TABLE I
IN VITRO PROCESSING OF THE Y^r PRECURSOR

Cellular fractions added	Percent of polypeptide species lost after 60-min incubation at 37°C	
	β-Galactosidase	87 kd Precursor
None		
−ATP	<2	<2
+ATP	<2	5
Supernatant		
−ATP	<2	5
+ATP	<2	8
Membrane		
−ATP	<2	12
+ATP	<2	32
Membrane + Supernatant		
−ATP	<2	11
+ATP	<2	34

mechanism of precursor processing. These experiments suggest that the precursor can be modified by an ATP-dependent membrane component in vitro. Aliquots of a double-label Y^r supernatant fraction were incubated with supernatant and/or membrane fractions prepared from unlabeled cells. Incubations were performed in the absence or presence of 2 mM ATP since it has been recently demonstrated that several of the more interesting proteolytic enzymes are ATP dependent (Charette et al., 1981). In a typical experiment, incubations were at 37°C for 60 min and the reaction was terminated by heating at 50°C in SDS-gel sample buffer. The samples were fractionated by SDS-PAGE and analyzed as described (Fried, 1981). The effect of various cellular fractions and ATP on the recovery of β-galactosidase (an internal control) and the precursor are summarized in Table I. The various incubations did not affect β-galactosidase, a well-characterized stable protein. The precursor was not processed either by itself or when incubated with the supernatant fraction in the presence of ATP. In contrast, addition of membranes stimulated the loss of precursor and this was enhanced threefold by addition of ATP. No synergism was observed when supernatant and membrane fractions were both present. While these experiments demonstrated a specific loss of precursor, the appearance of the 30 kd membrane protein was not consistently observed. This may be due to the low sensitivity in the double-label analysis. On the other hand, material was found to accumulate at the top of the separating gel in a broad smear and could account for the loss of the precursor. The mature permease aggregates and runs in the same pattern when heated above 50°C (Fried, 1981). It is possible that intermediates in the processing of the precursor in vitro may be more susceptible to aggregation at 50°C. Experiments are in progress to determine the fate of the precursor in the in vitro system.

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FUSION AND DISASSEMBLY OF SENDAI VIRUS MEMBRANES WITH LIPOSOMES

ANNE M. HAYWOOD AND BRADLEY P. BOYER
Departments of Pediatrics and Microbiology, University of Rochester, Rochester, New York 14642 U.S.A.

The mechanism of Sendai virus penetration (fusion of the viral membrane followed by disassembly) is being studied by using liposomes in place of the host cell. Fusion of Sendai virus membranes with liposomes after a 2-h incubation at 37°C was previously described (1). Sendai viruses were also previously shown to be enveloped by receptor-containing liposomes as in the ingestion step of endocytosis (2). Investigation of the initial steps of viral fusion with liposomes composed of phosphatidylcholine (PC), cholesterol, gangliosides, and phosphatidylethanolamine (PE) showed that the fusion of the viral membrane with the liposome occurs at the leading edge of the developing "endocytic vacuole," where there is a region of membrane with a small radius of curvature (3). Membrane fusion establishes the continuity of the viral and liposomal membranes. Viral disassembly with distribution of the viral glycoproteins on the surface of the liposome and deposition of the viral ribonucleoprotein

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(RNP) inside the liposome follows as a second step. The present paper shows that liposomes containing only PC, cholesterol, and gangliosides also fuse with Sendai virus membranes and that the course of viral disassembly into these liposomes is variable.

