A major theme underlying schizophrenia research is the hypothesis that structural and chemical brain abnormalities account for the abnormal mental function and behavior which characterize this syndrome. The discovery and understanding of these abnormalities will lead, it is hoped, to new and improved treatments and/or prevention. The goal of this report is to provide directions by which neuropathology and neuroimaging can contribute to the elucidation of brain abnormalities in the schizophrenic syndrome.

Attempts to demonstrate neuroanatomical and neurochemical abnormalities in the brain of schizophrenic patients can be placed in two broad categories. The first, the study of living patients with schizophrenia, involves neuroimaging techniques. The second, the study of the brain of deceased patients, is traditionally referred to as neuropathology. This report deals with neuroimaging and neuropathology as two approaches to research on the schizophrenic syndrome.

**Neuroimaging**

Neuroimaging techniques can be further subdivided into those that elucidate structure and those that demonstrate function. Techniques that provide an assessment of structure include computed tomographic (CT) scanning and magnetic resonance imaging (MRI) (also termed nuclear magnetic resonance or NMR). An even broader array of techniques is available for the study of function. These include positron emission tomography (PET), single photon emission computed tomography (SPECT), regional cerebral blood flow (rCBF), computerized electroencephalography (CEEG), magnetoencephalography (MEG), and nuclear magnetic resonance spectroscopy (NMRS).

**CT Scanning.** Since 1976, over 40 reports of CT studies of patients with schizophrenia have appeared in the literature. The vast majority of these studies have found structural abnormalities in the brain of schizophrenic patients as compared with controls. These abnormalities include large cerebral ventricles (lateral and third), more prominent cortical markings suggesting wider sulci (especially prefrontally), and decreased size of the anterior vermis of the cerebellum. Although the magnitude of these differences is small, they do support the notion that there are demonstrable structural abnormalities in the brains of some schizophrenic patients.

Attempts to demonstrate neuroanatomical and neurochemical abnormalities in the brain of schizophrenic patients can be placed in two broad categories. The first, the study of living patients with schizophrenia, involves neuroimaging techniques. The second, the study of the brain of deceased patients, is traditionally referred to as neuropathology. This report deals with neuroimaging and neuropathology as two approaches to research on the schizophrenic syndrome.

**Recommendations.** Obviously, further work needs to be done to...
understand fully the significance of the structural abnormalities in schizophrenia demonstrated on CT scan. Although CT has the disadvantage of ionizing radiation, it is still relatively safe, fast, and economical for screening large numbers of psychiatric patients. Studies with larger numbers of patients would go a long way toward answering questions of symptoms, prognosis, and the like so that there is a fuller understanding of the significance of these findings.

**MRI Scanning.** MRI scanning is an alternative approach to CT for demonstrating structural abnormalities. There are several advantages of MRI scanning that make it an attractive technique. One clear advantage is that patients receive no ionizing radiation from this procedure. This means that patients can be studied on multiple occasions without any radiation dangers. Numerous studies have established the safety of this technique. A second major advantage is that individual nuclei can be visualized and measured. This may allow for a more precise localization of structural abnormalities in the brains of schizophrenic patients.

Because of the apparent safety of MRI, it is often the imaging method of choice in children. In fact, studies in children have demonstrated increased resolution and sensitivity of MRI over CT scanning in post-infectious encephalomyelitis, gray matter heterotopia, intracranial masses, and various diseases of the central nervous system. MRI has even been extended to the human fetus in utero to monitor fetal and placental aging and function. MRI has also demonstrated brain abnormalities in patients with psychotic behavior in whom CT scans were normal and in whom a metabolic abnormality was subsequently found such as Wilson’s disease, adrenoleukodystrophy, or systemic lupus erythematosus.

MRI is frequently compared to CT scanning in terms of resolution and sensitivity. At this time, MRI has the following decided advantages for imaging the central nervous system:

- Better resolution: MRI resolutions of approximately 0.03 mm³ voxels are now being reported.
- MRI has a higher sensitivity to soft tissue contrast.
- MRI is capable of multiplanar imaging (axial, sagittal, coronal, and oblique).
- MRI monitors the chemical structure of biological water through the water T₁ and T₂ relaxation times.
- MRI has no bone artifact and is not so susceptible to brain-cerebrospinal fluid edge effects as CT and is, therefore, quantitatively more accurate for brain volume measurements. This is particularly important for use in conjunction with PET studies so that accurate tissue volumes can be obtained and related to measurements of molecular concentrations in various structures, such as the caudate nucleus.
- MRI has no ionizing radiation.
- MRI gives superior views of the posterior fossa than does CT, allowing a better view of the cerebellum and the mesencephalic tegmentum.

The sensitivity of MRI to the intracellular chemical structure of water may have significant implications for future applications of MRI. In fact, a preliminary study suggests that MRI is sensitive to altered intracellular water structure in brain areas of focal seizure activity. The potential for sodium imaging with a 4.0 mm resolution has also been demonstrated as has fluorine imaging with an in-plane resolution of 0.6 mm. Recently, preliminary studies have also demonstrated the feasibility of lithium NMR imaging in brain. Although the sodium, fluorine, and lithium NMR imaging capabilities are intriguing and should be researched vigorously, none of these nuclei have the sensitivity or resolution capabilities at present that hydrogen possesses.

New approaches are being rapidly developed to enhance the resolution and sensitivity of MRI even further. One rapidly developing area is image enhancement. Image enhancers are, in general, molecules that contain either paramagnetic or ferromagnetic atoms, which secondarily influence the relaxation properties of any molecules that come in close contact with the image enhancers.

The first type of image-enhancing agent is paramagnetic cations, which increase the relaxation rate for nuclei that undergo dipole-dipole interactions with the paramagnetic cation. The two types of paramagnetic image enhancers most commonly used are either transition metals such as manganese or lanthanides such as gadolinium. The other class of paramagnetic contrast agents is based on organic nitroxide spin labels such as derivatives of piperidine.

The paramagnetic metals are too toxic to be used as free cations for image enhancement. Therefore, the paramagnetic cations are usually chelated to organic molecules. The use of paramagnetic cations as image enhancers was first suggested by Lauterbar. Iron, manganese, chromium, and gadolinium chelates have all been demonstrated to be effective image-enhancing agents in...
animals. Gadolinium-DPTA has also been successfully used as an image enhancer in the human brain.

