bodies against antigens available only in femtomole quantities, even in the early steps of the purification of mixtures. Although immunization of spleen cells in vitro is an effective way of defining the maximum repertoire of potential antibody responses in a given animal or strain (*12*), practical limitations, including the poor survival capacity of clones generated in vitro, have largely prevented the use of this technique for construction of antibody "libraries." The addition of factors produced by thymic cells (in this study provided as conditioned medium from heterologous thymus cell suspensions) aids in the generation of antibody-secreting lymphoblasts in vitro (*4*) and in the survival of specific hybridomas derived from such cultures (*2*). For antigens available in extremely short supply, fusion frequencies must be maximized so that the relatively small number of clones responding to a given antigen will have a sufficient probability of survival to be recovered in the form of viable hybridomas. The latter consideration led to the choice of the UCR-M3 cell line in our studies because these cells have an excellent fusion rate and produce stable hybrids, although they have the disadvantage of being secretors of the MPC-11 myeloma IgG.

Many biologically active antigens would probably be amenable to studies similar to those we described. Together with other high-efficiency methodology such as HPLC and gas-phase protein sequencing (*13*), this would appear to provide a new approach to the isolation and chemical characterization of peptides present in only picomole quantities per gram of living tissues. The approach would be to prepare a relatively purified preparation of the antigen (having unambiguously detectable biological activity in a specific bioassay), to produce highly specific monoclonal antibodies against that antigen as shown by the bioassay, and then to purify the antigen by large-scale affinity chromatography; this would be followed by HPLC, ascertaining the amino acid composition on less than 100 pmole of the material (*14*), and determining the amino acid sequence on 1 nmole or less by gas-phase sequencing.

RICHARD A. LUBEN
Division of Biomedical Sciences and Department of Biochemistry, University of California, Riverside 92521

PAUL BRAZEAU
PETER BÖHLEN
ROGER GUILLEMIN
Laboratories for Neuroendocrinology, The Salk Institute, La Jolla, California 92037

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1. One hypothalamus fragment (rat or pig) yields, by our extraction methods, 150 to 500 fmole of GRF. These numbers as well as others in the text referring to molar quantities of pure GRF are based on quantitative amino acid analyses of GRF recently isolated from human tumors. On the basis of physicochemical and biological evidence, materials from all three sources are assumed to be similar in structure and specific activity. One GRF unit is defined as the weight of an in-house preparation of purified hypothalamic rGRF (GRF reference standard) known to produce half-maximal stimulation of secretion of growth hormone in the bioassay (*3*).
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Colloidal Solution of Water in Organic Solvents: A Microheterogeneous Medium for Enzymatic Reactions

Abstract. *To simulate in vitro the conditions under which enzymes act in vivo, enzyme molecules have been entrapped in hydrated reverse micelles of a surfactant in organic solvents. In this system the catalytic activity of one of the enzymes studied (peroxidase) became much higher than in water, and the specificity of the other enzyme (alcohol dehydrogenase) was dramatically altered.*

In vitro studies of enzymes are usually conducted in water. In the living cell, however, enzymes mostly act on the surface of biological membranes or inside them. Even the diffusion-free enzymes of plasma have a medium whose physical parameters (dielectric permeability, polarity, viscosity, and so on) and chemical composition only resemble those of the aqueous solutions used in most in vitro studies. Moreover, properties of water near an interface are vastly different from those of "bulk" water (*1*). For these reasons it is considered by many that traditional enzymologic studies of the behavior of enzymes in aqueous solutions provide an imperfect picture of biological reality (*2*).

To simulate in vitro the conditions of enzyme action in vivo, enzymatic reactions are often performed in mixtures of water and organic solvents with a high concentration of a nonaqueous component (*3*). However, such homogeneous media are also far from ideal. To provide a more realistic compromise between classical enzymology and the enzymology of complex biological systems, immobilized (covalently bound, adsorbed, or matrix-entrapped) enzymes have been developed (*4*).