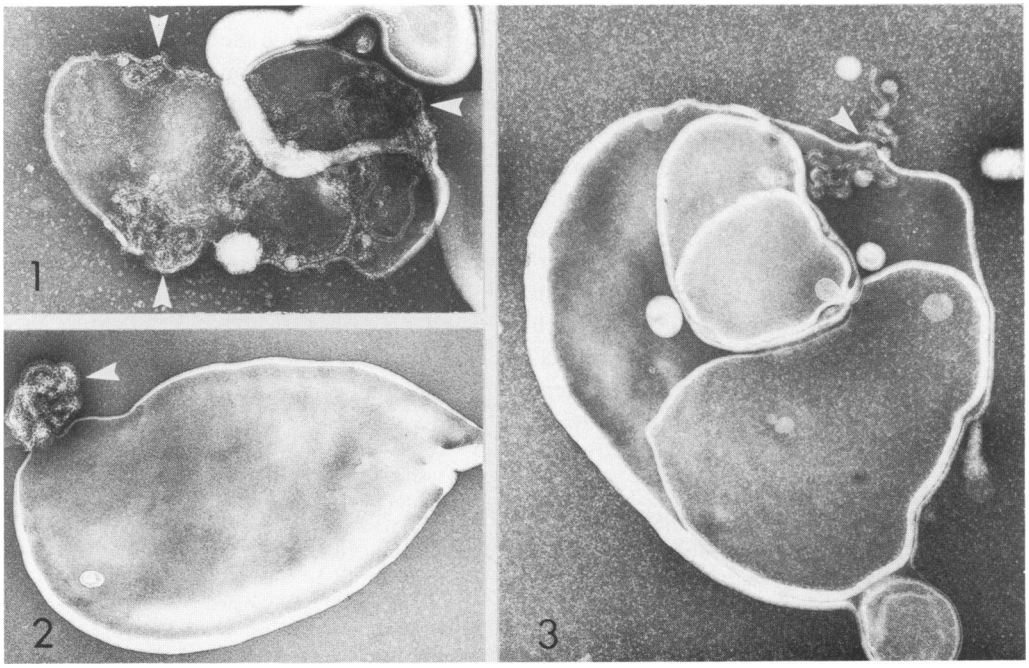
MATERIALS AND METHODS

Egg phosphatidylcholine (PC) was obtained from Avanti Biochemicals (Birmingham, AL) and cholesterol from Sigma Chemical Co. (St. Louis, MO). Each gave one spot when 0.8 μmol was chromatographed with chloroform-methanol-concentrated NH_4OH (60:25:4, vol/vol/vol) and with chloroform-methanol-glacial acetic acid-water (65:25:2:4, vol/vol/vol/vol) on silica gel G thin-layer chromatography (TLC) plates. Bovine brain gangliosides from Koch-Light Laboratories were purified by chromatography with chloroform-methanol-water (60:40:9, vol/vol/vol) + 20 mg KCl/100 ml on silica gel G TLC plates. Chromatography of the purified gangliosides with chloroform-methanol-2.5 M NH_4OH (60:35:8, vol/vol/vol) + 20 mg KCl/100 ml on silica gel G plates confirmed that they contained five ganglioside species and no contaminating lipids. Liposomes were made from 1 μmol of PC, 0.66 μmol of cholesterol, and gangliosides containing 0.1 μmol of *N*-acetylneuraminic acid. The lipids were hydrated in 0.15 ml of a buffer containing 137 mM NaCl, 2.7 mM KCl, 8 mM Na_2HPO_4, and 1 mM KH_2PO_4. Virus was prepared and electron microscopy was carried out as previously described (3). Virus (8,192 hemagglutinating units) was adsorbed to the liposomes at 0–4°C for 1 h and then incubated at 37°C for 2 h.

RESULTS AND DISCUSSION

Figs. 1–3 show liposomes containing only PC, cholesterol, and gangliosides which have fused with Sendai virus membranes. Gangliosides aid fusion because they serve as Sendai virus receptors (4), and because the resultant receptor-ligand binding causes envelopment and formation of regions of liposomes with increased curvature where fusion occurs (3). Gangliosides may play additional roles in membrane fusion. Virus binding could cause a local high concentration of gangliosides, and at high concentrations gangliosides leave the bilayer phase (5). Viral neuraminidase will release the negatively charged neuraminic acid from gangliosides which will result in a decreased negative charge on the external surface of the bilayer. Some ganglioside species have been shown to cause red cell fusion (6).

