in this study. Consistent with our findings, manipulation of 5-HT has been shown to affect fear (or anxiety) (*20*) and aggression (*21*) in animals and humans (*22*, *23*).

The distinct behavioral and neuronal deficits in the heterozygote suggest that only those brain regions that have intrinsically low levels of the α-CaMKII gene expression would be most affected by the lower gene dosage. These regions may include the serotonergic nuclei. In contrast, the behavioral abnormalities of the homozygote (in which both copies of the gene are absent) become widespread (Table 1). Such gene dosage effects may exist in human psychiatric diseases, in particular, those involving personality traits associated with increased aggression and decreased fear (consistent with increased risk-taking behaviors).

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# Thalamic Abnormalities in Schizophrenia Visualized Through Magnetic Resonance Image Averaging


Nancy C. Andreasen,* Stephan Arndt, Victor Swayze II, Ted Cizadlo, Michael Flaum, Daniel O'Leary, James C. Ehrhardt, William T. C. Yuh



**Schizophrenia is a complex illness characterized by multiple types of symptoms involving many aspects of cognition and emotion. Most efforts to identify its underlying neural substrates have focused on a strategy that relates a single symptom to a single brain region. An alternative hypothesis, that the variety of symptoms could be explained by a lesion in midline neural circuits mediating attention and information processing, is explored. Magnetic resonance images from patients and controls were transformed with a "bounding box" to produce an "average schizophrenic brain" and an "average normal brain." After image subtraction of the two averages, the areas of difference were displayed as an effect size map. Specific regional abnormalities were observed in the thalamus and adjacent white matter. An abnormality in the thalamus and related circuitry explains the diverse symptoms of schizophrenia parsimoniously because they could all result from a defect in filtering or gating sensory input, which is one of the primary functions of the thalamus in the human brain.**


Schizophrenia is a disorder characterized by a multiplicity of signs and symptoms, no single one of which is present in all patients. Patients have a mixture of cognitive and emotional disturbances in a variety of functional systems such as perception, language, inferential thinking, and emotional expression and experience. Nevertheless, the fact that this illness is recognized throughout the world suggests that there must be some central feature that gives the disorder conceptual unity. When Bleuler named the disorder "schizophrenia" early in the 20th century, he identified such a fea-


N. C. Andreasen, S. Arndt, V. Swayze II, T. Cizadlo, M. Flaum, D. O'Leary, The Mental Health Clinical Research Center and Department of Psychiatry, College of Medicine and University of Iowa Hospitals and Clinics, Iowa City, IA 52242, USA.
J. C. Ehrhardt and W. T. C. Yuh, Department of Radiology, College of Medicine and University of Iowa Hospitals and Clinics, Iowa City, IA 52242, USA.

*To whom correspondence should be addressed.

ture: the fragmenting or splitting apart of the cognitive and emotional functions of the mind (*1*). Although not all patients given this diagnosis have delusions, hallucinations, or disorganized speech, all share a fragmentation of the mind: a catastrophic experience involving the loss of control over thoughts and emotions, the capacity to integrate experience and expression, and the sense of personal autonomy.

In the search for the neural substrates of the symptoms of schizophrenia, links have been noted between hallucinations and temporal lobe abnormalities, thought disorder and hippocampal abnormalities, and negative symptoms and prefrontal abnormalities (*2*). A central problem with this work has been the use of small samples, owing to the labor-intensiveness of the methods used, and a related difficulty in finding abnormalities consistent across studies.

We propose a parsimonious explanation for the multiplicity of signs and symptoms: abnormalities in midline structures that mediate attention and information processing, particularly the thalamus and related midline circuitry. To support this explanation, we provide evidence from magnetic resonance (MR) imaging. Fully automated techniques for image analysis of MR scans were used to generate an "average brain" from a sample of normal individuals and a sample of patients suffering from schizophrenia (*3*). The "average brain" is used to compare the two groups and to identify regions where they differ. This strategy provides an efficient alternative to current methods for analysis of MR imaging data, which involve manually tracing structures on prespecified areas of each individual scan.