The magnetic resonance properties of 12 paramagnetic piperidine nitrooxyls have been reviewed recently. One nitrooxide spin label has recently been demonstrated to be a hypoxia-sensitive NMR image enhancer. Immunospecific NMR image enhancers have also been described. The potential application of immunspecific NMR image enhancers to neuroscience research seems promising.

No technology, including MRI, is without its drawbacks. The major disadvantage of MRI scanning is that the approach is time consuming (up to 45 minutes). Studying patients with schizophrenia who are either uncooperative or who have movement disorders such as tardive dyskinesia can be difficult or impossible. An additional disadvantage which is not relevant to schizophrenia is that patients with metallic material in their brains (such as a metal clip for a surgically repaired aneurysm) cannot be studied with an MRI scan. Despite these limitations, MRI scanning is an approach with great potential for research into the structural abnormalities of schizophrenia.

Recommendations. MRI scanning is probably not the structural imaging approach of choice for screening large populations of psychiatric patients. Time constraints and the cooperativeness of psychiatric patients may mitigate against its use. However, in selected populations where detailed neuroanatomical information is valuable (e.g., in conjunction with PET or SPECT imaging or for measurements of individual nuclei such as amygdala or hippocampus), MRI should clearly be chosen. Moreover, when cumulative radiation effects are an issue (i.e., longitudinal studies with repeated measures or in high-risk children or fetuses), again MRI is a superior technique for structure.

PET Scanning. The development by Louis Sokoloff and his colleagues at the National Institute of Mental Health of the $^{14}$C-deoxyglucose method of measuring regional cerebral glucose utilization by means of quantitative autoradiography was a major factor in the application of PET to the study of brain neurochemistry in living subjects. Sokoloff's work stimulated Ido, Wolf, Reivich, and their colleagues at Brookhaven National Laboratory and the University of Pennsylvania to extend the autoradiographic technique to humans. To do so, they labeled deoxyglucose with the positron-emitting tracer fluorine-18, which made possible the use of PET to demonstrate in humans that physiological functions such as seeing, hearing, or moving led to corresponding increases in glucose metabolism in visual, auditory, and motor cortex, respectively. Since the initial efforts at demonstrating the utility of using deoxyglucose to assess brain functions in living subjects, there have been a number of studies in schizophrenic patients. Several groups of investigators have found a relative decrease in the metabolic rate of glucose in the frontal lobes of schizophrenic patients, but other groups have failed to confirm this finding (for review, see Buchsbaum and Haier 1987). Although the results have not yielded a pathognomonic lesion in schizophrenic patients, they have demonstrated the feasibility of the approach and suggested where further studies might look.

Obviously, PET scanning is not limited to glucose metabolism. It can be used to measure oxygen consumption, the transformation of L-dopa into dopamine (an example of neurotransmitter synthesis), and to image and quantify neurotransmitters. Thus, the processes of neurotransmission as well as substrate metabolism can be studied in the living human brain with PET scanning.

One of the major neurochemical hypotheses in schizophrenia is that there is increased dopaminergic activity in the brain of schizophrenic patients. Neuropathological studies (to be discussed in greater detail later in this report) suggest that increases in dopamine receptors may represent this proposed increased dopaminergic activity. The development of $^{11}$C-N-methylspiperone, which binds to dopamine receptors, allows this hypothesis to be tested directly by PET scanning in drug-naive schizophrenic patients. Initial results with $^{11}$C-N-methylspiperone in humans indicate that dopamine type-2 receptors can be visualized primarily in the basal ganglia. Moreover, increases in these receptors in the basal ganglia were demonstrated in drug-naive schizophrenic patients relative to controls. These results confirmed earlier neuropathological studies with similar increases in the basal ganglia. Unfortunately, a similar study using a different dopamine type-2 receptor ligand, $^{11}$C-raclopride, has not demonstrated increases in dopamine receptors in drug-naive schizophrenic patients as compared with controls.

Differences between the two studies include: (1) the use of different D$_2$ dopamine receptor radioligands, one reversibly (raclopride)
and the other irreversibly bound (N-methylspiperone) (when considering the time spans of the studies); (2) differences in the manner in which competitive inhibition was achieved (one study blocked the receptors with a different ligand before the administration of the tracer dose, while the other blocked the receptors by administration of a lower specific activity of the tracer ligand); (3) possible differences in the effects of endogenously secreted dopamine on the competitive binding of the different radioligands; (4) possible differences in the precision and accuracy of quantification of tracer concentrations within the regions of interest, including the effects of patient positioning errors, spatial resolution limitations, low signal/noise ratios, and propagated statistical errors; and (5) the effects, if any, of internalization of ligand/receptor complexes.

Recommendations. Since normal persons and patients meeting standardized criteria for schizophrenia are heterogeneous, it would be particularly useful to study the same normal persons and patients using the two different methods to help reconcile differences. It would also be of great importance to determine whether increased D2 dopamine receptors precede or follow the development of symptoms of schizophrenia. It is also too soon to tell whether D2 dopamine receptors are elevated in other types of mental illness than schizophrenia, although preliminary evidence suggests that D2 dopamine receptors are elevated in patients with manic-depressive illness. Further studies are also needed in regions other than the caudate/putamen. The important role of the limbic system in emotional behavior makes it a region of interest for PET studies in patients with schizophrenia; the hippocampus and amygdala are areas of special interest. Differences in the affinity state of receptors also need to be examined.

Other receptor systems of interest in schizophrenia are the D1 dopamine and the opiate receptor systems. The latter can be examined with the radioactive tracer drug 11C-labeled 4-carbo-methoxyfentanyl (carfentanil), a narcotic with a potency more than 7,000 times greater than morphine, or the recently developed opiate receptor agonist, 11C diprenorphine. In human beings, PET imaging reveals opiate receptors in high concentrations in the limbic system, amygdala, and cingulate gyrus, as well as the caudate nucleus, putamen, medial thalamus, and frontal and parietal cortex. Few opiate receptors are found in the sensory-motor cortex and visual cortex. Second messenger receptors, as well as classical receptors, can also be measured by PET. The effects of selective D1 or D2 agonists on second messengers could also be determined.

