Elvehjem homogenizer instead of a nitrogen pressure vessel. The tissue was first cut with scissors into small pieces of about 2 mm square, and then homogenized at 2300 rpm (20 up-and-down strokes, clearance 0.03 mm) in 5 volumes of the homogenizing medium which was afterwards adjusted to 8 volumes. 2. Nuclei and mitochondria were spun down at 22,000 × g for 15 min in a MSE-18 centrifuge; the microsomes by centrifugation at 105,000 × g for 90 min (Spinco-L, rotor No. 30). 3. KAMAT and WALLACH[8] used Ficoll of density 1.096 for the separation of plasma membranes from microsomes. We determined

[1] D. M. NEVILLE, J. biophys. biochem. Cytol. *8*, 413 (1960).
[2] P. EMMELOT, C. J. BOS, E. L. BENEDETTI and PH. RÜMKE, Biochim. biophys. Acta *90*, 126 (1964).
[3] M. TAKEUCHI and H. TERAYAMA, Expl. Cell. Res. *40*, 32 (1965).
[4] R. COLEMAN, R. H. MICHELL, J. B. FINEAN and J. N. HAWTHORNE, Biochim. biophys. Acta *135*, 573 (1967).
[5] J. M. GRAHAM, J. A. HIGGINS and C. GREEN, Biochim. biophys. Acta *150*, 303 (1968).
[6] H. M. BERMAN, W. GRAM and M. A. SPIRTES, Biochim. biophys. Acta *183*, 10 (1969).
[7] T. K. RAY, Biochim. biophys. Acta *196*, 1 (1970).
[8] V. B. KAMAT and D. F. H. WALLACH, Science *148*, 1343 (1965).
[9] J. MOLNAR, G. MARKOVIC, H. CHAO and Z. MOLNAR, Arch. Biochem. Biophys. *134*, 524 (1969).
[10] D. W. McKEEL and L. JARETT, J. Cell Biol. *44*, 417 (1970).

the densities of Ficoll to be used by preliminary experiments on a discontinuous gradient; the densities were 1.066 for brown adipose tissue, and 1.048 for white adipose tissue. The yield of the method of KAMAT and WALLACH[8] was about 1 mg of plasma membrane proteins from 3 g of brown adipose tissue and 1 mg of plasma membrane proteins from 20 g of white adipose tissue.

The proteins were measured according to LOWRY et al.[11]. Mg^{2+}-Na^{+}-K^{+}-ATPase (EC. 3.6.1.3) activity was measured according to MODOLELL and MOORE[12]. This assay does not discriminate between the Mg^{2+}-ATPase and the Mg^{2+}-Na^{+}-K^{+}-ATPase. 5'-nucleotidase (EC. 3.1.3.5) activity was measured according to HEPPEL and HILMOE[13], inorganic phosphorus (Pi) according to TAUSSKY and SHORR[14] and NADH-diaphorase (EC. 1.6.99.3) activity, spectrophotometrically (Beckman DB-G Spectrophotometer), according to STRITTMATTER[15]. In certain experiments attempts were made to increase the yield of enzyme activity by sonicating the fractions (see Table).

Electron microscopy. Pellets obtained after recentrifugation of the sedimentation zones were fixed with 4% glutaraldehyde in 0.1 *M* phosphate buffer for 3 h. After glutaraldehyde fixation, the pellets were removed from the cellulose tubes, divided in strips when possible and postfixed in 2% osmium tetroxide. Dehydration was performed through alcohols and embedding in Epon. Ultrathin sections were stained with lead citrate and examined in a Philips EM 300 electron microscope.

Results. The marker enzymes characterizing the plasma membrane and the microsomal fractions have been measured. As shown in the Table, the Mg^{2+}-Na^{+}-K^{+}-ATPase specific activities of brown and white adipose tissues were respectively 6.0 and 4.2 times more concentrated in the plasma membrane fraction than in the microsomal fraction, while 5'-nucleotidase was found to be 5 times more concentrated in the plasma membranes than in the microsomes in both tissues. The NADH-diaphorase was about 2.5 times more active in the sonicated microsomes than in the sonicated plasma membranes.