MR data were collected with a 1.5-T GE Signa Scanner. The three-dimensional SPGR sequence was used with the following scanning parameters: 1.5-mm coronal slices, flip angle 40°, repetition time 24 ms, echo time 5 ms, two excitations, field of view 26 cm, matrix 256 × 192. Postacquisition processing was done with locally developed software (*4*).

The imaged brains were then linearly transformed with a "bounding box" technique that included six points: the most anterior and posterior, the most right and left lateral, and the most superior and inferior (*5*). Each imaged brain was stretched or compressed in three dimensions, creating three scaling factors: $S_x$, $S_y$, and $S_z$. After each brain was transformed to the same three-dimensional space, brains were subsequently averaged on a pixel by pixel basis. Before averaging, signal intensity was normalized with a histogram equalization process (*6*). Signal intensity values were then averaged separately for each of the two groups, normal controls and schizophrenic patients, by calculation of the mean and standard deviation for each equivalent pixel within the bounding box. Thus, an "average brain" was generated for the schizophrenic group and for the normal group. This average brain can be visualized and resampled three-dimensionally in the same manner as an individual MR data set. It has the advantage, however, of providing a concise numeric and visual summary of the group as a whole.

In order to submit the average brains to statistical analysis, we used the strategy of comparing groups by examining differences between the schizophrenic and normals as displayed in subtracted images. This approach to determining whether the two groups differ anatomically and to identifying the specific regions where they differ is analogous to methods used for many years for the analysis of positron emission tomography (PET) data (*7*). A variety of techniques are currently in use for the analysis of differences in PET images; most rely on some type of "*t* statistic" (*8*). In this case, we chose instead to display the differences as an effect size (ES) map, considering it a better indicator of the extent to which the two groups actually differ in a biologically significant way. An image-based ES shows the extent of difference between images in terms of the average intersubject variability. Thus, the image presents a descriptive picture of the size of group differences (*9*). The statistical image was built from the ES for group differences voxel by voxel.