PET represents a theoretically "limitless" technology to brain functioning in living subjects. Radiochemists using cyclotrons can synthesize radioactive compounds for the study of amino acids, proteins, enzymes, receptors, neurotransmitters, drugs, and the like. After these compounds are injected into human subjects, any neuronal system can, in theory, be studied. This approach offers staggering promise for the neurochemical elucidation of neuropsychiatric disorders including schizophrenia.

Are there drawbacks and limitations to PET scanning? Since the signals for PET come from positrons that emit radioactivity, there are limitations on how often a subject can be studied with this technique. Also, positron-emitting compounds must be synthesized in cyclotrons, which are large and expensive. Moreover, the radiochemists who are essential for the development of radioactive ligands are expensive to employ as well. In addition, the analysis of the data may require complicated mathematical models making interpretation difficult at times. Lastly, there are theoretical limitations which suggest that PET scans may not adequately quantify very small structures (on the order of 4 mm or less), such as interesting limbic nuclei which are thought to be relevant in the schizophrenic syndrome.

Despite these limitations, PET scanning is a major new tool that should be extremely useful in research in schizophrenia. It should probably be developed in those regional centers with space and money to support the cyclotrons and radiochemists that are essential for these projects. In addition, it would be useful to have specific teams of neurochemists working in conjunction with clinicians who can suggest which neurochemical and neurobehavioral hypotheses are most worth pursuing.

SPECT. A second neuroimaging approach to assess brain function involves using radioactive compounds that use single photon emitters. This technique is called single photon emission computed tomography (SPECT). Although SPECT has been available for a number of years, refinements of its detection systems and new ligands make this technique a practical neuroimaging approach to the brain with the capacity
to visualize subcortical receptors and/or neurotransmitters and substrates. In addition, SPECT allows for blood flow determinations while receptors are being measured.

Although SPECT cannot currently resolve structures as well as PET, it does have resolving capacities of the same order of magnitude (roughly, 8 mm). It has several advantages which include not requiring a cyclotron or needing a radiochemist on site. Radiochemists can synthesize single-photon-emitting compounds and literally mail them to distant sites for subsequent use. Since these compounds do not decay as rapidly as those used in PET scanning, the mathematical analyses of the data may be less complicated. Since the amounts of radioactivity are somewhat less than those encountered with PET, more frequent studies in the same subject are possible. The bottom line with SPECT is that it is a less expensive alternative to PET which does not require a radiochemist or cyclotron. As such, it may be useful in any hospital, not just regional medical centers as may be the case with PET. This technique has thus far received scant attention in schizophrenia research.

**Recommendations.** Schizophrenia research groups that wish to study function by imaging but cannot afford cyclotrons or radiochemists (by virtue of space or money) should consider SPECT. It may prove ideal for smaller hospitals and research groups. It also deserves serious consideration for longitudinal studies or any studies where reducing cumulative radiation risks is a consideration (i.e., high-risk children).

**CBF Imaging.** Regional CBF, assessed with inhalation of $^{133}$xenon, is a relatively inexpensive neuroimaging technique that allows for assessment of cortical functions in neuropsychiatric patients and controls. The first application of in vivo functional imaging in schizophrenia was the pioneering studies of Ingvar and Franzen, in which an intracarotid injection of radioactive xenon was used as a tracer of cerebral blood flow. Initial findings of diminished prefrontal blood flow “at rest” have been difficult to replicate. However, recent efforts have emphasized specific cognitive activation paradigms during the CBF procedure. With this approach, there is evidence that behavior-specific hypofunction of prefrontal cortex characterizes many patients with schizophrenia, particularly those who are intellectually impaired. Xenon inhalation, a noninvasive modification of the technique used by Ingvar, has shown that schizophrenic patients who were attempting to perform a cognitive task involving the dorsolateral prefrontal cortex failed to increase blood flow into this region as did normal controls. Other investigators using verbal (right and left hemispheres and spatial hemispheric) tasks found an abnormal pattern of hemispheric functioning in schizophrenic patients with increased blood flow in the left hemisphere greater for the spatial than the verbal task.

**Recommendations.** The advantages of the xenon inhalation method are its noninvasiveness, its sensitivity to cognitive activation, its relatively low cost, its low radiation dosimetry, and its high time resolution. Disadvantages include limited spatial resolution and an inability to visualize subcortical structures. It remains, however, a useful technique for studying cortical functions in schizophrenic patients.

**CEEG.** The conventional electroencephalogram (EEG) based on paper recordings has generally not revealed significant findings in patients with schizophrenia. Quantitative analysis of the EEG, such as spectrum analysis, has shown differences, including increased delta (slow wave activity), increased beta (fast activity), and reduced alpha frequency.

Many electrophysiological studies have demonstrated differences in average evoked potentials between schizophrenic patients and normal persons during sensory activation processes, conditioning, and habituation. Findings in schizophrenic patients occur in the following: early components, such as the so-called $P_{20}$ potential; middle components related to selective attention, such as the $N_{140}$ component; and late components related to cognitive processing, such as the $P_{300}$ component. Studies of many of these findings have been equivocal because of the great difficulty in obtaining artifact-free recordings from this patient group.

The last few years have seen the development of computer-based systems to display multi-lead surface EEG recordings in the form of brain maps (CEEG). The goal is to localize neuronal activity in regions of the brain. It must be emphasized, however, that the inhomogeneity of the brain and surrounding tissues makes it difficult to use the electrical activity recorded from the scalp surface to locate the underlying neural activity. Only recently have methods been proposed to attempt to make possible tomographic measurements of regional neuronal activity. This approach is at the stage of basic research and is not yet applicable to patient studies.
The findings from the more usual EEG and evoked potential studies indicate that there are electrophysiological differences in the brains of schizophrenic patients. It is to be hoped that more sophisticated approaches being developed may be more productive in the study of schizophrenia, perhaps helping to distinguish subgroups of patients or to localize abnormalities to particular brain regions. Electrophysiology remains one of the few noninvasive techniques for investigating brain function in relation to behavior. While its spatial resolution limits the degree of localization possible, the excellent temporal resolution relative to behavior is the best of any available method, which makes such an approach particularly useful in conjunction with other neuroimaging methods. While commercial interests in so-called brain-mapping instruments have perhaps oversold the present capability of this approach, one should not allow the commercial propaganda to cloud the potential utility of the approach.

