tides, particularly the MSf (0.068 cycle per day) tidal constituent (*3*). That is, the wind-forced and tidally forced motions interact nonlinearly, and so some tidal energy has leaked into the eigenfunction described here. This may at least partially explain the modification to the Ekman spiral at depth that was described earlier. For both the clockwise and anticlockwise rotating components of the eigenfunction, at all frequencies for which the eigenfunction is coherent with the wind the magnitude of the phase is close to 90°, so the lag in time is a function of frequency. At the frequency for which the eigenfunction is most coherent with the wind ($\simeq -0.12$ cycle per day), the lag time is about 2 days, which corresponds to about three inertial periods at 49° latitude.

These results suggest that a substantial portion of the observed low-frequency fluctuations in the Strait of Georgia were, on average, interpretable as a quasi-steady, classical Ekman spiral. The low frequency of the fluctuations relative to the inertial period of 16 hours suggests that the quasi-steady assumption was valid. The presence of significant tidal currents in the strait suggests that the distribution of turbulence with depth was more uniform than if only the wind-forced flow had been present; thus the assumption of constant eddy viscosity had a better chance of being approximately satisfied. The fluctuating nature of the flow made the assumptions of infinite expanse and no horizontal variations reasonable approximations. More importantly, by examining the fluctuations over a number of cycles instead of examining the mean flow, we obtain a result that is an average of a number of separate realizations.

In general, contemporary wisdom states that Ekman's theory is not sophisticated enough to explain actual flow fields. The results presented here show that Ekman's theory can in certain cases describe in some detail the vertical structure of wind-forced flows, even in hydrographically complicated regions such as coastal straits.

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A Poliovirus Neutralization Epitope Expressed on Hybrid Hepatitis B Surface Antigen Particles

F. DELPEYROUX, N. CHENCINER, A. LIM, Y. MALPIÈCE, B. BLONDEL, R. CRAINIC, S. VAN DER WERF, R. E. STREECK*

The hepatitis B virus (HBV) envelope protein carrying the surface antigen (HBsAg) is assembled with cellular lipids in mammalian cells into empty viral envelopes. In a study to evaluate the capacity of such particles to present foreign peptide sequences in a biologically active form, in-phase insertions were created in the S gene encoding the major envelope protein. One of the sequences inserted was a synthetic DNA fragment encoding a poliovirus neutralization epitope. Mammalian cells expressing the modified gene secreted hybrid particles closely resembling authentic 22-nanometer HBsAg particles. These particles reacted with a poliovirus-specific monoclonal antibody and induced neutralizing antibodies against poliovirus. The results indicate that empty viral envelopes of HBV may provide a means for the presentation of peptide sequences and for their export from mammalian cells.

VARIOUS PARTICLES ARE PRESENT in the blood of humans infected with the hepatitis B virus (HBV), including 45-nm particles corresponding to complete virions and spherical or tubular particles, about 22 nm in diameter, corresponding to empty viral envelopes (*1*). The 22-nm spherical particle is a complex structure containing lipids and about 100 molecules of protein, including a major protein carrying the surface antigen (HBsAg) encoded by the S gene, and a minor protein encoded by the pre-S region and the S gene (*2, 3*). Both the major and the minor protein are found in glycosylated and nonglycosylated form. The pre-S region codes for an immunodominant epitope of HBV (*4*), but only the 226-amino-acid sequence of the major envelope protein is needed for the assembly of the 22-nm particles and their secretion from mammalian cells (*5*). HBsAg particles from chronic carriers of HBV are currently used as vaccine against hepatitis B (*1*); they are also synthesized and secreted by mammalian cell lines transfected by vectors carrying the S gene (*6, 7*). Related particles have been prepared from yeast (*8*).

We have developed a cellular system for synthesizing HBsAg and have used it for studying the structural requirements for formation and secretion of 22-nm particles by creating in-phase insertions of variable length and sequence in different regions of the S gene (*9*).