Before the image subtraction analyses,

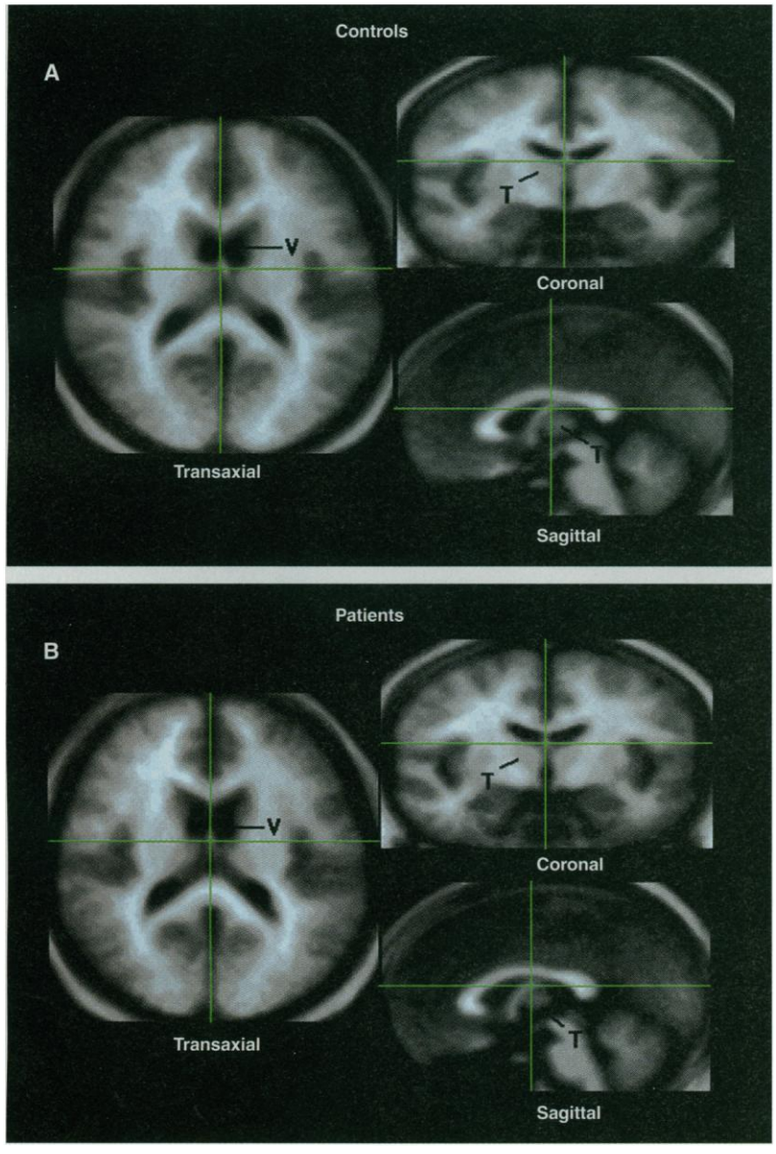


**Fig. 1.** Three orthogonal views of the "average brains" from (**A**) 47 healthy male volunteers and (**B**) 39 male patients suffering from schizophrenia. Green cross hairs display the coordinates of the location three-dimensionally. T, thalamus; V, ventricles.

the total volume of tissue and cerebrospinal fluid (CSF) was compared for the schizophrenic patients and normal control subjects. The patients had a mean brain tissue volume of 1263 ± 91 $cm^3$, as compared to 1327 ± 114 $cm^3$ in controls ($t = 2.90$, $P < 0.006$); CSF volume in patients was 131 ± 32 $cm^3$, as compared to 108 ± 29 $cm^3$ in controls ($t = 3.48$, $P < 0.001$). Thus, patients have more CSF and less brain tissue; results remained the same after covarying height. These results confirm earlier reports indicating reduced brain size and increased ventricular size in schizophrenia (*10*). A fundamental question raised by these studies is whether the reduced brain size and increased area of CSF-filled space results from a diffuse abnormality or whether it is a consequence of specific regional abnormalities. There appears to be a subtle but visually detectable difference in ventricular size, seen especially on the transaxial view (Fig. 1). The most noticeable regional difference is in the thalamus, which appears to be smaller, especially as visualized on the midsagittal view.

The major abnormalities identified when the images are subtracted and portrayed as ES maps are in two areas: the thalamus, and white matter tracts adjacent to it (Fig. 2, A and B). These abnormalities are primarily in the right hemisphere and are seen on all three orthogonal planes. Differences in signal intensity occur primarily in lateral thalamic regions, but may also be seen in medial areas. Differences are also present in white matter regions in the frontal lobe and to a lesser extent in the parietal and temporal lobes. An area of difference is also seen in the posterior (occipital) regions; this reflects the differences in brain size, which produces an aliasing ("nose wrap") artifact in the controls because of their larger amount of brain tissue. Thus, this type of image analysis appears to be sensitive to group differences and may offer an efficient and empirical method for analysis of MR data.

As a check on the possibility that these results could be due to some unknown artifact, the 48 normal males in this study were subtracted from a sample of 44 normal females, collected in an ongoing study of gender differences in the normal brain (Fig. 3) (*11*). In contrast to the control-patient images, no major differences are seen, apart from the rim of color in the skull and scalp, which reflects larger male head size. No differences are seen in brain parenchyma, apart from the posterior region, which reflects an aliasing artifact in the males because of their larger heads.