Three patterns of disassembly are seen after a 2-h incubation of Sendai virus with liposomes containing only PC, cholesterol, and gangliosides. The first pattern is seen in Fig. 1 where apparently three viruses have fused and disassembled into a moderate-sized liposome. The viral ribonucleoproteins are inside the liposome and the viral glycoprotein spikes are distributed over the surface of the liposome. This liposome will have received a considerable contribution of viral lipids from at least three viruses, so its final composition will be different from its original composition. The second pattern is seen in Fig. 2 where a virus has fused with a liposome but not disassembled. The third pattern is seen in Fig. 3 where the fused viral and liposomal membranes have not maintained their continuity, so that part of the viral RNP has been released through the membrane. Howe and Morgan also noted partial RNP release after fusion of Sendai virus with erythrocytes (7). The patterns seen in Figs. 2 and 3 have not been noted after incubation for 2 h with liposomes that have egg PE in their composition. This difference may be due to a lipid requirement for viral disassembly, i.e., egg PE could assist the packing of the viral proteins into the liposomal



FIGURES 1–3 Liposomes were made and "infected" as described in materials and methods. Arrows indicate positions of viral RNPs ×53,200.

membrane. Alternatively, the different patterns could be due to physical differences in individual virions such as those that determine the ability of a virion to cause hemolysis (8).

In summary, Sendai virus can fuse with liposomes containing only PC, cholesterol, and gangliosides. This suggests that gangliosides may have a role in membrane fusion in addition to their receptor function. In liposomes of this simple composition, the course of disassembly is variable.

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HUMAN BLOOD PLATELET AGGREGATION-INHIBITORY TARGET SITES ASSUMED TO INVOLVE MEMBRANE PHOSPHOLIPIDS

R. P. QUINTANA, A. LASSLO, AND M. DUGDALE,
Departments of Medicinal Chemistry and Medicine, University of Tennessee Center for the Health Sciences, Memphis, Tennessee 38163 U.S.A.

In an extensive study identifying structural features associated with the inhibition of adhesion-release-aggregation chain reactions in human blood platelets, we employed a series of carbamoylpiperidine derivatives with systematic and gradual changes in their molecular constitution, allowing meaningful interpretation of biological response variances. Current indications are that the entities conceptualized and synthesized in our laboratories (1–6) inhibit platelet aggregation (culminating at 5 μM concentrations) by penetrating the lipid bilayer of the platelet membrane and by interacting, as cations, with negatively charged phospholipids (e.g., phosphatidylserine and phosphatidylinositol) within the bilayer's inner segment (7). Following such penetration, those cations could be interfering with phospholipase-A_2 activation by counteracting stimulus-induced mobilization of Ca^{++} ions and Ca^{++}-dependent phospholipase-A_2 activity (8), thereby rendering platelets less susceptible to aggregation reactions. Our studies, employing our mono- and bis(carbamoylpiperidino)alkanes and -aralkanes as molecular probes, suggest platelet aggregation-inhibitory target sites spaced at 8 Å, and yield exacting information on the influence of hydrophobicity, planarity and geometric isomerism, as well as on the significance of interatomic distances between, and the charge levels of, the amino functions primarily instrumental in consummating interactions leading to the inhibition of human blood platelet aggregation.

To confirm these interpretations, the effects of our carbamoylpiperidine derivatives are being evaluated at surface pressures estimated to occur in actual platelet membranes (34 mN m^{-1})(9), on monomolecular films of phosphatidylserine whose location in the inner segment of the platelet membrane's lipid bilayer is acknowledged, on those of phosphatidylcholine, which is known to be a constituent of the bilayer's outer leaflet, and on related pure and mixed monolayer systems; e.g., cholesterol/phospholipid in ratios of 0.5, paralleling those observed in platelet membranes (10).

MATERIALS AND METHODS

A Payton aggregometer (Payton Associates Inc., Buffalo, NY) with a dual channel recorder (PF10H0-D) and a Coulter counter ZBI (Coulter Electronics Inc., Hialeah, FL) were employed in our platelet aggregation determinations (11); the inhibitory potency of our compounds was classified: 0 ≤ 10%, 1+ = 11%–19%, 2+ = 20%–29%, 3+ = 30%–39%, 4+ = 40%–49%, 5+ = 50%–59%, 6+ ≥ 60%. Interfacial and surface tension values were determined (12) on a Kahlsico duNouy instrument TE03 (Kahl Scientific Instrument Corp., El Cajon, CA). Surface pressure and potential measurements (13) are being carried out in a recent version of our previously conceptualized (14) Wilhelmy-type surface balance system, equipped with a dual pen Omniscribe B-5000 (Houston Instrument Div., Bausch and Lomb Inc., Austin, TX).

Please address correspondence to Dr. Lasslo.

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