Fusion of microsomal vesicles*

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Summary: Studies which indicate the fusion of rat liver microsomal vesicles show that the rate of fusion of microsomal vesicles, as revealed by electron microscopic examinations, is dependent on the fusion temperature and the amount of detergent present in the microsomal suspension.

The importance of membrane fluidity for fusion of a variety of systems involving whole cells[1], isolated membranes[2], and phospholipid vesicles[3,4] has recently been suggested. However, quantitative experimental data are not yet available to allow complete evaluation of the role of membrane fluidity in the fusion reaction, and further studies are required to clarify the complex influence of the physical state of the lipids for membrane fusion.

In this paper, we report experiments which demonstrate a relationship between the extent of fusion of rat liver microsomal vesicles and the fusion temperature as well as the amount of detergent present in the microsomal suspension.

Materials and methods. Female Wistar rats (AF/Han.) weighing about 180 g and fasted for 12 h were used. Liver microsomes were prepared essentially by the method of Schneider[5] modified as described earlier[6].

For fusion experiments, microsomes (20 mg protein) were mixed at 0 °C either with 133 ml 0.1 M sucrose buffered with 0.01 M Tris-HCl pH 7.4, or with 133 ml of the same solution containing in addition 0.03% (w/v) Triton X 114 (Triton/protein ratio of 2). After 10 min of incubation (0 °C), the suspensions were centrifuged at 105,700 × g (max) for 60 min (Spinco rotor 30) at 0 °C or 37 °C, respectively. The resulting pellets of the 4 samples were cooled in ice and subsequently fixed overnight at 4 °C in 1% OsO_4 in twicedistilled water, then stained for 1 h in 0.5% aqueous uranyl acetate before dehydration through graded acetones and propylene oxide, and were embedded in Epon/Araldite[7]. Ultrathin sections were contrasted with methanolic uranyl acetate and basic lead citrate and examined in a Phillips 300 electron microscope.

Protein was assayed by the biuret method of Bode et al.[8]. The phospholipids were extracted with chloroform/methanol 2:1 (v/v)[9], separated by 1-dimensional TLC[10] and determinated by inorganic phosphate analysis[11].

Phospholipid content and composition of microsomes sedimented at different temperatures

Components	Microsomes sedimented at 0 °C Specific content*	%	37 °C Specific content*	%
Total phospholipids	883 ± 51	100	906 ± 56	100
Lysophosphatidyl choline	14 ± 3	1.6	14 ± 3	1.5
Sphingomyelin	35 ± 3	4.0	37 ± 3	4.1
Phosphatidyl choline	507 ± 38	57.4	519 ± 40	57.3
Phosphatidyl serine + phosphatidyl inositol	123 ± 8	13.9	125 ± 10	13.8
Phosphatidyl ethanolamine	204 ± 15	23.1	211 ± 14	23.3

Both analyses were always carried out with the same microsomal preparation. *nmoles phospholipid-P/mg protein; n = 5.

Results and discussion. Figure 1 illustrates typical electron microscopic appearance of microsomal pellets obtained as described in 'Materials and methods' at different temperatures and in the absence or presence of detergent. As observed many times previously, the untreated microsomes which were sedimented at 0 °C (figure 1a) appear at this magnification as closed, single membrane-limited vesicles ranging in size from 90 to 360 nm, some of which bear attached ribosomes.

Microsomes sedimented at temperatures (around 37 °C) above the lipid phase transition temperature [12–14] show general preservation of the vesicular structure, although the profiles are essentially more irregular (figure 1b). The most striking finding, however, is the occurrence of numerous very large closed vesicles (about 1500 nm in diameter) containing several large and smaller smooth surface vesicles, indicating that fusion of microsomes occurs under these experimental conditions.