Decreased thalamic size has been our most consistently replicated finding in earlier studies, in addition to increased ventricular size (*12*). Thalamic abnormalities in

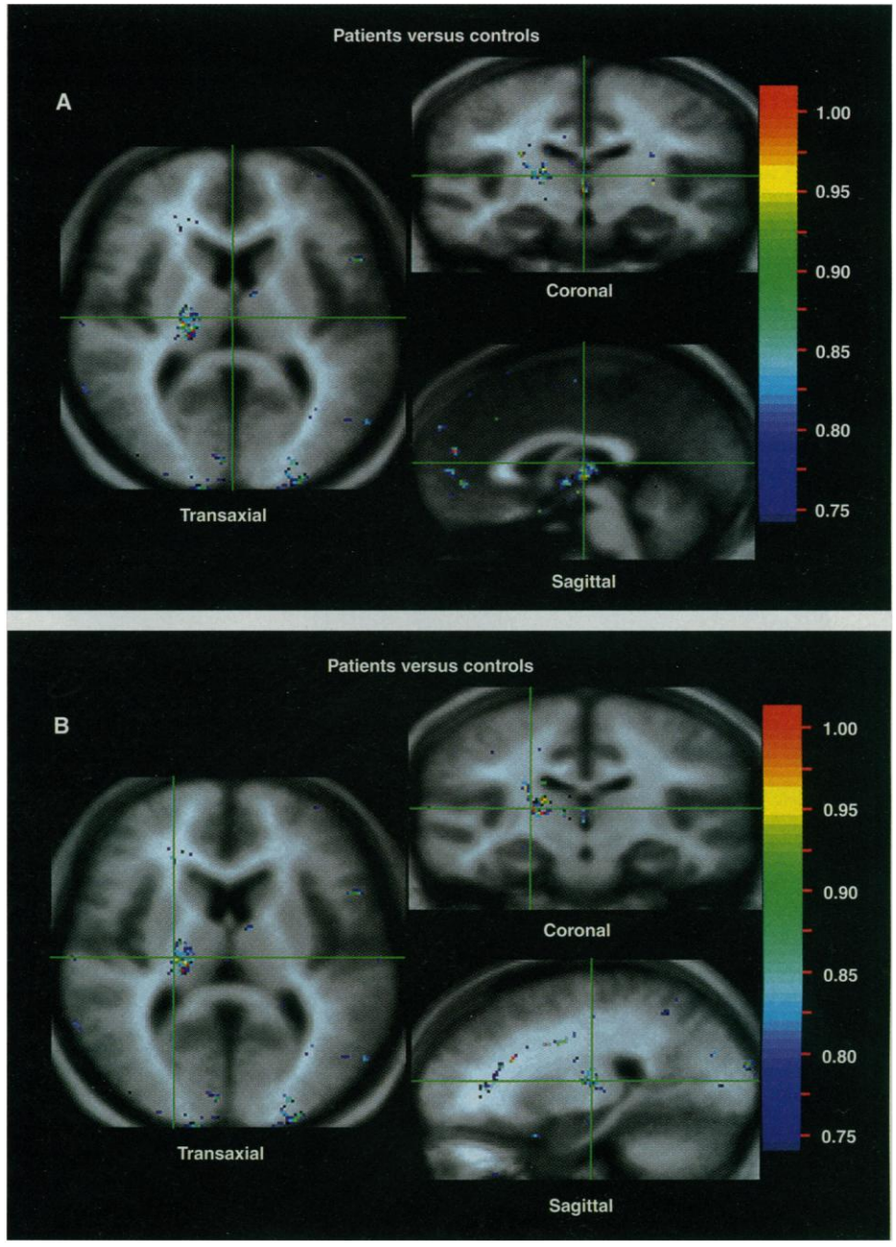


**Fig. 2.** Three orthogonal views showing an ES map on subtracted images. The average brains from patients have been subtracted from controls. In these images effect size is color-coded with a red-green-blue visualization scale, with an ES map varying from 0.75 SD (blue) to 1 SD (red) or greater. (**A**) shows a midline view, whereas (**B**) shows the location of differences in a more lateral view

schizophrenia have been previously reported in the neuropathological literature; five previous studies have shown either decreased size or neuronal loss (*13*). The thalamic abnormalities noted in this study cannot be localized specifically, owing to the inherent limits of resolution of the MR sequences used. While they may involve the medial dorsal regions of thalamus (which have been most frequently studied in neuropathological literature), the abnormalities seen in the lateral thalamus are more prominent. The medial dorsal nucleus is of interest because it projects to the prefrontal cortex, whereas lateral nuclei project to parietal and temporal association regions (*14*). Other studies have also reported abnormalities in midline attentional circuits either downstream or upstream of the thalamus, such as the cingulate gyrus or the pontine reticular activating system (*15*).