Pellets of the plasma membrane and microsomal fractions of brown adipose tissue prepared according to the method of KAMAT and WALLACH[8] are presented in Figure 1 and 2. The plasma membrane fraction (Figure 1) is composed mostly of circular membranous profiles of varying sizes ranging from 0.08 to 0.3 µm. Several profiles appear composed of 2 or 3 superposed sheets of membranes. The microsomal pellet of the same tissue (Figure 2) is composed mostly of single-walled vesicles of small diameter (about 0.15 µm) interspersed with granular material, possibly of ribosomal origin. The lack of good morphological distinction between plasma membranes and microsomes obtained with the method of KAMAT and WALLACH[8] contrasts with the biochemical studies which indicate distinct differences between these two populations of membranes. This difference may be explained by the strong homogenization of the tissue which may disrupt the plasma membranes into small fragments. These fragments can seal up and become difficult to distinguish from the small vesicles of the microsomal fraction.

Discussion. MCKEEL and JARETT[10] showed that the Mg^{2+}-Na^{+}-K^{+}-ATPase specific activity was especially high in the plasma membranes of white adipose tissue, while this enzyme was not completely absent in the microsomes. With brown and white adipose tissues, our separation procedure provides plasma membranes highly enriched in Mg^{2+}-Na^{+}-K^{+}-ATPase activity compared with the microsomal fraction.

The 5'-nucleotidase has been used as a marker of the plasma membranes isolated from liver[4-7]. In our experiment, 5'-nucleotidase activity is distributed between plasma membrane and microsomal fractions in the same proportion as ATPase. In liver[2], ascites cells[8] and white adipose tissue[10], NADH-diaphorase is considered as a microsomal enzyme, but it has also been found in small amount in the plasma membranes. This suggests that part of the NADH-diaphorase may be an intrinsic enzyme of the plasma membranes[2,8,10]. Our data are in agreement with these observations. Our results show a great similarity between the plasma membranes of brown and white adipose tissues. Using another procedure and starting from isolated white fat cells, MCKEEL and JARETT[10] obtained a comparable distribution of the marker enzymes between the plasma membranes and the microsomes[16,17].

Résumé. Les membranes plasmatiques des tissus adipeux brun et blanc sont isolées de la fraction microsomale selon une modification de la technique décrite par KAMAT et WALLACH[8]. Les résultats de l'analyse enzymatique démontrent un degré de purification satisfaisant des membranes plasmatiques et des microsomes.

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[11] O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, J. biol. Chem. *193*, 265 (1951).

[12] J. B. MODOLELL and R. O. MOORE, Biochim. biophys. Acta *135*, 319 (1967).

[13] L. A. HEPPEL and R. J. HILMOE, J. biol. Chem. *188*, 665 (1951).

[14] H. H. TAUSSKY and E. SHORR, J. biol. Chem. *202*, 675 (1953).

[15] P. STRITTMATTER, J. biol. Chem. *234*, 2665 (1959).

[16] Our sincere thanks are due to Miss C. WELTER for excellent technical assistance.

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Inhibition of DNA Synthesis by Lidocaine and Procaine

We wish to report that exposure of Ehrlich ascites cells to lidocaine or procaine resulted in a preferential inhibition of DNA synthesis (Figures 1 and 2). The relationship of this observation to the anesthetic action of these drugs is unclear. It has been suggested[1], however, that local anesthetics interact with lipid components of membraneous cellular structures and it is interesting that nascent mammalian DNA has been shown to be associated with a unique cellular component, probably the nuclear membrane[2], see also[3-6]. It may therefore be that the inhibition of DNA synthesis observed by us is a result of the complexing of lidocaine and procaine with membraneous structures thereby interfering with the site of DNA synthesis.