Preparations of Triton X 114 treated and at 0 °C sedimented microsomes (figure 1c) are characterized by membranes arranged both in long linear arrays and in concentric lamellar striations of 2 or more membrane layers like extra membrane whorls enriched from *E. coli*[15]. Some intact microsomal vesicles are also still present. In addition, flocculent material and some small homogeneous densities are located between the membrane structures and these are assumed to represent partly already solubilized lipoprotein micelles and partly albumins and globulins which were released out of the vesicles.

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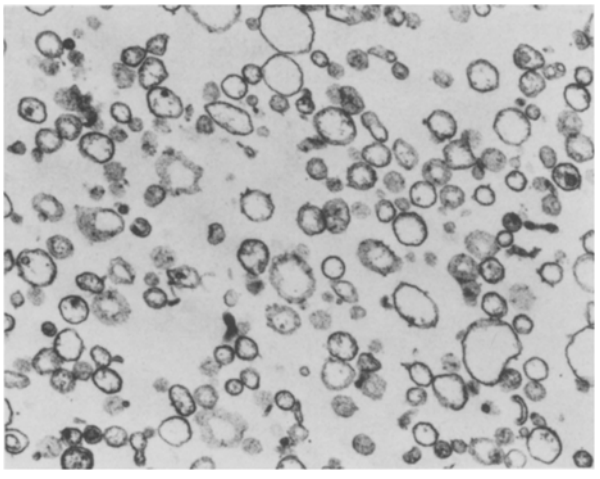
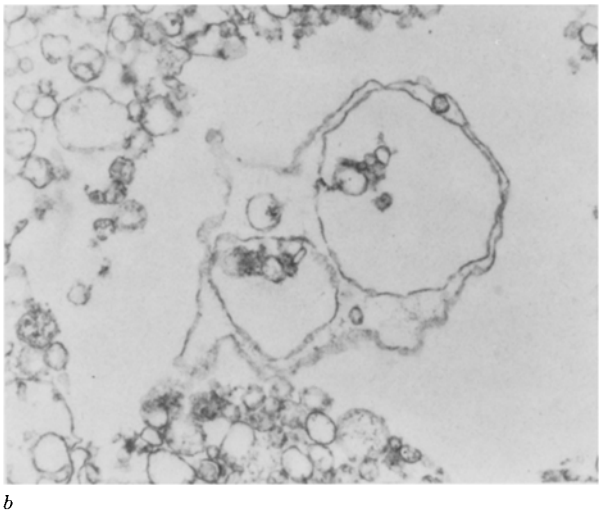


a



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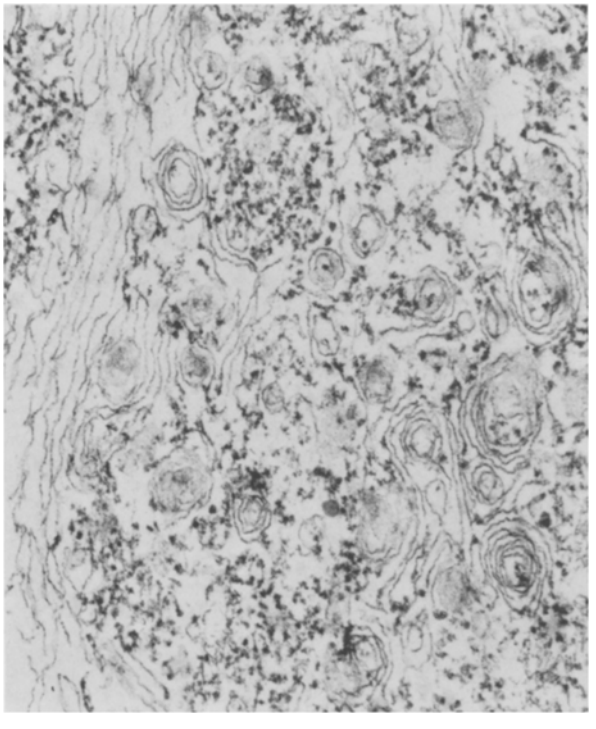