These findings are consistent with the role that the thalamus plays in modulating overall brain function and its possible relation to schizophrenia. The thalamus serves as the major way station that receives input from the reticular activating system, structures involved in emotion and memory such

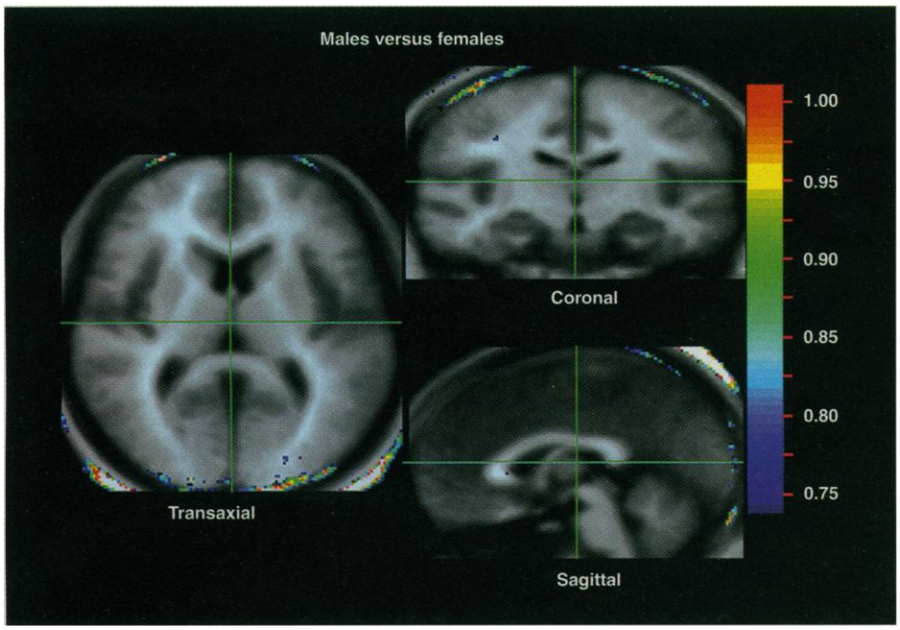


**Fig. 3.** Three orthogonal views showing an ES map on subtracted images from the 47 healthy male volunteers and 44 healthy female volunteers.

as the amygdala, and cortical association areas. Thus, it plays a significant role in filtering, gating, processing, and relaying information. An abnormality in this structure could explain most of the psychopathology in schizophrenia, which can be readily understood as the result of abnormalities in filtering stimuli, focusing attention, or sensory gating (*16*). A person with a defective thalamus is likely to be flooded with information and overwhelmed with stimuli. That person may consequently experience the striking misperceptions that we refer to as delusions or hallucinations or may withdraw and retreat and display negative symptoms such as avolition.

The thalamic abnormalities are coupled with an area of difference in the white matter that may implicate tracts connecting the thalamus with the prefrontal cortex, a finding consistent with a large literature documenting frontal abnormalities in schizophrenia (*17*). An abnormality in temporal and parietal projections may also be present. The abnormalities occur primarily in the right hemisphere. A right-sided abnormality appears to run counter to the substantial literature suggesting deficits in left hemisphere brain regions, particularly those dedicated to language functions. Nevertheless, an abnormality in the right thalamus, and particularly in the lateral nuclei that project to temporoparietal association areas, is also consistent with both the clinical picture of the illness and with some existing literature. Several studies have indicated linked circuitry between frontal and right parietal regions, particularly when higher level cognitive tasks are performed (*18*). Right temporoparietal regions are also crucial for spatial orientation. An individual who cannot correctly link multimodal information in a spatial context is likely to feel confused, overwhelmed, and almost literally "lost in space."

