nearest neighbor instantaneous configurations both show that the five-coordination is very irregular.

Figure 2 shows how the anomalous pressure dependence of network ion diffusivities in the silicates can be removed by sufficient breakdown of the tetrahedral network structure. For clarity, we have omitted data points for the original SiO_2 system (which first showed the existence of anomalous pressure dependences of transport in such inorganic systems) (*2*). In the sodium trisilicate case, in which the network structure is only partly broken down, an anomalous pressure dependence of the silicon and oxygen mobilities remains, consistent with the observation of a negative pressure dependence of the viscosity in this system (*4*). The orthosilicate structure, on the other hand, shows the normal pressure dependence observed in ionic liquids (*2*). Results for the metasilicate composition are given elsewhere (*10*).

The outstanding difference between the network and the orthosilicate liquids is the volume that must be assigned to the system to produce a pressure of about zero. Particularly at the high temperatures of these calculations, the normal pressure density of the orthosilicate (and metasilicate) compositions is very low. This is consistent with extrapolations (*10*) to the temperatures of our study of precise density data obtained by Bockris *et al.* (*11*) at lower temperatures.

Network (or framework) compounds are the predominant materials of the earth's crust. It seems clear from these studies, supported by the direct experimental studies of viscosity (*4*), that all these framework minerals in the liquid state, and probably also in the defect crystal state, will show anomalous pressure dependences of the framework ion mobilities. This characteristic is of interest in relation to the problem of modeling planetary evolution and even that of interpreting creep-dependent crustal displacements during the current epoch of the earth's geological history. Most planetary bodies pass through stages in their evolution in which they are molten, and in which the interior temperature may be somewhat closer to that chosen for the present study than is the case with the earth at present. While we are not competent to pursue this matter in any depth, it seems that the interior viscosity of a molten SiO_2-rich planetary body would be much smaller than the viscosity at the surface, since it seems that, at least to pressures of 150 kbar, increasing pressure acts in the same way as increasing temperature to decrease the viscosity. Experience with viscous liquids shows that, in general, pressure effects become much more pronounced as temperature decreases; hence, increases of several orders of magnitude in fluidity and a zone of maximum fluidity deep within the molten body are possibilities which should be considered in discussions of the hydrodynamics and long-term evolution of planets. From studies of aqueous systems (*12*) it seems that the pressure at which the viscosity minimum would occur would move to lower values as the temperature decreases.

By contrast, in liquid silicates poor in SiO_2, such as the olivine-type (orthosilicate) compositions believed to constitute the earth's mantle, behavior qualitatively similar to that for Na_4SiO_4 in Fig. 2 must be expected. In this case pressure and temperature will act in opposite directions at all pressures, giving less interesting profiles of viscosity (or creep rate) versus depth.

It is unfortunate that the time scale for the study of creep in stable (unsuperheated) crystals is too long for fruitful simulation studies at this time. However, the fact that simulation "samples" have no free surfaces (thanks to the periodic boundary conditions used) makes the study of fast-creeping superheated solids no less feasible than the study of supercooled liquids. Qualitative features of superheated solid behavior, such as the sign of the pressure dependences of creep rates, would probably be relevant to stable-state behavior, and we believe such studies would represent a worthwhile extension of the present work.

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Monoclonal Antibodies to Hypothalamic Growth Hormone–Releasing Factor with Picomoles of Antigen

Abstract. *Monoclonal antibodies specific for rat hypothalamic growth hormone–releasing factor (rGRF) have been produced by in vitro immunization of mouse spleen cells with less than 1 nanomole of rGRF in a partially purified preparation. Hybridoma supernatants were screened for anti-rGRF activity by use of a pituitary culture assay system that can detect growth hormone–releasing factor in the femtomole range. Such highly sensitive in vitro techniques permit the use of picomole quantities of an antigen in partially purified preparations for the isolation of monoclonal antibodies, which can in turn be used in biological studies and in immunochemical procedures for large-scale purification and isolation of that antigen.*

When specific high-affinity antibodies to a biologically active molecule are available, a variety of powerful techniques can be used to purify and characterize that molecule or study its physiological functions. A consistent problem, however, during the characterization of some biologically active molecules, such as neuropeptides present in brain tissue in amounts of only picomoles per gram, has been the difficulty of producing sufficient quantities of high-purity materials to be used as antigens to produce antibodies by standard in vivo immunization procedures. Although hypothalamic growth hormone–releasing factor is obtained in picomole amounts as a highly purified material, it has not been characterized yet, primarily because of its extremely low concentration (*1*) in the hypothalamus. We combined an in vitro immunization for hybridoma production (*2*) with a highly sensitive in vitro assay (*3*) to achieve the production of unlimited amounts of monoclonal antibodies to rat hypothalamic growth hormone–re-

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leasing factor (rGRF), using only picomoles of the antigen in a relatively crude preparation as the immunogen.