The plasmid pPAP (Fig. 1A) carries the SV40 early promoter and a modified S gene encoding an envelope protein (HBsPolioAg) that is 13 amino acid residues longer than HBsAg. The construction of this plasmid was achieved by inserting a 39-bp oligonucleotide into a naturally occurring Bam HI site; this led to duplication of amino acids 112–113 (Gly–Ser) and insertion of an 11-amino-acid sequence between the duplicated residues. The site of insertion into HBsAg is part of a hydrophilic domain of the viral envelope protein and is close to the region carrying most of the HBs antigenic determinants (*1*). We also constructed a plasmid termed pLAS, which is identical to pPAP except for the insertion in the Bam HI site (Fig. 1A). The inserted sequence represents amino acid residues 93–103 of capsid protein VP1 of poliovirus type 1 (Mahoney strain). The corresponding synthetic peptide mimicks a neutralization epitope of infectious poliovirus (*10*) and can be recognized by a neutralizing monoclonal antibody (C3) (*11*).

Clones of mouse L cells transfected by

F. Delpeyroux, N. Chenciner, A. Lim, Y. Malpièce, R. E. Streeck, Unité des Applications du Génie Génétique (G3), Institut Pasteur, 75724 Paris Cedex 15, France.
B. Blondel and R. Crainic, Unité de Virologie Médicale, Institut Pasteur, 75724 Paris Cedex 15, France.
S. Van der Werf, Unité de Virologie Moléculaire, Institut Pasteur, 75724 Paris Cedex 15, France.

*To whom correspondence should be addressed.

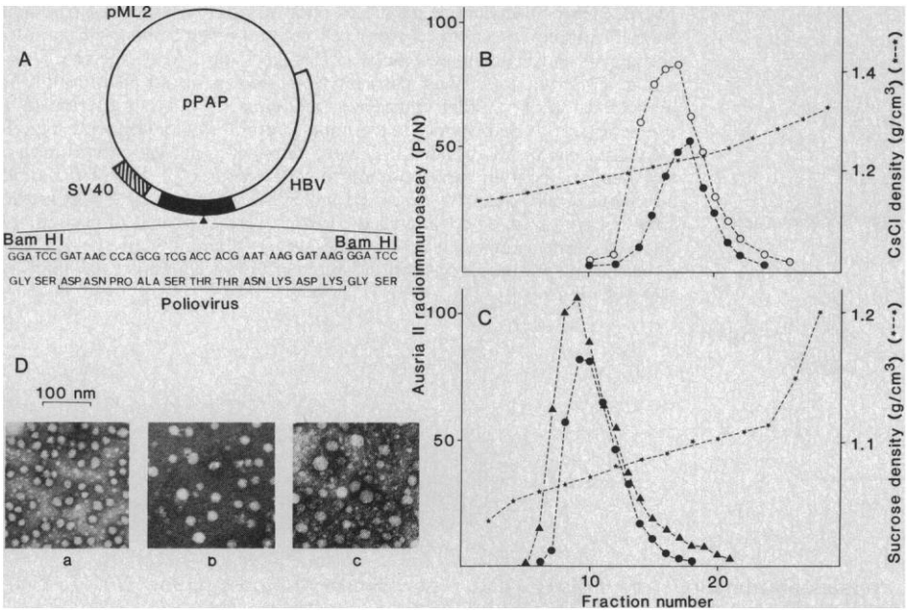

Fig. 1. Characterization of HBsPolioAg. (A) Plasmid pPAP carrying the β-lactamase gene and the colE1 origin from pML2 (*20*), the SV40 early promoter (hatched) and a segment of the HBV genome (subtype ayw, nucleotides 43–1984) including the S gene (filled area) (*1*). A synthetic DNA fragment, flanked by Bam HI sites, encoding an 11-amino-acid sequence of type 1 poliovirus VP1 (Mahoney strain) was inserted into the Bam HI site within the S gene. The Bam HI site at position 1400 of the HBV genome was eliminated by partial Bam HI cleavage, filling-in, and religation. pLAS is identical to pPAP except for the insertion. (B) CsCl density gradient centrifugation of HBsPolioAg (●) and HBsAg (○) particles from the cell culture supernatant of cellular clones transfected by pPAP and pLAS, respectively. (C) Sucrose gradient centrifugation of HBsPolioAg particles (●) and of authentic 22-nm HBsAg particles from human serum (▲). Curves with asterisks in (B) and (C) indicate the CsCl and sucrose densities, respectively. (D) Electron microscopy of HBsAg and HBsPolioAg particles stained with 2% phosphotungstic acid: (a) HBsAg from human serum (HEVAC B vaccine); (b) HBsAg from clone LAS; (c) HBsPolioAg from clone PAP. Mouse L cells were transfected according to (*21*) except that carrier DNA was omitted. Ten micrograms of pLAS or pPAP were cotransfected with 2 μg of pW carrying a neomycin resistance gene (*22*). Supernatants of isolated colonies growing in selective medium (400 μg/ml of G418) were assayed for HBsAg (Ausria II, Abbott). HBsAg particles were purified by centrifugation through two successive CsCl gradients (*23*) and one sucrose gradient (*7*).