Recently, a new mode of studying protein function has been introduced. It has been proposed that enzymes be dissolved not in water but in a colloidal solution of water in an organic solvent. In such systems many enzymes retain their catalytic activity; examples are chymotrypsin, trypsin, lysozyme, ribonuclease, pyrophosphatase, peroxidase, alcohol dehydrogenase, lactate dehydrogenase, pyruvate kinase, cytochrome c,

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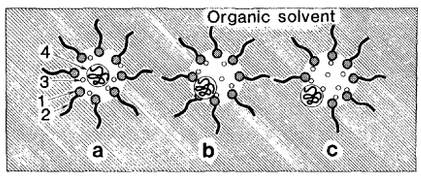
catalase, some forms of cytochrome P-450, and so forth (*5–9*). For lipolytic (interfacial) enzymes, heterogeneous media of this kind have been used for some time (*10*).

Research on "micellar enzymology" is still in its infancy, but there are some methodological aspects that will, in our opinion, stimulate such studies in the future. First, it is easy to prepare a colloidal system containing an enzyme; the enzyme (lyophilized or as a concentrated aqueous solution) is added, with stirring or shaking, to a solution of a surfactant in an organic solvent, where it is incorporated in an appropriate way into reverse micelles (*11, 12*). For example, hydrophilic protein molecules (such as many plasma enzymes) can avoid direct contact with the organic medium, as shown in (a) in the insert. Interfacial



enzymes (such as lipases) can interact with the surface layer of the micelle (b) or penetrate it, while typical membrane enzymes may come into contact with the organic solvent (c) (*13*). Second, the composition of such a microheterogeneous medium can be varied over a wide range with respect to the lipid surfactant, the organic solvent, and the degree of hydration of the reverse micelle. As the degree of hydration increases, the aggregation number and size of the reverse micelles grow (*11*). This provides a great many possibilities for choosing the optimal conditions for a particular enzyme (*14*). Third, the structure of micelle-entrapped protein and the kinetics of enzymatic reactions can be followed by routine spectroscopic techniques, since colloidal solutions of enzymes transmit light (*5–9*). Fourth, enzymes in reverse micelles can be studied at sub-zero temperatures without freezing (*7*).

It is already known that enzymes can be solubilized in organic solvents by use of surfactants without the loss of their catalytic activity (*5–9*). We now report that a micelle-entrapped enzyme may have a dramatically altered specificity. As an example, consider the oxidation of aliphatic alcohols to the respective aldehydes—$RCH_2OH + NAD^+ \rightarrow RCHO + NADH + H^+$—catalyzed by alcohol dehydrogenase from horse liver (*15*). The apparent second-order rate constant ($k_{cat}/K_{m,app}$) for substrates of the type $H(CH_2)_nOH$ is maximal for octanol ($n = 8$) in aqueous solution and for butanol ($n = 4$) in colloidal solution (Fig. 1).

Moreover, enzymatic catalysis may be more effective in a micellar medium than in water. For example, the value of k_{cat} for peroxidase oxidation of pyrogallol in reverse micelles of Aerosol OT in octane

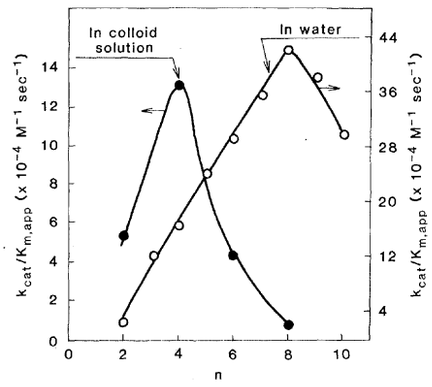


Fig. 1. Dependence of the second-order rate constant for oxidation of aliphatic alcohols of the $H(CH_2)_nOH$ type, catalyzed by alcohol dehydrogenase from horse liver, on the length of the hydrocarbon fragment in a molecule of the substrate (n). Experimental conditions: 25°C; (●) colloid system consisting of 0.1*M* Aerosol OT plus 8.5 percent (by volume) aqueous buffer (0.02*M* phosphate, *p*H 8.8) plus octane; (○) water (0.02*M* phosphate, *p*H 8.8). Measurements were made with the enzyme saturated with NAD^+. The initial steady-state rate of formation of NADH was followed spectrophotometrically (340 nm). The kinetic assay was carried out as described in (*15*).

