ible trace. The patterns show that these conditions are sometimes met over only a narrow range (less than a pH unit), evidence that sole reliance upon tests at arbitrary pH intervals could result in the exclusion of optimum or critical conditions for separation.

3) A trace of the change of mobility with pH is a distinguishing characteristic of a protein, the trace being a type of two-dimensional analysis or "fingerprint," analogous to an absorption spectrum and with similar value for identification or molecular structural information. In Fig. 2 is emphasized, for example, the sharp contrast that the long sweeping (impure) albumin trace makes with other protein traces and with the gently curving esterase trace. Each configuration reflects such fundamental molecular conditions as amino acid composition of the protein, the attachment of other ionizable groups to the protein, and molecular shape and size—structural factors that determine relations between pH and mobility in gel media. Each pattern can give considerable enlightening information on many proteins simultaneously.

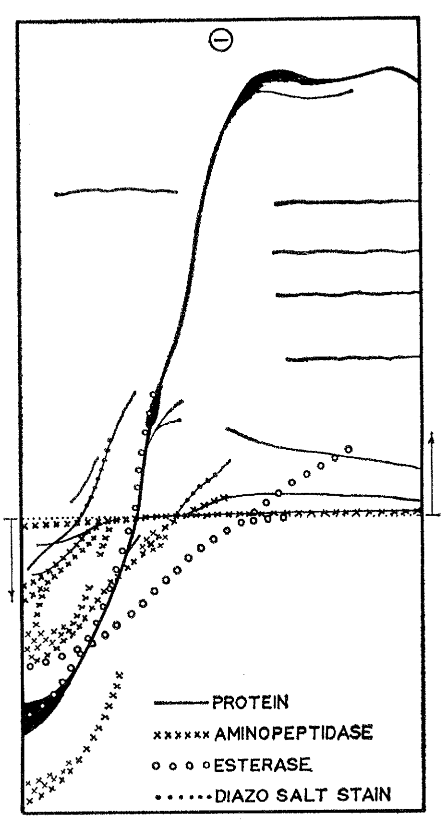

Fig. 2. Composite drawing for transverse-gradient starch-gel electrophoresis of human serum, bringing out pH-mobility relations among protein and enzyme traces (6); taken from Fig. 1 and other patterns. (The horizontal solid-line segments coarsely locate several rather broad and diffuse protein traces.)

4) The patterns can provide information on intermolecular associations. Even for a complex protein mixture like serum, Fig. 2 shows that most of the enzyme traces move quite independently of one another and bulk proteins (freely crossing over). On the other hand, at least two aminopeptidase traces do follow closely along corresponding protein traces, suggesting association, and one esterase trace clings to the albumin trace over almost its entire cathodic-anodic sweep.

5) The patterns can provide information on optimal pH conditions for isolating a given enzyme (or other protein) with minimum contamination by other enzymatic activity and with maximum specific activity. Thus the major esterase activity is well separated from both aminopeptidase and bulk protein in a pH region somewhat left of center in the patterns illustrated.

Transverse gradient electrophoresis offers a fundamental advantage in that a single unidirectional run can submit a mixture to an entire series of different conditions of fractionation. In contrast, the isoelectric spectra method provides only one condition, isoelectric point. Conventional two-dimensional methods provide two conditions, a different pH for each of the two dimensions (which actually involves two successive right-angle runs).

While the method as described has manifest effectiveness for qualitative separations, the technique has additional potential for quantitative measurements of pH-mobility relations. Other types of gradients containing agents that differentially affect electrophoretic mobility might be even more useful. To our knowledge, this technique has not been described or performed previously. Bitancourt and Nogueira (5) have simultaneously made chromatographic separations at four discrete pH conditions on a paper sheet, but they did not provide a continuous pH gradient.

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6. Two cathodic traces on the aminopeptidase (AP) pattern of Fig. 1 do not represent AP activity. As apparent on the original gels, these traces are yellow-orange rather than the red-orange characterizing true AP activity and arise from direct staining of protein by the diazo salt. (Previously we found that, while this background stain occurs typically for normal and most abnormal serums, it is diminished or absent in some abnormal serums.) Notable here is the selectivity with which two minor protein traces are stained (one even more strongly than by naphthalene black), while the major protein zone is quite ignored.
7. Aided by grants T-77 and T-252 from the American Cancer Society, PHS grant No. CA-06730, and gifts from Mollie E. Swinney. For assistance, I thank Charlotte Vivonia and Jean M. Hilburn (for work done at Los Angeles Veterans Administration Center) and E. R. May.