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3. Subjects included 47 healthy normal male volunteers recruited from the community and 39 male patients suffering from schizophrenia recruited for admission to the University of Iowa Mental Health Clinical Research Center and diagnosed by DSM-IIIR criteria according to the Comprehensive Assessment of Symptoms and History [N. C. Andreasen, M. Flaum, S. Arndt, *Arch. Gen. Psychiatry* **49**, 615 (1992)]. The mean age of the patients was 30.3 years. The majority had been chronically ill (mean duration of illness, 51.5 months), although five were first admissions. Lifetime duration of hospitalization was 7.7 months, and mean age of onset was 21.4 years. Most had received neuroleptic treatment at some time in the past, but eight were neuroleptic naïve or nearly so (that is, had not received more than the equivalent of 20 mg of haloperidol over their lifetime). None had been receiving medications known to affect MR scans, such as steroids, or had any recent alcohol or drug abuse. Patients were excluded if they had a lifetime history of serious head trauma, neurological illness, or a serious medical or surgical illness. Mean parental educational level was 12.7 years. The comparison group consisted of healthy volunteers recruited from the community by newspaper advertising and matched on relevant measures such as age, height, and parental education. Subjects were excluded if there was any history of psychiatric, neurologic, or medical illness, or a family history of schizophrenia. Their mean parental educational level was 13.8 years. All subjects gave informed consent.
4. This software, BRAINS (Brain Research: Analysis of Images, Networks, and Systems), is described in the following: N. C. Andreasen *et al.*, *J. Neuropsychiatry Clin. Neurosci.* **4**, 125 (1992); N. C. Andreasen *et al.*, *ibid.* **5**, 121 (1993); N. C. Andreasen *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 93 (1994). The BRAINS package includes utilities for tissue classification, surface and volume rendering, edge detection, volume measurement, resampling with simultaneous visualization in multiple planes, and automated measurement of sulcal-gyral surface anatomy. The initial step in image analysis involved removing the brain from the skull by edge detection techniques and manual tracing. Data were then converted to a three-dimensional data set by volume rendering. The pixels representing CSF were "washed off" with the use of a threshold based on training classes and histograms. The data for each subject were then realigned and resampled with the anterior commissure–posterior commissure line in the transaxial plane and the interhemispheric fissure coronally, in order to ensure comparability of head position across all subjects.
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$$ES_i = \frac{\overline{X}_{iP} - \overline{X}_{iC}}{SD_{pooled\,i}}$$

where $\overline{X}$ is the group mean for patients (P) or controls (C). Because the ES is closely tied to the amount of variance, it is more sensitive to detection of differences in areas of low variance and less sensitive in areas of high variance. Consequently, in Fig. 2 no areas of effect size are seen in the lateral ventricles because of the large variance in their boundaries in both groups. The sample size of the voxels used in ES calculations also varied around tissue-CSF borders, because only voxels containing brain tissue (that is, not CSF) were used in these calculations, and the exact brain tissue–CSF boundaries varied from subject to subject.
10. The BRAINS software provides automated estimates of intracranial components (that is, brain tissue, CSF) in cubic centimeters, on the basis of principles of tissue classification and pixel counting [G. Cohen *et al.*, *Psychiatric Research: Neuroimaging* **45**, 33 (1992); S. Arndt *et al.*, *NeuroImage* **1**, 191 (1994). Earlier reports of ventricular enlargement are summarized in R. E. Gur and G. D. Pearlson [*Schizophr. Bull.* **19**, 337 (1993)].
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# TECHNICAL COMMENTS

## Identifying Species of Origin from Prehistoric Blood Residues

In the archaeological world, some excitement (*1*) and controversy (*2*, *3*) has been generated by reports (*4*–*6*) that hemoglobin from blood residues could survive intact for tens of thousands of years on stone tools and that the species of origin could be identified. One proposal (*4*) suggested that hemoglobin recovered from blood residues on artifacts could be crystallized by a simple procedure and the species of origin determined by microscopic examination of the crystals. Such a technique would be of great interest in establishing ancient hunting patterns, animal migratory movements and in analysis of human blood residues (*1*–*6*).