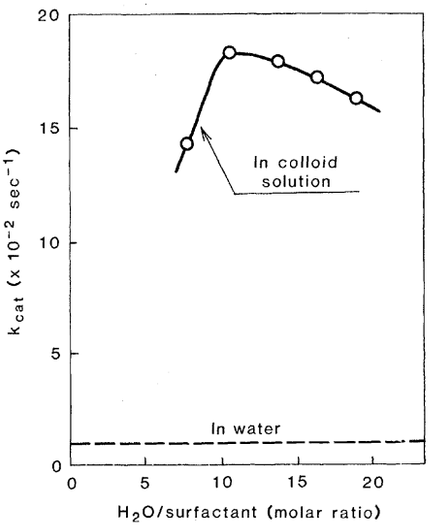


Fig. 2. Dependence of the first-order rate constant for peroxidase oxidation of pyrogallol on the content of water solubilized in 0.1*M* Aerosol OT plus aqueous buffer (0.02*M* phosphate-borate-acetate, *p*H 7.0) plus octane at 26°C. For comparison, the value of k_{cat} in the same buffer (dashed line) is shown. The initial steady-state rate of formation of purpurogallin was followed spectrophotometrically (420 nm). The kinetic assay was carried out as described in (*5, 16*).

is about 20 times higher than in water (*16*) (Fig. 2).

Such micellar effects on the catalytic activity and specificity of enzymes may be due to (i) microenvironmental effects on the reactivity of enzyme groups or substrate molecules (*17*), (ii) conformational alterations of the protein structure (*18*), and (iii) partition of the substrate or other molecules involved in the reaction between the aqueous and organic phases as well as the surface layer of the micelle (*19*). Assessment of the contribution of each of these mechanisms will require further study. Even at this stage, however, our data demonstrate the distorted (alcohol dehydrogenase) or "anemic" (peroxidase) behavior of an enzyme in water compared to that in a colloidal solution.

In addition, solubilization of enzymes in organic solvents has potential applications outside enzymology. For example, micellar solutions and water-in-oil microemulsions may be useful as reaction media in organic synthesis (to shift chemical equilibria compared to those in aqueous solution), in analyses for water-insoluble reagent, and as a means of modifying proteins with water-insoluble compounds (*20*).

KAREL MARTINEK
A. V. LEVASHOV
YU. L. KHMELNITSKY
N. L. KLYACHKO
I. V. BEREZIN
Chemistry Department, Moscow State University, Moscow 117234, USSR

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18. The occurrence of conformational changes in the structure of micelle-entrapped protein is supported by ultraviolet and circular dichroism spectral data (*8, 16*). The alterations in the protein conformation may be due to microenvironmental effects and direct interactions between the protein and surfactant molecules, as is frequently the case in aqueous solutions [C. Tanford, *Adv. Protein Chem.* **23**, 121 (1968); J. Steinhardt and J. A. Reynolds, *Multiple Equilibria in Proteins* (Academic Press, New York, 1969); S. Lapanje, *Physico-chemical Aspects of Protein Denaturation* (Wiley, New York, 1978); E. Mori and B. Jirgensons, *Biochemistry* **20**, 1630 (1981)].
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Neuromagnetic Localization of Epileptiform Spike Activity in the Human Brain

Abstract. *Local paroxysmal discharges of epileptic tissue within the human brain, which may be electrically recorded as voltage spikes in the electroencephalogram, also generate extracranial magnetic fields. These fields were assessed by means of recently developed neuromagnetometric techniques. Surface measurements of magnetic spike field strength in the region of the focus appear sufficient to establish the location, depth, orientation, and polarity of currents underlying the paroxysmal discharge.*