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Tabashir: An Opal of Plant Origin

Abstract. *A specimen of tabashir, a variety of opal found in bamboo, contained more water but smaller amounts of alkalis and alkaline earths than most opals. It consisted of particles of about 100 angstroms in diameter, linked together in clumps, which appeared in fractured surfaces as irregularities. The tabashir was amorphous, but its microstructure differed from that of silica gel and amorphous opal of inorganic origin.*

Many plants, such as grasses, *Equisetum*, bamboo, and certain tropical trees, absorb large quantities of dissolved silica, and as a result, solid silica is deposited in the cell walls of the plants (*1*). This silica, which can be isolated from the plant tissues as particles of the same dimensions as cells, has been identified as opal by optical and x-ray techniques (*2*). The particles are known as opal phytoliths, and, although long neglected in mineralogical studies of soils, they have been frequently reported in recent years (*3*).

In contrast to the small phytoliths, relatively large masses of silica have been found in the hollow stems of bamboo; this material is called tabashir (also tabasheer and tabaschir). Although recognized as a curiosity since antiquity, tabashir has not previously been examined by modern techniques, and little is known about it except that it is a porous, hydrous silica with a reported index of refraction of 1.11 to 1.18 (*4*, *5*). We have examined tabashir and compared its structure with that of opal phytoliths, silica gel, and opal of inorganic origin.

A single piece of tabashir weighing

1.2 g was obtained from bamboo grown in Burma (*6*). The specific gravity of the tabashir is 1.93, appreciably less than that of common opal, which tabashir resembles in other ways: it has a milky appearance, is translucent, and shows conchoidal fracture. Roughly parallel bands of different optical densities can be seen within the tabashir in transmitted light (Fig. 1*a*). The tabashir is optically isotropic. Its index of refraction as determined by the immersion method in sodium light is 1.427 ± 0.002 in contrast to that previously reported (*4, 5*).

Grinding and dispersing the tabashir for electron optical studies produced fragments whose sizes were between 1 and 0.5 μ. Many of the larger fragments were sufficiently thin at their edges to show that they were highly porous (Fig. 1*b*). An examination of smaller fragments at high magnification (Fig. 1*c*) showed that they were composed of rounded, roughly spherical particles, about 100 Å in diameter, which commonly were linked together into chains or clumps. Although freshly fractured surfaces looked glassy-smooth in an optical microscope (Fig. 1*a*), shadowed replicas at higher magnification showed that they were irregular and rough (Fig. 1*d*). This roughness comes from asperities several hundred angstroms apart, and corresponds, not to the basic particles of 100 Å in diameter, but rather to the clumps into which they are grouped. Some surfaces were etched briefly in hydrofluoric acid, but their replicas showed no additional details. The structure of tabashir may be compared with that of opals of inorganic origin, which were recently examined by similar techniques (*7*) and shown to consist of closely packed spheres of uniform size ranging from 1000 to 4500 Å in diameter (Fig. 1*e*).

The x-ray diffraction pattern of tabashir showed an intense, broad band at 4.0 Å and three additional bands, which were barely discernible, at 1.95 Å, 1.46 Å, and 1.18 Å. Heating at 1000°C and 1150°C only reduced the small-angle scatter, but heating at 1250°C caused the tabashir to crystallize to a low-temperature form of cristobalite that was either slightly defective or contained a small amount of poorly crystallized tridymite (*8*). The tabashir heated at 1250°C had a specific gravity of 2.30 and a mean index of refraction in sodium light of 1.484.

The chemical composition of tabashir, in percent by weight, is: SiO_2, 85.82; $H_2O(+100°C)$, 8.28; H_2O