Recommendations. In short, CEEG is a safe, inexpensive neuroimaging technique with excellent temporal resolution. Its disadvantages include an inability to visualize subcortical structures. It has considerable research potential, especially with regard to cortical abnormalities in schizophrenia and in combination with other neuroimaging devices such as CBF or SPECT.

MEG. Magnetic fields associated with neuronal activation are less affected by the inhomogeneity of electrical conduction. Thus, it is theoretically possible to localize changes in magnetic fields in threedimensional volume elements within the brain from measurements made at the surface, a procedure called magnetoencephalography (MEG). In this approach, mapping the magnetic fields in specific volume elements may permit localization of regional neuronal activity. An example of this approach is the seven-channel SQUID system, which requires a very cooperative subject in a magnetically quiet environment. Repeated sensory stimulation must be used. Basic research in this area seems promising. Advances in superconducting technology should reduce the cost and allow larger multichannel systems to be built.

Recommendations. In summary, MEG is a safe but expensive neuroimaging technology which is very much in the developmental stage. It offers the promise of measuring subcortical electrical activity. Current drawbacks include its time-consuming nature, which makes its application to psychiatric patients problematic. Nevertheless, it is definitely worthy of further development as a neuroimaging research tool.

NMRS. Nuclear magnetic resonance spectroscopy (NMRS) is a technique that has the potential to assess brain functioning at the subcellular molecular/metabolic level. It involves generating physiological information about a variety of nonradiated atomic nuclei, many of which exist naturally and some of which can be administered to humans. Potential nuclei include $^{31}$P, $^{13}$C, $^{23}$Na, $^7$Li, and $^1$H. As this is a relatively new technology with great potential (as yet unrealized in psychiatry), detailed attention will be paid to its future directions.

The $^{31}$P-NMR spectrum of mammalian brain can be conveniently separated into the following three regions: (1) orthophosphate (5 to $-1.5$ ppm), (2) guanidophosphate ($-3.5$ to $-5$ ppm), and (3) polypolyposphate ($-5$ to $-23$ ppm). The orthophosphate region can be further subdivided into ionized ends ($-5$ to $-8$ ppm), esterified ends ($-8$ to $-14$ ppm), and middles ($-18$ to $-23$ ppm).

Contributing to the phosphomonester region are hexose 6-phosphates, triose phosphates, pentose phosphates, phosphoethanolamine, phosphocholine, inorganic orthophosphate (Pi), anomic sugar phosphates, and several signals that have not been characterized as to the source phosphate. Contributing to the phosphodiester region are glycerol phosphodiesteres (primarily glycerol 3-phosphoethanolamine and glycerol 3-phosphocholine), a broad resonance from phosphorylated glycolipids and glycoproteins, and several uncharacterized resonances. The guanidophosphate region contains resonances from phosphocreatine (PCr) and phosphoarginine.

In the polyphosphate part of the spectrum, the ionized ends region contains resonances from the $\gamma$-phosphate of nucleotide triphosphates and the $\beta$-phosphate of nucleotide diphosphates. The esterified ends region contains resonances for the $\alpha$-phosphate of nucleotide triphosphates, the $\alpha$-phosphate of nucleotide diphosphates, the nicotinamide adenine dinucleotides, and the uridine diphospho-sugars (galactose, glucose, mannose). The only resonance that makes a contribution to the middles region is the $\beta$-phosphate of nucleotide triphosphates. In mammalian brain, the predominant contributors to the
nucleotide triphosphate and nucleotide diphosphate resonances are adenosine 5'-triphosphate (ATP) and adenosine 5'-diphosphate (ADP).

The $^{31}$P-NMR spectrum contains information about the energy status of the brain from the resonances for PCr, ATP, ADP, and Pi. Resonances related to phospholipid metabolism are contained in the phosphomonoester and phosphodiester regions. In mammalian brain, the phosphomonoester region contains resonances predominantly from α-glycerol phosphate, phosphoethanolamine, and phosphocholine. These three metabolites in mammalian brain are found predominantly in the anabolic pathway of membrane phospholipid metabolism. The phosphodiester region contains predominantly the resonances of glycerol 3-phosphoethanolamine and glycerol 3-phosphocholine, which, in mammalian brain, are catabolic breakdown products of phospholipid metabolism. Therefore, the steady-state turnover of brain phospholipids (anabolism/catabolism) can be assessed by $^{31}$P-NMRS. Since neural membrane (especially synapto-osomal) structure, dynamics, and function are of vital importance to normal neurochemical, neurophysiological, and neuropharmacological function, $^{31}$P-NMRS has the potential to provide important insights into normal and altered brain function.

The in vitro $^{31}$P-NMR studies provide chemical conditions more favorable to $^{31}$P-NMR analysis than occur in the living brain, and a greater sensitivity and resolution is therefore achieved as compared to in vivo analytical approaches. The enhanced sensitivity and resolution of in vitro extract studies enables the characterization and quantitation of many different phosphorus-containing compounds. Previous in vitro $^{31}$P-NMR studies demonstrated a remarkable correlation with more classical assay procedures and, in addition, revealed previously uncharacterized metabolites and unrecognized metabolic relationships. To interpret $^{31}$P-NMR spectra correctly, the identities of the individual resonance signals must be carefully verified through the use of appropriate biochemical and spectroscopic procedures. The importance of this verification was recently demonstrated for a prominent $^{31}$P-NMR resonance at 3.84 ppm in mammalian brain which has now been identified as phosphoethanolamine. The identification was based on $^1$H and $^{31}$P-NMR findings (including pH titrations) at 4.7 and 14.1 Tesla, as well as thin-layer chromatography studies.

Pettegrew and co-workers previously demonstrated an elevated phosphomonoester resonance in rapidly dividing neuroblastoma clonal lines and have recently shown the elevation of a prominent phosphomonoester resonance in developing and degenerating brain, including Huntington’s and Alzheimer’s brain. The prominent phosphomonoester resonance has been identified as phosphoethanolamine. These previous studies are in agreement with earlier studies that demonstrated a relative abundance of phosphoethanolamine in developing rabbit brain. A relatively prominent phosphomonoester resonance exhibiting the appropriate $^{31}$P chemical shift also has been reported in human neonatal brain and childhood neuroblastoma using an in vivo MRS surface coil technique.