Most living cells maintain an electrical potential across their outer membrane; in the nervous system, it is this feature of the neuron that provides the basis for electrical signaling. When similar patterns of electrical activity are occurring in populations of cortical neurons, electrical potentials reflecting this activity may be recorded from the scalp (*1*). Under many conditions these same currents also generate extracranial magnetic fields (*2*). Synchronous firing of regional neuronal populations often occurs in healthy brain, the response to sensory stimulation being one common example (*3*). However, synchronous firing also marks certain neuropathologies, in particular the epilepsies (*4*). We now report that the measurement of extracranial magnetic fields produced by abnormal discharge in limited regions of the human cortex provides precise spatial information concerning the location, depth, and orientation of currents within the epileptogenic focus.

Focal (partial) epilepsy is a disorder characterized by the disposition of restricted regions of the cortex toward the production of synchronous paroxysmal discharges (*5*). Occasionally, this activity may spread beyond its normal boundaries to include larger areas of cortical tissue, resulting in a clinical seizure, or ictus. Between such ictal episodes, the epileptic focus often produces single, randomly occurring paroxysmal transients, termed interictal spikes. Interictal spikes may be recorded electrically over wide regions of the scalp, but precise electrical localization often requires recording from arrays of electrodes surgically placed on the cortical surface or implanted in the depth of the brain (*5, 6*).

Interictal spikes may be expected to be more sharply localized in the magnetoencephalogram (MEG) than in its electrical counterpart, the electroencephalogram (EEG), for several reasons. First, the magnetic field is influenced primarily by intracellular axial currents, which are confined to the region of the discharging cortex; the EEG, in contrast, is dominated by volume currents, which distribute the electrical potential throughout wide areas of bodily tissue (*2*). Second, the electrically resistive skull is transparent with respect to brain magnetic fields, whereas it distorts and smears volume-conducted brain electrical potentials (*2, 7*). For this reason, MEG recordings have been shown to have a spatial resolution more similar to electrical recordings made directly from the surface of the brain than to that of the scalp EEG (*3, 8*). Finally, MEG is an absolute rather than differential measure. Spatial derivatives of the magnetic field may be determined by comparing flux strength proximal to the scalp with that of a distal point on the same axis, unlike the EEG which requires reference to a secondary scalp location (*9*).

Neuromagnetic measurements were obtained from one child and one adult with focal (partial) seizure disorders (*10*). Previous examination of the scalp EEG for subject 1 revealed right temporofrontal (Sylvian) spike discharges; for subject 2 the discharge was in the left anterior temporal region. A dependent contralateral homologous EEG spike focus was also recorded in subject 1.

Brain magnetic fields were measured normal to the scalp in an unshielded environment by using a superconducting quantum interference device (SQUID) coupled to a second-derivative flux transporter with a coil diameter of 2.4 cm and separation of 3.2 cm. The probe assembly consisting of the SQUID and gradiometer was mounted in a fiberglass Dewar containing liquid helium (*11*). The lowest coil of the gradiometer was positioned tangentially 17 mm above the scalp over a rectangular matrix of 28 closely spaced points (2 cm), centered on the approximate EEG spike focus. For each point in the matrix, at least ten interictal spikes were recorded magnetically. During all magnetic measurements, concurrent EEG was recorded bilaterally from temporal electrodes in a bipolar configuration (*12*). Both the one channel of MEG and the six channels of EEG were amplified (Grass model 8-18 C electroencephalograph), band-pass filtered (MEG: 1 to 35 Hz; EEG: 1 to 70 Hz, −3 dB), and digitized on-line (PDP 11/34). Eight-second samples containing the magnetic and electrical spike activity were stored digitally on magnetic tape for further analysis. The principal features of the magnetic data were replicated on a separate occasion for each patient. During every recording session, magnetic measurements were also performed with the probe removed from the subject's head to control for environmental artifacts.

Data analysis was performed in three phases. First, the sampled MEG and EEG data were displayed in 8-second blocks on a high-resolution storage CRT display (Tektronix 611). The EEG was

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