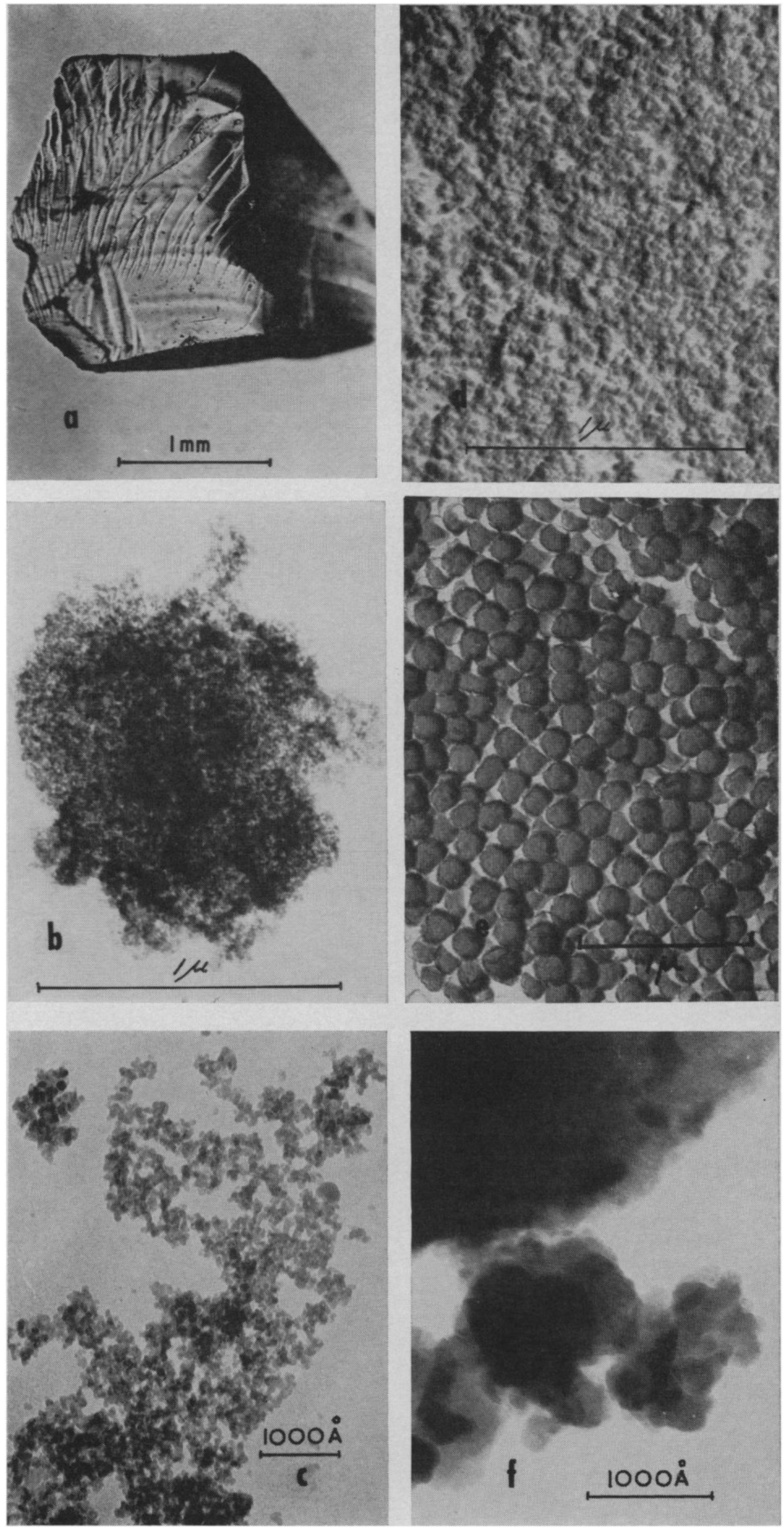

Fig. 1. *a*, Specimen of tabashir in transmitted light (× 23). *b*, Porous fragment of tabashir (× 45,000). *c*, Particles of dispersed tabashir (× 120,000). *d*, Replica of a fracture surface of tabashir (× 42,000). *e*, Replica of etched fracture surface of amorphous opal of inorganic origin—precious opal (× 25,500). *f*, Fragments of pure silica gel (× 187,500).

($-100°C$), 5.87; Na_2O, 0.002; K_2O, 0.039; CaO, <0.007; and MgO, 0.004. Tabashir contains more water than is commonly found in opals (*5*) but smaller amounts of alkalis and alkaline earths.

The statement (*4*) that tabashir is silica gel led us to examine a pure silica gel in the electron microscope. The gel, which was produced by drying a solution of monosilicic acid at 80°C, had no detectable alkalis or alkaline earths. Grinding produced fragments which ranged in size from a few microns to several hundred angstroms and showed no internal structure. The featureless structure of the irregular fragments and the tapering edge of a larger fragment are shown in Fig. 1*f*. Replicas of fracture surfaces had a slight texture but were smooth by comparison with tabashir. Silica gel therefore differs from tabashir and is homogeneous to the limit of resolution of the microscope (10 Å).