The basis for the proposed procedure appears to be an exhaustive study conducted by Reichert and Brown at the turn of the century (*7*), which established that hemoglobin from the blood of many vertebrates can be crystallized. These authors showed that when hemoglobin from a single species was crystallized, often several different crystal forms could be obtained, but within each crystal form the crystals were isomorphous (that is, each example had the same symmetry and unit cell dimensions). Further, it was shown that crystals from two different species were often easy to distinguish from one another, while those from similar species were often similar, sometimes differing only slightly in unit-cell dimensions (*7*, pp. 325–327). These results suggest that animal species might be identified by examination of crystals of their hemoglobin.

The proposed procedure (*4*), however, as applied to material obtained from prehistoric artifacts, has been challenged on two major fronts (*2*, *3*): First, does microscopic examination of crystals permit unambiguous identification of the species of origin? Second, does protein survive in the intact, correctly folded native state in quantities sufficient to provide well-formed crystals that would be large enough to analyze?

The first problem arises from a consideration of allowed crystal morphologies. There are 32 point groups (the symmetry of polyhedra), but protein crystals have only the symmetry of the 11 proper ones, that is, only those lacking operations of inversion and reflection. Likewise, of the 230 crystallographic space groups, only 65 are available for protein crystals. These limitations are severe in the face of the number of extant and extinct species having hemoglobin, and in many instances it may be impossible to distinguish crystals from different species by optical methods. For example, upon examination of the family *Canidae*, crystals of hemoglobin obtained from the blood of 10 distinct species of dogs, wolves, and foxes were studied by Reichert and Brown, who reported (*7*, p. 265) that "[a]ll members of the family furnished oxyhemoglobin crystals which closely resembled each other, so that the differences between species were not readily made out."

In order to identify the species of origin of a hemoglobin crystal unambiguously by the proposed technique (*4*), the minimum requirement is a complete and accurate analysis of the interaxial angles, and the axial length ratios of the unit cell. X-ray diffraction is the method of choice and can yield accurate information about space group and cell dimensions from a single well-formed crystal (*9*). A possible drawback is that the minimum protein crystal size for x-ray analysis is about 100 × 100 × 100 μm (*9*), which contains about 0.5 μg of protein. Optical analysis is much more painstaking, but may be possible with smaller crystals. This requires the use of an optical goniometer, with which one must measure the angles between crystal faces to an accuracy of about 10 arc minutes. One must also determine the relationship of optical axes to crystal faces and edges (*7*, pp. 146–148). From this information, one can often deduce the crystal system and determine the relative unit cell lengths. Usually, this would require several well-formed single crystals and, of course, they must all be of the same material.

Optical examination of crystals cannot, however, identify the space group. At best it can only provide crystal system (and perhaps point group), axial length ratios, interaxial angles, optical axes, and birefringence sign. This compounds the problem raised by the limited number of ways in which proteins can crystallize.

Armed with data obtained by either x-ray or optical analysis, one can then consult a compendium (*7*) of crystal forms obtained from the hemoglobin of known species and attempt to identify the species of origin. For several proteins [citrate synthase, for example (*10*)] crystals from species as divergent as chickens and pigs are isomorphous, and rigorous optical analysis would not distinguish between the two species of origin. The required measurements of crystal system, unit cell axial length ratios, and interaxial angles have not been reported (*4*–*6*), nor has it been demonstrated that the crystals obtained from putative blood residues on stone tools actually contained hemoglobin. One cannot be confident of the correctness of the species identification in these instances.

The other major problem arises from the amount of material required for crystallization and the fact that crystallization of proteins generally requires intact, correctly folded protein. While dry protein samples can retain biological activity for years, a large percentage of hemoglobin will be degraded in samples taken from ancient artifacts (*6*). In controlled experiments simulating burial conditions (*3*), blood protein was usually not detectable by sensitive chemical analysis after several weeks of contact with damp soil.

While the actual degree of protein degradation varies from sample to sample, it is possible to estimate the minimum amount of intact, native protein required for crystallization. Most protein crystals are about