The rGRF preparation used here was derived from approximately 600 rat hypothalami by means of gel filtration followed by three steps of high-performance liquid chromatography (HPLC). Approximately 60 μg of this preparation containing less than 1 percent rGRF—about 100 pmole—was taken to dryness in the presence of 400 μg of human serum albumin and dissolved in culture medium for immunization of spleen cells in vitro (*2*). Spleen cells were isolated from BALB/c mice (two spleens per immunization, with about 10^8 cells per spleen) and cultured in 20 ml of medium containing the entire amount of immunogen. The culture medium was a mixture of 10 ml of fresh Eagle's minimum essential medium (MEM) with 20 percent fetal bovine serum (Gibco) and 10 ml of the same medium previously conditioned by 48 hours of culture with mouse thymus cells (*4*). The spleen cell culture was continued for 4 days in an atmosphere of 5 percent CO_2 in air at 37°C; substantial activation of the culture occurred, as shown by the appearance of lymphoblasts. At end of the 4th day, nonadherent cells in the culture were removed, washed with serum-free medium and fused with the mouse plasmacytoma line UCR-M3, a strain derived from the MPC-11 clone 45.6TG1.7 Oua3 (*5*). Fusion was carried out with 50 percent polyethylene glycol 4000 at room temperature (*6*); the fused cells were distributed into 48 culture wells containing BALB/c spleen macrophages (*7*) and 1 ml of hypoxanthine-aminopterin-thymidine (HAT) medium per well (*8*). A similar immunization was carried out to produce a total of 96 cultures derived from immunization with approximately 120 μg of immunogen. Growth of HAT-resistant colonies was observed in all 96 culture wells, with a visually estimated yield of 10 to 100 viable hybridoma colonies per well.

For assay of anti-rGRF activity in hybridoma culture fluids, portions of a reference standard preparation of rGRF (*1*) having known biological activity, expressed as units per milligram, were incubated overnight with hybridoma supernatants at varying dilutions and then treated with precipitating antiserum to mouse immunoglobulins to remove antigen-antibody complexes. These samples were then quantitatively assayed for GRF activity with dispersed rat pituitary cells in monolayer culture in defined medium (*3*), the end point being the measurement by radioimmunoassay of the growth hormone released into the culture medium during a 3-hour incubation (Table 1). The assay system is sensitive to as little as 0.5 fmole of pure GRF (*3*). Such high sensitivity permitted the assay of very low levels of antibody to rGRF in the culture medium of the mixed hybridoma cultures, a necessity for detecting the products of even a single clone among the approximately 10^5 cells in a confluent 1-ml culture. Fifteen mixed hybridoma cultures of the original 96 had detectable levels of anti-rGRF activity; the seven cultures with the highest apparent activities were subjected to cloning by limiting dilution in 96-well culture plates (0.1 ml per culture) with 50 percent conditioned culture medium from UCR-M3 cells (*5*). Of approximately 350 clones isolated after 4 weeks, 24 were shown in bioassay to have anti-rGRF activity, and the six best clones were chosen for large-scale isolation of antibody.

Immunoglobulins against rGRF were isolated from large-scale cultures of hybridoma clones by ammonium sulfate precipitation (*9*). In addition, ascites fluid was obtained from each clone by injection of cells into pristane-primed mice (*10*). The isolated immunoglobulins were used to prepare affinity columns (*11*) for further purification of rGRF. Each of the six monoclonal antibodies examined was able to retain significant quantities of rGRF and to release the material in biologically active form under appropriate elution conditions (Table 2). Immunodiffusion studies with class-specific antiserums indicated that all six of the anti-rGRF hybridoma clones secreted immunoglobulin M (IgM); five of the six clones also secreted the IgG2b-k parental immunoglobulin from the UCR-M3 cells.

Our procedure of immunization in vitro may be regarded as of general applicability for producing monoclonal anti-

Table 1. Summary of GRF immunoprecipitation by hybridoma supernatants isolated from clones having anti-GRF activity. Culture media from hybridomas were diluted with Dulbecco's phosphate buffer saline and incubated with 40 to 80 units of GRF per milliliter. After 24 to 48 hours at 4°C, antigen-antibody complexes were precipitated by addition of mouse serum (2 μl per milliliter of incubation medium) and rabbit antiserum to mouse IgG and IgM (50 μl/ml), followed by incubation overnight at 4°C. The supernatants after centrifugation were assayed for GRF activity remaining (*6*). The antibody class of each hybridoma was determined by Ouchterlony immunodiffusion analysis. Blank spaces indicate that values were not determined.

Clone	Antibody class	Percentage of total GRF precipitated at indicated antibody dilution		
		1:15	1:30	1:150
3.10	IgG	98		85
3.12	IgM	95	95	
3.13	IgM	95	94	
4.2	IgM	95	100	
4.3	IgG	97		
4.4	IgM	88	93	
5.2	IgM	97		90
5.5	IgM	91	85	
6.3	IgM	98		94
6.11	IgG	97		93
7.3	IgM	92	85	

Table 2. Binding and elution of GRF from immunoaffinity columns derived from anti-GRF hybridomas containing antibodies to GRF. Ammonium sulfate–precipitated hydbridoma culture medium was used to furnish immunoglobulins for coupling to Sepharose 4B–CNBr, at a total protein to Sepharose ratio of 2 mg/g. Growth hormone–releasing factor (550 units) was added to 0.5 ml of the immunoadsorbent, which had been washed, in small columns, with 5 volumes of 10 m*M* phosphate buffer, *p*H 7.4. The complex was then washed with 1.5 ml of buffer, and the peptide GRF was eluted with 1.5 ml of 100 m*M* HCl. Both wash and eluate were assayed for GRF activity. Units of activity recovered were calculated with parallel-lines bioassay statistics. For definition of a GRF unit, see (*5*).

Clone	Units of GRF activity measured				Retained (%)	Recovered (%)
	Applied	Wash	100 m*M* HCl	Total measured		
3.12	550	69	504	573	88	104
3.13	550	48	504	552	91	100
4.2	550	119	231	350	66	64
4.4	550	47	428	475	90	86
5.5	550	80	441	521	85	95
7.3	550	48	458	506	91	92

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.

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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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