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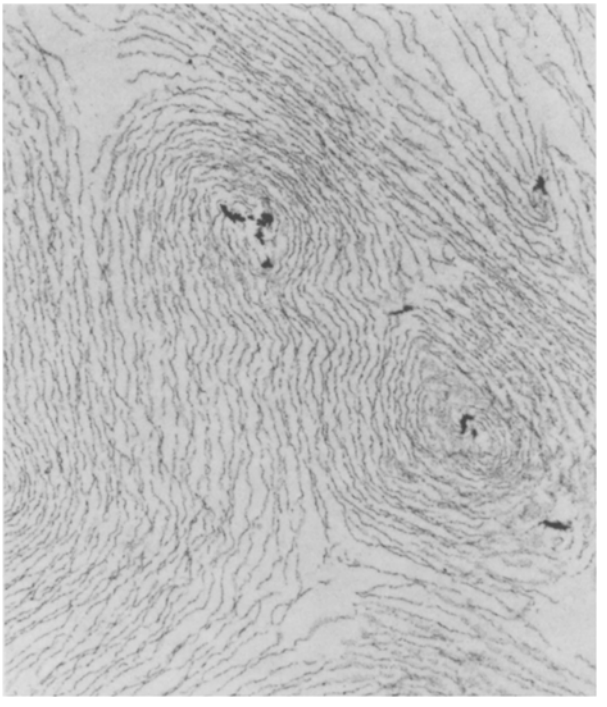

d

Fig. 1. Electron micrographs of microsomal pellets. Microsomes were sedimented as described in 'Materials and methods' at *a* 0 °C or *b* 37 °C in the absence and at *c* 0 °C or *d* 37 °C in the presence of 0.03% Triton X 114 (detergent/protein ratio of 2). *a* Original microsomes. *b* Giant vesicles formed by fusion of microsomes. *c* Long continuous membranes and multilamellar structures formed by fusion of microsomal vesicles. *d* Long continuous membraneous structures and membrane whorls formed by complete fusion of microsomal vesicles. × 22,000.

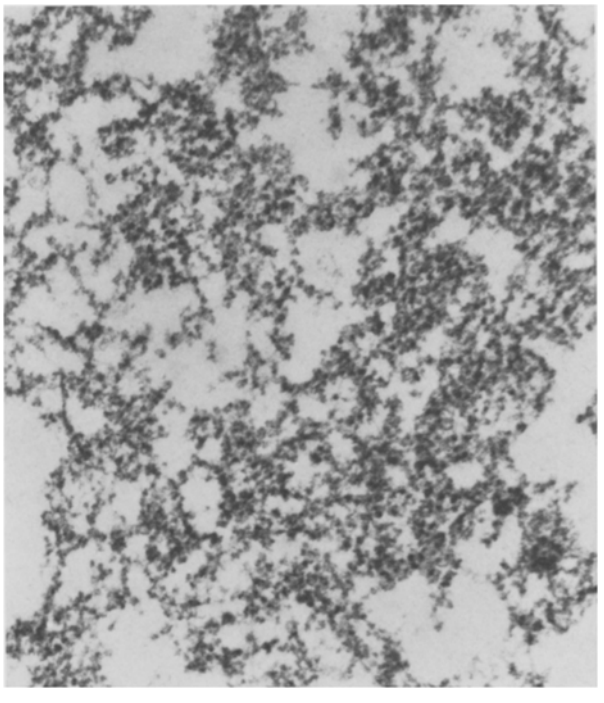

Fig. 2. Electron micrograph of microsomal pellet. Microsomes were sedimented as described in 'Materials and methods' at 0 °C, but in the presence of 0.06% Triton X 114 (detergent/protein ratio of 4). Solubilized rat liver microsomes. The microsomal vesicles are completely dissociated into flocculent material and small homogeneous densities. ×22,000.

The examination of Triton X 114 treated and at 37 °C sedimented microsomes (figure 1d) reveals large areas of extended membrane systems, most of which form whorls of 20 and more layers. Other regions clearly show long continuous membraneous structures. Microsomal vesicles could no longer be observed in this preparation; the ability to fuse completely among each other exists under these experimental conditions.