Separate $^{31}$P-NMR studies demonstrated phosphoethanolamine to be high in immature developing brain and both phosphomonoesters and phosphodiester to be elevated in degenerating brain of Alzheimer’s and Huntington’s disease patients. These studies suggested that the phosphomonoester and phosphodiester resonances could serve as sensitive markers of membrane phospholipid turnover for both in vitro and in vivo $^{31}$P-NMR studies of neuropsychiatric diseases such as schizophrenia.

$^{13}$C-NMRS of mammalian brain.

1. Natural Abundance $^{13}$C-NMR. The natural abundance $^{13}$C-NMR spectrum of rat brain has been obtained and demonstrates $^{13}$C-NMR chemical shifts of brain metabolites very similar to the chemical shifts of the pure chemicals. Readily identifiable resonances for amino acids, neurotransmitters and their derivatives include glutamate, glutamine, glycine, taurine, alanine, aspartate, N-acetylaspartate, and γ-aminobutyrate; for the phospholipid metabolites, phosphoethanolamine, inositol, and glycerol 3-phosphocholine; and for the energy-related metabolites, creatine and lactic acid. Therefore, the natural abundance $^{13}$C-NMR spectrum contains significant information about brain amino acid metabolism and neurotransmitter levels (glutamate, γ-aminobutyrate) and some information about phospholipid and energy metabolism.

2. $^{13}$C-NMR Using $^{13}$C-Enriched Substrates. $^{13}$C-NMR in combination with $^{13}$C-enriched substrates has proved to be a powerful and elegant analytical method to study selected areas of metabolism. The advantage of the $^{13}$C-NMR studies is that all metabolites that have been adequately labeled are simultaneously...
The potential of these kinds of ¹³C-NMR in vitro studies to be informative is great.

¹H-NMR spectrum. ¹H-NMR spectra have been obtained for tissues and tissue extracts. Identifiable resonances include those from amino acids, neurotransmitters, and their derivatives such as glutamate, glutamine, aspartate, N-acetyl-aspartate, glycine, alanine, valine, taurine, leucine, phenylalanine, tyrosine, proline, γ-aminobutyrate, and histidine; metabolites related to energy metabolism such as glucose, phosphocreatine, creatine, lactate, and acetate; metabolites related to phospholipid metabolism such as phosphocholine and phosphoethanolamine; and metabolites related to nucleotide metabolism such as adenosine, guanine, uracil, and cytosine. Because of the inherent sensitivity of ¹H-NMR and because methods exist to suppress the large water signal, ¹H-NMR studies of brain should be very informative.

²³Na-NMR and ⁷Li-NMR.

1. ²³Na-NMR. Fundamentally important to human biological processes are sodium transmembrane fluxes and intracellular sodium concentrations, including their modulation by means of exchange processes operating among the ion-coordinated ligands. These exchange processes serve to alter the chemical state of the ion and, hence, the position of delicately poised equilibria. Alterations in transmembrane sodium unidirectional rate constants with resultant alterations in intracellular sodium concentration have been implicated in a number of human diseases. A few such diseases which are diverse in clinical phenotype include manic-depressive disorder, essential hypertension, and cystic fibrosis, as well as cellular proliferative responses during mitogenesis and oncogenesis. Since sodium transmembrane fluxes are clearly dependent on the physiological integrity of the living cell and its membrane, the advantage of a noninvasive, nonperturbing technique for monitoring tissue sodium is obvious. Such a technique is ²³Na-NMR. A recent ²³Na-NMR study has demonstrated that a significant fraction of the sodium inside human erythrocytes is relatively immobilized. This immobilization of intracellular sodium probably results from a transient coordination of the cation to functional groups of proteins or lipids. This observation has two immediate implications. The intracellular immobilization of sodium is likely to be genetically regulated, and the degree of sodium immobilization will significantly affect sodium transmembrane flux and, secondarily, cellular physiology.

2. ⁷Li-NMR. Lithium has a demonstrated efficacy in the prophylactic treatment of manic-depressive illness, the acute treatment of mania, and the treatment of some patients with schizophrenia. The molecular basis for the biological action of Li⁺ is unknown at this time, although it has been postulated that Li⁺ competes for the binding sites of biological cations such as Na⁺, K⁺, Mg²⁺, and/or Ca²⁺ and that Li⁺ interacts with membrane phospholipids. Li⁺ has been demonstrated to inhibit the conversion of inositol phosphate to inositol plus phosphate in the phosphatidylinositol second messenger pathway. In addition, a recent ⁷Li-NMR study provided evidence that Li⁺ interacts with the membrane associated cytoskeleton in human erythrocytes. These observations taken together suggest that Li⁺ is interacting with important regulatory sites on the cytoplasmic surface of cellular membranes. These regulatory sites may be phosphorylated sites of polymers or enzymes. Therefore, ⁷Li-NMR would appear to be a very informative analytical approach to the intracellular chemistry of Li⁺.

Solid-state NMR of model membranes and brain tissues.

1. Solid-state ³¹P-NMR. In recent years there has been increasing interest in NMR of solids. This interest is the result of a series of instrument advances including mini-computer-based digital techniques, high-power radio frequency pulse sequences, high-power proton decoupling, cross-polarization and magic-angle sample spinning. For many years, the main applications of NMR were molecular structure analyses of liquid samples. The increasing application of NMR of solid samples represents, however, a return to the early experiments in the field of NMR. The first successful NMR experiments were carried out independently in late 1945 by Purcell, Torrey, and Pound and by Bloch, Hansen, and Packard. The Purcell group detected proton NMR in solid paraffin; the Bloch group detected proton NMR in liquid water. Bloch and Purcell received the Nobel Prize for Physics in 1952 for their observations. Up to about 1952, studies of solids dominated the field of NMR. However, the reports of observations of chemical shifts of ³¹P resonances in several compounds, of ¹⁴N resonances in several ions, and of ¹⁹F resonances in several compounds led to the development of high resolution NMR in liquids. Since the molecular motions in liquids result in very narrow
lines compared to those in solids, much smaller chemical shifts could be detected. This enabled the domination of high resolution NMR in liquids. The broad NMR lines in solids generally precluded the detailed chemical shift measurements possible in liquids until the recent advances, mentioned above, enabled the spectroscopist to mimic for solids the line-narrowing molecular motions in liquids.

