that it is faster and easier to perform. We foresee no problems in using this technique for large numbers of cells.

The potential sensitivity of this separation technique is as good as or better than that of any other technique, since we can detect the presence of very small numbers of beads (not shown). In addition, we found that cells that contain such small numbers of beads can be readily separated on the basis of their differential magnetophoretic mobility. The separated cells were viable and grew again to confluency.

Plastic microspheres are widely used in a number of areas—for instance, as chromatographic fixed phases, as immunoprecipitants, for cell labeling, as supports for cell culture, and as vehicles for drug delivery. Addition of the magnetophoretic property to the particles could improve all of the associated techniques. The microspheres used in these various areas, however, differ widely in size. The method of preparation described here is, to the best of our knowledge, the only one capable of yielding sizes that cover this whole range, from tens of nanometers to tens of micrometers. With this development, expansion of the uses of the magnetic particles to these areas is now feasible.

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Reversible Cerebral Atrophy in Recently Abstinent Chronic Alcoholics Measured by Computed Tomography Scans

Abstract. *Eight chronic alcoholics received repeated computed tomography scans. Four, who maintained abstinence and functionally improved, showed partially reversible cerebral atrophy. Two nonabstinent patients and two abstinent patients who had completed functional improvement before the first scan showed no change in atrophy.*

Pathological (*1*) and pneumoencephalographic (*2*) data and studies using computed tomography (CT) scans (*3*), indicate that chronic alcoholism is associated with cerebral atrophy. Chronic alcoholism is also associated with a number of functional deficits in cerebral (*4*) and cerebellar function (*5*) which can show recovery when abstinence is maintained over periods of weeks to many months (*6*). Rapid recovery has been attributed to resolution of the alcohol withdrawal syndrome, which is considered a biochemical disorder (*7*). However, no mechanism has been proposed for the functional improvement observed weeks to months after the last drink. This gradual functional improvement suggested a structural rather than a purely biochemical basis for the recovery. Using the benign diagnostic technique of CT scanning (*8*), we noted a measurable decrease in the degree of cerebral atrophy in repeated CT scans in four of eight chronic alcoholics. We propose that this reversible atrophy represents a form of morphological plasticity in the central nervous system (CNS).

The CT scans were evaluated clinically by G.W. and subsequently evaluated by R.H., who was unaware of patients' clinical status. These evaluations were identical. In four cases, less atrophy, particulary cortical atrophy, was seen on the second scan. In another four cases no difference was seen between the first and second scans. Reversible atrophy was noted only in those patients who abstained from alcohol, showed clinical improvement, and had their initial CT scan before demonstrable clinical improvement was complete (Fig. 1).

The eight patients in this series are the first available for repeat clinical and CT