We then compared tabashir with opal phytoliths, which we separated from oats by destroying organic matter with nitric and perchloric acids (*2*). Grinding and dispersing the phytoliths produced a heterogeneous mixture of small fragments. In the electron microscope many of these fragments were similar to tabashir, but the basic particles were rather larger and more irregular. There were in addition some larger, irregularly shaped fragments up to 1000 Å in size with a homogeneous appearance.

Although tabashir and opal phytoliths have rather similar physical properties (including specific gravity and index of refraction), opal phytoliths contain greater proportions of alkalis and alkaline earths (*2*). This difference in purity is reflected in a different reaction to heating; both forms crystallized to the low-temperature form of cristobalite, but in the opal phytoliths the crystallization occurred at 1050°C (*2*), 200°C below the crystallization temperature of tabashir.

Electron diffraction from tabashir, opal phytoliths, and silica gel gave only a diffuse ring corresponding to a lattice spacing of 4.0 Å. However, the carbon supporting film gives weak, diffuse rings at 2.0 Å and 1.1 Å, which could conceal any similar but weak diffraction from silica fragments. Although the patterns from opal phytoliths and silica gel were indistinguishable, the pattern from tabashir was always sharper than either of them. The x-ray diffraction patterns from the three varieties of silica differed slightly. Thus in addition to the spacing at 4.0 Å, tabashir gave three diffuse bands, opal phytoliths gave one (at 1.97 Å), and silica gel gave none.

Because the additional bands appeared in x-ray patterns of tabashir and opal phytoliths but not in electron-diffraction patterns, we can infer that they were not produced by a homogeneous structure of disordered or microcrystalline cristobalite. Electron diffraction is the more sensitive technique, but the x-ray diffraction as carried out here was more selective because generally the pattern was obtained from a single fragment a few microns in size. The very low intensity of the extra x-ray bands relative to that at 4.0 Å is therefore in keeping with the interpretation that they were produced from a small fraction of the sample rather than from homogeneous, long-range ordering or incipient crystallization.

Although tabashir, opal phytoliths, silica gel, and many opals of inorganic origin are amorphous, the two forms of opal of plant origin can be distinguished from the others in the electron microscope. Because the electron optical microstructure of tabashir differs so markedly from that of amorphous opal of inorganic origin, tabashir appears to deserve a special place among the varieties of opaline silica.

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Hyperbaric Oxygenation: The Eye as a Limiting Factor

Abstract. *In dogs exposed to 100 percent oxygen at 3 atmospheres absolute pressure for more than 4 hours, a characteristic retinal lesion, manifested as the cytoid-body change, occurs. The selectivity of this injury suggests that the eye may be used as a sensitive indicator and as a site for study of oxygen toxicity. The cytoid body, an entity of disputed genesis, was produced experimentally for the first time.*

In recent years, through collaboration between the engineer and the medical scientist, thresholds have been crossed freeing man for finite periods from his dependence on the earthly atmosphere. These advances, enabling man to probe into space, to extend his reaches undersea, and to use hyperbaric oxygenation in medicine, are dependent upon the establishment and maintenance of a life-sustaining milieu, independent of and isolated from the surrounding environment. Oxygen is a major component of this milieu. Yet, paradoxically, a crucial limiting factor in the exploitation of this advance is man's intolerance to oxygen at high ambient pressures or concentrations. In our studies the eye has been a consistent and early indicator of oxygen toxicity, and we have observed a new pathologic manifestation of this injurious effect.

Our data are part of a comprehensive study of the applications and limitations of hyperbaric oxygenation in medicine (*1*). Using standard Navy diving and decompression techniques (*2*), we placed mature, healthy mongrel dogs individually, without anesthesia and without restraint, in a 1-m^3 chamber and exposed them to 100 percent oxygen. Oxygen inflow was regulated at the inflow valve to maintain a pressure of 30 $lb/in.^2$ gauge pressure, equivalent to approximately 3 atm absolute pressure, while the chamber was being vented at 0.014 m^3 per minute. Soda lime was used in the chamber to extract carbon dioxide. Each animal was subjected to a single exposure ranging from 210 to 362 minutes, regardless of adverse effects, which were predominantly manifested as seizures. These seizures developed at irregular intervals with abrupt onset and spontaneous cessation and in no instance led to paralytic sequelae or evidence of neurologic damage. For 6 to 13 days following exposure, the ani-