2. \(^{31}\text{P}-\text{NMR studies of selected membranes.}\) To date, numerous solid-state \(^{31}\text{P}-\text{NMR studies have been performed on model membranes and biological membranes.}\) These studies demonstrate that solid-state \(^{31}\text{P}-\text{NMR can clearly differentiate the bilayer and hexagonal II phases.}\) Calcium has been shown to induce bilayer to hexagonal II phase transitions in model membranes. A recent study has also demonstrated that \(\text{Al}^{3+}\) can induce the bilayer to hexagonal II phase transition in model membranes and mammalian brain. These findings are of particular biological importance as the hexagonal II phase structure has been shown to enhance membrane fusion and membrane vesicle formation, and similar membrane alterations may contribute to the pathogenesis of Alzheimer’s disease.

The application of solid-state NMR to the study of brain tissue will provide insights into membrane organization and function that are not available with any other technology. The solid-state NMR studies will be complementary to freeze fracture electron microscopy studies of membranes.

**Two-dimensional Fourier transform NMR.** Two-dimensional Fourier transform NMR (2D-FT NMR) techniques are powerful analytical approaches for determining molecular structure and conformation in solution. A few examples to which these techniques have been successfully applied include the 65-residue protein hirudin, the 85-residue protein HPr from *Escherichia coli*, the alkaloid gephyrotoxin (C\(_{19}\)H\(_{26}\)NO\(_4\)), the coenzyme B\(_{12}\) (\(\text{M}_r = 180\)), the antibiotic desertomycin (\(\text{M}_r = 1192\)), the polynucleotide d(C-G-C-A-G-A-G-C-T-C-G-C-G), and the enkephalin analogues. In addition, the molecular conformations that are derived from the 2D-FT NMR experiments can be used as starting points for computer-calculated energy minimization studies yielding refined molecular conformations. Therefore, it seems certain that 2D-FT NMR techniques will continue to make major contributions to understanding molecular conformation in solution. The determination of molecular conformation by 2D-FT NMR techniques is likely to find many applications in future neuroscience research.

**NMR microscopy (micro-imaging).** The first NMR image of a single cell was reported by Aguayo, Blackband, Schoeniger, Mattingly, and Hentermann. This experiment was performed on a Bruker 9.4 Tesla NMR spectrometer in a collaborative effort between Johns Hopkins and Bruker Medical Instruments. This initial attempt yielded a resolution of 10 x 13 \(\mu\)m for a slice width of 250 \(\mu\)m, which was sufficient to identify intracellular structures in ova obtained from *Xenopus laevis*. The potential of imaging single cells opens up many exciting applications of this technology to schizophrenia research.

**Recommendations.** Increased research support is needed to develop in vivo NMRS in humans. \(^{31}\text{P-}, \, ^{1}\text{H-},\) and \(^{23}\text{Na-NMR studies, in particular, hold great promise for providing molecular and metabolic insights into brain function that are not possible with other existing technologies. These NMRS studies will be complementary to and synergistic with PET studies. Ideally, both NMR and PET studies should be conducted on the same subjects. Again, the NMRS studies should be longitudinal as well as cross-sectional in order to assess the very important contributions of maturational, development, and aging to the pathophysiology of schizophrenia.**

Increased research support is needed for in vitro NMRS studies of cells, tissues, and tissue extracts in patients with schizophrenia as well as appropriate animal models of schizophrenia. These in vitro studies will provide very valuable and detailed molecular structure, dynamics, and metabolic insights that are only possible with the resolution and sensitivity obtainable with in vivo studies. These studies should include high-resolution one- and two-dimensional multiple quantum studies of liquid and solid-state samples. Again, both longitudinal and cross-sectional studies are needed.

**Neuropathology**

Schizophrenia has been referred to as "the graveyard of neuropathology." This quote, from Dr. Fred Plum, has been repeated out of context with consequences for schizophrenia research that have been the opposite of the original intent. Plum did not suggest that neuropathological research in schizophrenia be abandoned. Rather, he suggested that where traditional neuropathology had failed, newer neuropathological approaches might succeed.
Perhaps the best example of success with modern neuropathological techniques in neuroscience has been the elucidation of the neurochemical aspects of the neuropathology of Parkinson's disease. In pioneering the neurochemical approach to neuropathology, Hornykiewicz demonstrated decreased concentrations of dopamine in the pars compacta of the substantia nigra in Parkinson's disease. This discovery paved the way to the development of l-dopa and carbidopa, which remain the most effective treatments for this syndrome.

The relative success of this approach in Parkinson's disease stands in contrast to the results of traditional neuropathology in schizophrenia. At least a partial explanation for this contrast is that the absence of pigment from the substantia nigra in Parkinson's disease gave a valuable clue as to where to look. Clues such as this may be vital to a neuropathological approach to schizophrenia. Although the clues may not be so obvious as a loss of pigment, they may exist from neuroimaging and neuropharmacological research. For example, if neuropsychological testing and/or regional cerebral blood flow suggests that the dorsolateral prefrontal cortex is abnormal in schizophrenia, then neuropathological research should perhaps be focused in that region. Similarly, if the dopamine hypothesis of schizophrenia suggests that dopamine type-2 receptors are important in the psychosis of this syndrome, then neuropathological research should focus on regions where these receptors are located such as the caudate, putamen, and nucleus accumbens. Obviously, there are many clues such as these which may guide neuropathological approaches.

Neuropathological approaches, in a fashion similar to neuroimaging, can provide assessments of structure and function. Structure can be delineated by traditional neuropathology, neuronal morphometrics, and electron microscopy. Function can be studied by post-mortem neurochemistry and in vitro DNA and RNA hybridization. Approaches such as autoradiography and immunocytochemistry can provide data on both structure and function. A review of the results of these approaches to date, with their advantages and disadvantages, follows.

**Traditional Neuropathology.** The history of the neuropathology of schizophrenia includes many "findings" dating back to the 19th century. There has never been a shortage of findings in the traditional staining of fixed schizophrenic brain tissue. Unfortunately, the lack of a pathognomonic lesion or reproducibility from one study to the next has plagued this approach. Even the most recent descriptions of periventricular gliosis suffer from a lack of neuroanatomical specificity and controversy as to reproducibility.

**Recommendations.** If there is a future for this traditional approach, it probably needs to be combined with a more modern method of quantification such as is available with neuronal morphometrics. At the least, studies should focus on particular neuroanatomical structures implicated in the pathophysiology of the syndrome.