In addition, it should be noted that divalent cations as Ca^{++} or Mn^{++}, which have been shown to induce fusion of cells[16] and artificial membrane systems[17], are not required for fusion of microsomes.

The table shows that the sedimentation of microsomes at 37 °C had no apparent effect on the specific phospholipid content (per mg microsomal protein) and the qualitative composition of the microsomal phospholipids consisting of lysophosphatidyl choline, phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl serine + phosphatidyl inositol, and sphingomyelin. The values for microsomes given in the table are in good agreement with those previously found in our and in other laboratories[18–20]. Therefore, the fusion at 37 °C is surely not induced by lysophosphatides which arise from endogenous phospholipids by a phosphatide acyl-hydrolase.

More probable is, however, that an increase in the fluidity of the membrane lipids, as directly observed at the lipid phase transition (around 25 °C) by electron spin resonance, X-ray diffraction and NMR studies[12–14], is one of the prerequisites for the fusion of microsomal vesicles at 37 °C.

Helenius and Simons[21] have summarized evidence that when small amounts of detergent are added to biological membranes, some of it will be incorporated into the membrane. This is in agreement with sedimentation experiments carried out in our laboratory in order to study the relationship between fusion and solubilization of microsomal vesicles[22]. It is, therefore, reasonable to assume that incorporation of small amounts of detergent into the microsomal membranes without disrupting them, has a marked effect on the physical state of the membrane matrix. Furthermore, membranes containing detergent within the hydrophobic core should be more fluid than native membranes, because the viscosity of Triton X 114 (2.6 poise)[23] is lower than the apparent effective viscosity of the membrane fluid phase (3–10 poise)[24–27]. Consistent with this view, and in good agreement with our interpretation of the fusion of microsomes at 37 °C, is our observation that the ability of microsomal vesicles to fuse completely among each other exists in the presence of 0.03% (w/v) Triton X 114 (Triton/protein ratio of 2). However, the microsomal membranes are solubilized if the Triton X 114/protein ratio is increased to 4 (figure 2).

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Photoelectric properties of the 'yellow strips' of social wasps

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Summary. The yellow strips on the cuticle of social wasp workers become photoconductive following irradiation with light, the effect being perfectly reversible.

The influence of light on the behavior of social wasps has been studied by several investigators[2–7] and it is now well-known that sunlight plays a central role in the diurnal activity of these insects. We studied the photoelectric properties of the cuticle of various species of wasps collected from different countries, and, for comparative purposes, also the photoelectric properties of the honeybee cuticle. We concentrated mainly on the brown and yellow cuticular strips on the dorsal surface of the abdomen, because these are most frequently exposed to light. As is known[7], several yellow strips and spots occur on the cuticle of Vespinae and Polistinae. These are interspersed with brown or dark strips and together give the wasp its characteristic color. From an earlier study we suspected that the yellow strips are in some way connected with photoconduction because they shine in the darkness of the natural nest and also fluoresce under UV-illumination.

In the early pupal stages the yellow strips are not easily differentiated from the other strips, but become clearly distinguishable before eclosion. In *Vespa orientalis* and several other hornets, 2 of the abdominal segments, namely the 3rd and 4th, are especially suitable for photoconductivity determinations because their anterior half is brown and their posterior half is mostly yellow. In other species, the colored abdominal segments may differ in number, size, pattern, etc.

The photovoltaic potential and the photocurrent were measured with a Keithley digital electrometer Model 616 whose sensitivity is in the range of 10^{-1}–10^{-11} A and 10^{-2}–10^{+2} V and 10^{5}–10^{12} Ω. Hookup of the cuticle strips to the electrometer was accomplished via copper wire, 0.05–0.1 mm in diameter, both ends of which were smeared with a small amount of colloidal silver paint. Test insects were various hornets and wasps, both pupae and adults, and the measurements pertained to the