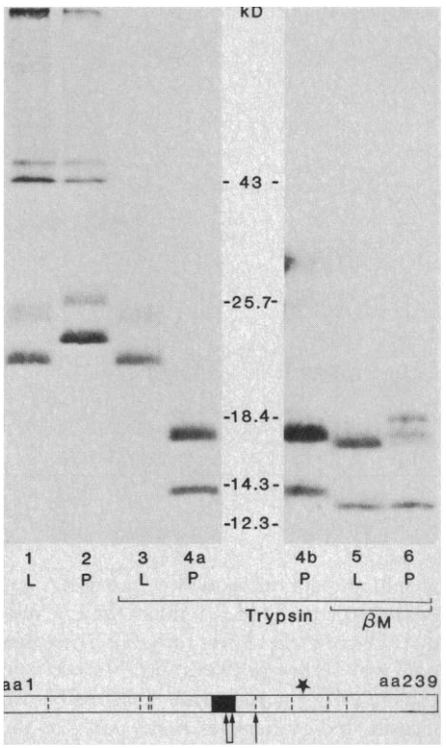

Fig. 2. Effect of trypsin treatment on polypeptides in HBsAg and HBsPolioAg particles. An autoradiograph of two 15% SDS-polyacrylamide gels (*24*) of [^{35}S]methionine-labeled polypeptides immunoprecipitated by a rabbit antiserum to human HBsAg particles (Behring) are shown before and after trypsin digestion. L, partially purified HBsAg particles; P, partially purified HBsPolioAg particles. 1 and 2, nontreated controls; 3, 4a, and 4b, trypsin treatment under nonreducing conditions; 5 and 6, trypsin treatment in the presence of 1% β-mercaptoethanol. 1 to 4a and 4b to 6 were run on separate gels; 4a and 4b are otherwise identical. The positions of prestained polypeptide molecular size markers (Bethesda Research Laboratories) run in the same gels are indicated. The scheme represents the 239-amino-acid HBsPolioAg with the poliovirus VP1 segment given in black. The double arrow indicates the trypsin cleavage site (or sites) in the VP1 insertion, the single arrow the position of Arg-122 of HBsAg. Dotted lines indicate the positions of the other Lys and Arg residues in HBsAg and the star, the site of glycosylation (Asn-146).

pPAP or pLAS express HBsAg at high frequency as determined by radioimmunoassay (85% of the clones tested). Particles purified from the culture medium of cellular clones (PAP, LAS) transfected by pPAP or pLAS, respectively, have about the same density in CsCl (Fig. 1B). They do not differ significantly from human HBsAg particles according to sedimentation in sucrose (Fig. 1C), but appear to be more variable in diameter (Fig. 1D). The polypeptides immunoprecipitated from LAS particles are resolved into two bands by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) (Fig. 2, lane 1) which correspond to glycosylated (25 kD) and nonglycosylated (22 kD) HBsAg (*1, 9*). The polypeptides immunoprecipitated from PAP particles (HBsPolioAg) by antiserum to HBsAg (Fig. 2, lane 2) are also present in the two forms. The difference of 1.5 kD between the apparent molecular sizes of HBsAg and HBsPolioAg (Fig. 2, lanes 1 and 2) is in agreement with the molecular size of the inserted sequence. These results demonstrate that the insertion prevents neither the specific protein-lipid interactions required for the assembly of envelope particles nor the glycosylation and the secretion of the particles from the cells into the medium.