**Neuronal Morphometrics.** The number of neurons in a human brain has been estimated at somewhere between 15 and 50 billion, a number so large that it has defied an exact determination. With the use of computers that can measure optical density, it has now become possible to count automatically neurons that have been appropriately stained. This technique, neuronal morphometrics, also allows for determination of cell size, direction, and/or similar assessments of the size and shape of brain structures (e.g., the amygdala and the lateral ventricles). Although cell counts and sizes of structures can be determined without a computer, the computer approach makes the task of screening large numbers of neurons and structures much easier and more practical.

Studies of this type have yielded several promising leads in the neuropathology of schizophrenia, including apparent abnormalities in the size of the amygdala, internal globus pallidus, hippocampus (all reduced), ventricular enlargement, thinning of the temporal cortex in the parahippocampal gyrus, and subtle abnormalities in the distribution of neurons in the frontal and cingulate cortex. Each of these findings will require further replications. Moreover, should they be replicated, the clinical relevance remains to be determined.

**Recommendations.** The major rate-limiting factors in the development of this promising field are a shortage of post-mortem schizophrenic brains and neuropathologists. Improving donations and attracting neuropathologists to this area of research should be high priorities for any brain research groups interested in schizophrenia. This approach offers considerable promise as a research tool for the neuropathology of schizophrenia. Recently, several new computer-based analytical techniques have been de-
developed to permit objective quantification and the avoidance of subjective bias. If properly applied, these techniques provide high interobserver reliability.

Other methodological limitations of these neuronal morphometric studies include artifacts from fixation such as shrinkage. The usual problems of age, gender, race, prior neuroleptic treatment, and the like are compounded by the relatively small numbers of brains in studies of this type.

**Electron Microscopy.** This approach has been used only rarely in schizophrenia research. Subcellular structures undergo considerable post-mortem degradation, making this technique most useful for surgical biopsy material or for visualization of relatively resistant materials such as viral particles. Since the former is relatively rare in schizophrenia, the major use for this approach in schizophrenia should probably be to test viral hypotheses.

**Recommendations.** Unless new important improvements in technique become available, this approach should probably not be vigorously pursued outside of viral studies. The latter would warrant further electron microscopy studies if new findings suggest its relevance to schizophrenia.

**Post-Mortem Neurochemistry.** The most productive area of neuropathology for schizophrenia has been post-mortem neurochemistry, with over 60 articles in the last 2 decades. Although post-mortem intervals, neuroleptic treatment; age, race, and gender differences; manner of death; and diagnosis pose problems for this approach to the brain, none of these factors are insurmountable.

Many post-mortem neurochemical substances—some peptides and receptors, for example—are remarkably stable post-mortem. Patients with other neurologic and psychiatric disorders treated with neuroleptics help control for the treatment variable. Age, race, gender, and manner of death can each be dealt with by matched controls. And, lastly, suitable scales using chart and family interviews allow for adequate diagnoses.

In the many post-mortem neurochemical studies to date, it has been possible to measure catecholamines, indoleamines, peptides, amino acids, enzymes, receptors, binding sites, and the like. The most reproducible finding has been increased D2 receptors in the caudate, putamen, and nucleus accumbens of schizophrenic patients versus controls. Unfortunately, the significance of this finding remains controversial. It is unclear whether these increases are primary to the illness or secondary to prior neuroleptic treatment, as only a handful of patients have been drug-naive. Nevertheless, regardless of cause, this finding is highly suggestive for the importance of the caudate, putamen, and nucleus accumbens in schizophrenia, as these regions may be either a factor in psychotic symptoms and/or a locus for neuroleptic treatment. As such, this could be the type of clue (as was seen in pigment loss in Parkinson’s disease) that directs future research to the striatum and its innervations. Already, these post-mortem studies have helped to stimulate studies of drug-naive schizophrenic patients with dopamine receptor type-2 ligands using PET scanners. Interestingly enough, post-mortem data were surprisingly close to those seen with PET: receptor numbers of 10-20 pmol/g in the former and 17 pmol/g (SEM = 2.5) in the latter.

Interestingly enough, many of the other neurochemical abnormalities reported in schizophrenia also occur in the basal ganglia and nucleus accumbens. One such finding, increased norepinephrine and its metabolite, 3-methoxy, 4-hydroxyphenylglycol, in the nucleus accumbens and other limbic structures in paranoid schizophrenic patients, further highlights the potential importance of the basal ganglia (nucleus accumbens) and the limbic system.

**Recommendations.** In short, post-mortem neurochemistry is a major technique for testing neurochemical hypotheses of schizophrenia. Although it has been used with success already, its future could be vastly enhanced with greater access to post-mortem samples. This may be aided by pre-mortem assessments and recruitment for future post-mortem brain donations.

**In Vitro DNA and RNA Hybridization.** This approach, which has been successfully used at the basic science level, involves demonstrating nucleic acids in cells with specific radioactive genetic probes. Although yet to be applied to post-mortem schizophrenic brain specimens, it has been successfully applied in other neurological disorders. It will most likely require relatively short post-mortem intervals, but it is as specific a technique for demonstrating neurochemical abnormalities as can be imagined. The approach is a molecular biological approach applied to a section of tissue rather than extracted nucleic acids in a test tube. It should be pointed out, moreover, that the lat-
is perfectly feasible in post-mortem specimens as well, provided post-mortem conditions are favorable.

Recommendations. Insofar as schizophrenia has a genetic component, or that there is an abnormal protein product, this approach offers great promise as a neuropathological technique. Further work needs to be done to determine what post-mortem factors enhance or prevent its application.

Autoradiography. Autoradiographic localization of drug and neurotransmitter receptors and other binding sites within the brain by light microscopy is another approach to the study of schizophrenia, and provides both anatomical and chemical information. One can measure binding sites with a high degree of sensitivity and anatomical resolution. That is, one can determine where as well as which chemical processes are involved. Thus, many important biochemicals can be measured in the brain, and their involvement in schizophrenia and other neuropsychiatric disorders can be assessed.