In an attempt to establish whether the inserted poliovirus sequence was exposed at the surface of the particles and to examine whether it had created conformational changes in the envelope protein, we studied the protease sensitivity of HBsAg and HBsPolioAg particles (Fig. 2). HBsAg particles are highly resistant to trypsin under nonreducing conditions (*2*) (Fig. 2, lane 3) whereas HBsPolioAg is completely cleaved at a unique site (or several closely spaced sites) to yield polypeptides with apparent molecular sizes of 17.4 and 14.3 kD (Fig. 2, lanes 4a and 4b). The sizes of the fragments are compatible with cleavage within the inserted sequence. In the presence of a reducing agent, HBsAg is cleaved exclusively at Arg-122 (*12*) to yield fragments of 16.6 and 13.2 kD (Fig. 2, lane 5) whereas HBsPolioAg is cleaved into 17.4- and 13.2-kD fragments (Fig. 2, lane 6; the 18.4-kD polypeptide is a partial digestion product). This indicates that the 14.3-kD fragment obtained under nonreducing conditions from HBsPolioAg (Fig. 2, lane 4) contains Arg-122 and is 10 to 12 amino acid residues longer at the NH_2-terminus than the 13.2-kD fragment. This confirms that cleavage of the HBsPolioAg particles under nonreducing conditions had occurred at one or both of the Lys residues in the inserted sequence (Fig. 1A).

These results demonstrate that the inserted peptide sequence is readily accessible to protease, that is, the sequence is exposed on the surface of the hybrid envelope particles. In type 1 poliovirion (Mahoney) the corre-

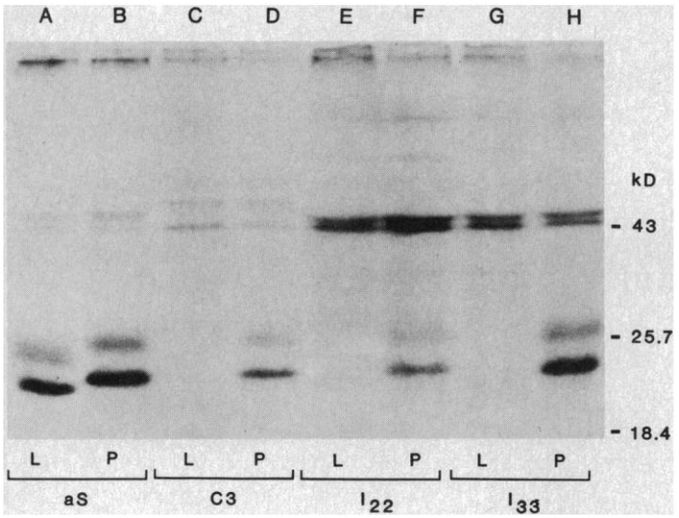

Fig. 3. SDS-polyacrylamide gel electrophoresis of polypeptides of envelope particles immunoprecipitated from cell culture supernatants with various antisera. L and P designate cellular clones LAS and PAP, respectively. aS, rabbit antiserum to HBsAg particles from human serum (Behring); C3, monoclonal antibody that neutralizes poliovirus (*11*); I_{22}, antiserum to a synthetic peptide corresponding to amino acids 93–104 of poliovirus type 1 (Mahoney strain) VP1, linked to bovine serum albumin; I_{33}, antiserum to a free synthetic peptide corresponding to VP1 residues 72–104 of the same poliovirus strain. Sizes of prestained molecular weight markers run in the same gel are given. The ^{35}S-labeled particles were incubated in phosphate-buffered saline containing 2% bovine serum albumin with the antisera indicated and immunoprecipitations were performed with protein A–Sepharose (Pharmacia).

sponding peptide sequence has a less exposed structure, which renders the Lys residues inaccessible to trypsin (*13*). The other potential cleavage sites in the hybrid HBsPolioAg particles are inaccessible to trypsin, indicating that these parts of the HBsAg molecule remain in a highly structured organization. Some conformational change, however, must have occurred in the major HBs antigenic region. This was indicated by the reduction (about 20-fold) of binding of antibody to HBsPolioAg particles as determined by radioimmunoassay, with HBsAg particles being used as reference and silver staining being used after SDS-PAGE for protein determination (*14*).

Immunoprecipitation of HBsAg and HBsPolioAg by different antisera is shown in Fig. 3. While both particles react with antibodies to HBsAg (Fig. 3, A and B), HBsPolioAg particles are specifically immunoprecipitated by the monoclonal antibody C3, which neutralizes poliovirus (Fig. 3, C and D), and by two different antisera to synthetic oligopeptides which include the inserted sequence (*15*) (Fig. 3, E to H).

To evaluate the immunogenic properties of the hybrid particles, we immunized mice with HBsAg or HBsPolioAg (Table 1). Ascites were created intraperitoneally by injecting tumorigenic cells not producing antibodies to obtain ascitic fluid immunogenically similar to mouse serum (*16*). Vaccination with HBsAg led to a high titer of antibodies reacting with human HBsAg particles (mouse 1). However, mice immunized with HBsPolioAg responded only weakly to HBs antigens (mice 2, 3, and 4). This is in agreement with the observation that the HBs antigenic determinants are partially distorted in the hybrid particles. The inserted poliovirus VP1 sequence, in contrast, was immunogenically reactive and in all mice induced antibodies recognizing synthetic peptides carrying this sequence (Table 1) as well as the entire VP1 protein of type 1 poliovirus (as determined by Western blot). In addition, the antisera obtained exhibited specific affinity for infectious and heat-denatured virions as shown by immunoprecipitation (Table 1). Moreover, all antisera had a significant titer of poliovirus neutralizing antibodies (Table 1). The best titer obtained was low by poliovirus standards and further work to improve the titer is needed.

We also found that the HBsPolioAg particles were immunogenic in rabbits: after injection of two doses of HBsPolioAg (10 to 40 μg each) three out of four animals had antibodies immunoprecipitating and neutralizing infectious poliovirions.

The potential of the HBsPolioAg particles to elicit neutralizing antibodies recognizing a rare epitope common to infectious and heat-denatured poliovirions (*10*) shows that the antibody-binding activity and immunogenicity of the corresponding amino acid sequence is expressed, at least in part, at the surface of the HBV envelope particles. This short sequence forms a peak on the capsid of poliovirus (*17*) and may therefore have an autonomous structure. In the HBsPolioAg particles the surrounding sequences of the HBsAg could further assist the inserted poliovirus sequence in maintaining its stability or flexibility.

Table 1. Immunogenic response to HBsAg and HBsPolioAg. BALB/c mice (8 weeks old) were immunized with either 2 μg of HBsAg (mouse 1) or 30 μg of HBsPolioAg (mice 2 to 4) by intraperitoneal injection using a 50% emulsion of Freund's complete adjuvant, repeated 2 weeks later with Freund's incomplete adjuvant. After 3 weeks each mouse received an injection of Sp2/0-Ag14 mouse myeloma cells (*25*) followed immediately by an intravenous booster injection without adjuvant. Mouse 5 was mock-immunized without antigen. Ascitic fluids were collected after 2 weeks and were analyzed by the following assays. Titers of antibody to HBsAg were determined by using the AUSAB radioimmunoassay (Abbott). Binding to the synthetic peptide corresponding to amino acid residues 93–104 of poliovirus VP1 was determined by enzyme-linked immunosorbent assay (ELISA) as described by Voller *et al.* (*26*). Ascitic fluids (1:80 dilution) were incubated in peptide-coated wells (0.5 μg in each) and fixed antibodies were screened by means of peroxidase-labeled goat antibodies to mouse immunoglobulins (Cappel). Positive sera are those showing an absorption of at least three times the absorption of the control (mouse 5). Heated or infectious ^{35}S-labeled virions incubated with ascitic fluid (1:2 dilution) were immunoprecipitated with *Staphylococcus aureus* (*27*). The recorded figures represent percentage values of immunoprecipitated radioactivity. The titer of neutralizing antibodies in each serum was measured by a standard plaque reduction assay on Vero cells, with an input of 100 plaque-forming units of Mahoney type 1 poliovirus. The reciprocals of the dilutions of sera (log_2) giving 50% plaque reduction were computed from regression curves of mean values obtained in three experiments.