In vitro labeling receptor autoradiography involves the following steps. First, tissue is obtained either from animals or from humans at autopsy or surgery. The tissues are frozen in such a way (rapidly) as to minimize freezing artifacts, and 10–20 μm sections are cut in a cryostat microtome. These sections are thaw-mounted onto slides, dried at room temperature, and then stored at −20 °C for long periods of time, up to months. Receptors in these slide-mounted tissue sections are labeled with radioactive ligands by an in vitro incubation under well-studied conditions. The binding sites examined by this approach are usually thorough characterization previously by biochemical techniques. After washing and drying the labeled slide-mounted tissue sections, the autoradiogram is generated by apposing sheet film or emulsion-coated coverslips to the labeled tissue. After appropriate exposure, the emulsions are developed and the autoradiograms are viewed. The images can then be analyzed by computerized image analysis instruments, and quantitative data can be obtained. Image analysis is an important and relatively recent addition to this approach. Its usefulness lies in the fact that it reduces the time spent in quantification by an order of magnitude and it makes quantification more reliable. Since the relationship between tissue radioactivity and grain density in an emulsion is not linear, the use of appropriate radioactive standards and a standardized analysis of autoradiograms is essential. Because of advances in computer technology, the analysis of the images can be successfully carried out with a microprocessor.

Because receptors for drugs and neurotransmitters tend to be preferentially localized to neurons, changes in receptors tend to reflect some abnormality or loss of neurons. Hence, receptor mapping by autoradiography has some special usefulness and utility in neuropathology. Receptor mapping has been carried out profitably in several diseases, including amyotrophic lateral sclerosis and Huntington’s chorea.

It is clear from biochemical experiments that there are changes in receptors in schizophrenia, and the application of autoradiographic techniques to tissue obtained from schizophrenic patients should therefore prove useful. Autoradiography will show more precisely where these changes occur and may help identify novel neuropathological changes in schizophrenia. This approach could identify new specific neurotransmitter systems that are involved in schizophrenia.

Recommendations. The technical aspects of receptor and binding site autoradiography need to be improved, even though significant advances have been made. Image analysis instruments should be improved and made even more “user friendly.” Autoradiographic standards need to be improved so that they are more readily available and more similar to brain tissue in their quenching characteristics. Binding ligands for new sites and improved ligands for known sites are needed. Iodinated ligands are especially helpful since autoabsorption seems to be less of a problem than with tritiated ligands.

Although autoradiography has been applied to a number of neurologic disorders, to date it has been used only once in a study for schizophrenia (α2-receptors in the locus ceruleus). The rate-limiting factor appears to be availability of samples. While this approach is far superior to any other for difficult to define or to dissect neuroanatomical regions (such as cerebral cortex, locus ceruleus, or raphe neurons), brain banks have not traditionally offered specimens collected in an appropriate fashion for this technique. An additional advantage of this technique is that large areas, whole coronal sections, can be screened for a particular neurochemical or binding site, while adjacent sections can be screened with a related or different ligand. If there is...
a disadvantage to this technique, it is that once a neurochemical abnormality is identified, tissue homogenate neurochemical studies are easier to perform on larger numbers of samples. Nevertheless, this is an extremely valuable neuropathological technique that has been underutilized thus far in schizophrenia research.

**Immunocytochemistry.** Although immunocytochemistry can provide information on both structure and function, it has different advantages and disadvantages compared to autoradiography. One major advantage is that it can be applied to fixed specimens of which there is probably a larger supply of samples. Unfortunately, only the hardiest of antigens survive formalin fixation so that other preservatives are more useful. Its major disadvantages are secondary to quantification and expense.

The issue of quantification is a complicated one. The ability to stain without interassay variability is a problem for immunocytochemistry. Nevertheless, it can be used as a technique for quantitating the number of neurons that react to the stain. When applied in the latter situation, the approach is useful for quantification allowing for assessment of structures and function.

**Recommendations.** Immunocytochemistry has its advantages for structures that are difficult to define or dissect in human brains such as cerebral cortex or raphe neurons. The cost of antibodies, however, prohibits the application of this technique as a screening device for a whole human brain. Moreover, prompt dissection and fixation to stop chemical reactions is necessary with immunocytochemistry depending on the antigen being studied. Nevertheless, it, like autoradiography, is a valuable neuropathological technique underutilized to date in schizophrenia research.

**Conclusions**

Schizophrenia research has entered an era of brain research. As can be readily ascertained from this report, there is a broad choice of approaches available for the direct study of the brain of living subjects (neuroimaging) and deceased subjects (neuropathology) which can give valuable information about both structure and function. Unfortunately, these approaches are not cheap. Moreover, money is not the only problem.

Although cyclotrons and scanners are costly, neurochemists to create ligands may be even harder to find. Alternatives such as SPECT should be explored as well. Techniques such as NMRS and MEG, which are relatively less developed, will require substantial investments in chemistry and physics before they will yield results in clinical studies. Attracting expert personnel to work on these problems is a major challenge, but one that seems well worth the effort.

Similar problems with techniques and personnel exist for neuropathology. This field cannot contribute to schizophrenia research without specimens. Attracting neuropathologists, expanding brain banks, and starting new brain collections for fixed and frozen materials is not a simple matter. Brain banks which distribute specimens to investigators have been invaluable in the last decade. New collections for specific research groups need to be promoted as well. Wherever possible, pre-mortem exams of targeted populations would greatly enhance neuropathological studies. These studies will require time and money, but again, the effort seems to this Panel to be one worth making.

Insofar as schizophrenia is a syndrome that involves the brain, it seems logical that neuroimaging and neuropathology can make significant contributions to understanding its causes and to developing new treatments. With these goals in mind, it seems clear that major investments should be made in neuroimaging and neuropathology to improve existing techniques, to develop new approaches, and to attract new and essential personnel to schizophrenia research.

**Suggested Readings**


Cohen, M.M.; Pettegrew, J.W.; Kopp, S.J.; Minshew, N.; and Glo-


An Invitation to Readers

Providing a forum for a lively exchange of ideas ranks high among the Schizophrenia Bulletin's objectives. In the section At Issue, readers are asked to comment on specific controversial subjects that merit wide discussion. But remarks need not be confined to the issues we have identified. At Issue is open to any schizophrenia-related topic that needs airing. It is a place for readers to discuss articles that appear in the Bulletin or elsewhere in the professional literature, to report informally on experiences in the clinic, laboratory, or community, and to share ideas—including those that might seem to be radical notions. We welcome all comments.—The Editors

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