Mouse number	Mouse antiserum to	Anti-HBsAg titer (IU)	Antipoliovirus activity			
			Synthetic peptide recognition	Immunoprecipitation (%)		Neutralizing titer (log_2)
				Infectious virions	Heated virions	
1	HBsAg	100.00	−	3	3	0
2	HBsPolioAg	0.01	+	89	96	4.86
3	HBsPolioAg	0.00	+	71	35	2.87
4	HBsPolioAg	0.10	+	90	87	3.89
5	Placebo	0.00	−	3	2	0

Protein epitopes correspond to specific arrangements of amino acid residues, and some such epitopes are contained in short continuous segments of peptide sequences (*18*). Our results show one example of a segmental (continuous) epitope that keeps at least a part of its biological activity when genetically incorporated into the middle of a highly structured membrane protein. To evaluate the generality of the system it will be necessary to study the integration of other sequences into the S gene encoding different epitopes and the variation of the site of insertion into the HBsAg protein.

As previously suggested by Valenzuela *et al.* (*19*), HBsAg particles could be useful as immunogenic carriers of foreign antigens for the preparation of vaccines. Our approach may also be useful for studying the biological activity of other peptides incorporated into the surface of an ordered multimolecular complex. The constitutive expression and secretion of the hybrid envelope particles by established cell lines provide an efficient system for the production of such structures.

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Calcium and Sodium Channels in Spontaneously Contracting Vascular Muscle Cells

MICHAEL STUREK AND KENT HERMSMEYER*

Electrophysiological recordings of inward currents from whole cells showed that vascular muscle cells have one type of sodium channel and two types of calcium channels. One of the calcium channels, the transient calcium channel, was activated by small depolarizations but then rapidly inactivated. It was equally permeable to calcium and barium and was blocked by cadmium, but not by tetrodotoxin. The other type, the sustained calcium channel, was activated by larger depolarizations, but inactivated very little; it was more permeable to barium than calcium. The sustained calcium channel was more sensitive to block by cadmium than the transient channel, but also was not blocked by tetrodotoxin. The sodium channel inactivated 15 times more rapidly than the transient calcium channel and at more negative voltages. This sodium channel, which is unusual because it is only blocked by a very high (60 μM) tetrodotoxin concentration but not by cadmium, is the first to be characterized in vascular muscle, and together with the two calcium channels, provides a basis for different patterns of excitation in vascular muscles.

ELECTRICAL EXCITATION OF VASCULAR muscles is characterized by a range of depolarization patterns that includes responses with unusually rapid onset and very long duration (*1*). Electrical spikes of a typical spontaneously active vascular muscle cell cause phasic contraction, while sustained depolarization causes tonic contraction (*1, 2*). These diverse forms of activation may be due to differential activation of various ion channels. Because vascular muscle excitation is likely to be calcium dependent (*1–3*), and because multiple types of calcium channels exist in other cell types (*4*), vascular muscle cells might be expected to have several types of calcium channels. The presence of sodium channels might provide additional combinations of inward current in these cells.

Voltage clamp data that could characterize different ion channels in vascular muscle have been technically difficult to obtain until the development of tight-seal pipette recording (*5*). With tight-seal recording from whole cells (*6*), we determined the voltage-dependent, inward-current channels in vascular muscle cells that might distinguish them from other cell types. For our studies, we used isolated, single vascular muscle cells that have been characterized electrophysiologically and pharmacologically (*7*). We did not use subcultured cell lines because they lose membrane excitability, pharmacological identity, and the ability to contract (*8*). Rather, we did all of our experiments with primary cultures of azygos venous muscle cells from neonatal rats (*9*); these vascular muscle cells contract spontaneously and show membrane properties (for example, 50 mV spikes and −45 mV resting potentials) appropriate for the blood vessel from which they were isolated (*7*).

Vascular muscle cells were studied in solutions that suppress outward potassium currents; cesium and EGTA were included in the recording electrode solution and barium and tetraethylammonium (TEA) were included in the external solution (*10*). For Na^+ current, only half of the Na^+ was replaced by TEA in extracellular solutions. Calcium currents were studied only after external sodium chloride was completely replaced by TEA chloride to characterize the smaller Ca^{2+} currents (Fig. 1, A and B).

Voltage clamp analysis in Na^+-containing

Department of Pharmacology, Cardiovascular Center, University of Iowa, Iowa City, IA 52242.

*To whom requests for reprints should be addressed.