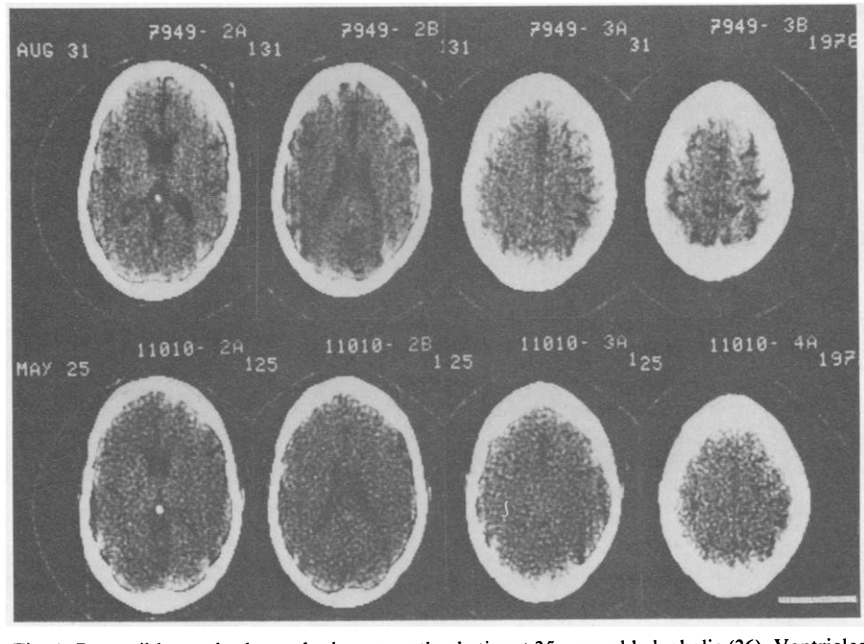

Fig. 1. Reversible cerebral atrophy in a recently abstinent 35-year-old alcoholic (*26*). Ventricles are dark areas in the central portion of pictures at left. The typical walnut-shell appearance of cortical sulcal atrophy is seen in pictures at right. The top row shows four contiguous CT scan cuts taken 4 weeks after the patient's last drink and demonstrates enlarged ventricles and numerous and enlarged cortical sulci. The bottom four CT scan cuts taken 8 months later show a reduction in ventricular size with a more marked reduction in size and number of visible cortical sulci. Variation in the photographic quality of the two scans cannot account for the observed changes. In a normal patient of the same age, ventricles are much smaller and sulci are not visible on CT scans. The calibration line represents 20 mm on standard Polariod photographs and 7.8 cm in actual tissue.

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Table 1. Clinical data and repeated CT scan measurements in eight chronic alcoholics. The degree of clinical impairment on admission ranged from mild (+) to moderate (++) or severe (+++); S, one or more withdrawal seizures on admission. Clinical improvement ranged from none compared to admission status (0) to mild (+), moderate (++), or marked (+++). In those patients who abstained, the time from the last drink to plateau in clinical improvement is indicated. Patient 2 showed moderate improvement for 4 weeks and then mild improvement over 20 weeks.

No.	Age (years)	Sex	Heavy drinking (years)	Clinical impairment on admission	Clinical improvement		Weeks after last drink		Ventricles (mm)		Sulci (mm)	
					Degree	Weeks	Scan 1	Scan 2	Scan 1	Scan 2	Scan 1	Scan 2
1	35	M	14	+++	+++	33	4	37	24	20	12	7
2	59	M	40	+++ S	++	4	11	97	23	19	14	12
					+	20						
3	67	M	15	+++	+++	13	2.5	72	25	23	14	12
4	35	M	10	+++ S	++	18	5.5	32	16	16	10	7
5	40	M	20	+++	+++	2	5	61	30	29	5	4
6	55	M	15	++	+++	7	8	36	28	28	12	12
7	53	M	15	+	0*		4	47	17	17	14	14
8	46	F	20	+S	0*		3	33	30	30	8	8

*Continued drinking.

examinations from a longitudinal study of chronic alcoholics with mental impairment. We have noted a greater degree of cortical as opposed to ventricular atrophy in chronic alcoholics (*9*), including the patients reported in this study. None of these patients had significant liver disease (ascites, jaundice, or evident hepatic encephalopathy), a history of severe head trauma requiring hospitalization, obvious malnutrition, or other evidence of generalized disease.

Attempts have been made to introduce standardized measurements for ventricular and sulcal size (*10*). Our modified measurements (Fig. 2) were employed in an attempt to quantify changes in cerebral atrophy between early and later CT scans from the same patient. While we recognize that no tomographic cut from one set of scans will be identical to that from another set even in the same patient, we believe that because multiple contiguous cuts are included in the measurement, valid comparisons can be made. Cerebral atrophy as used here is defined merely as loss of cerebral tissue as seen on the CT scan; the concept of irreversibility is not included in this definition.

Table 1 summarizes data for these patients. Clinical improvement was sometimes (cases 1 to 4) but not always associated with a decrease in cerebral atrophy, with percentage of decrease in cortical atrophy greater than that for ventricular atrophy. The two exceptions were cases 5 and 6, who showed marked improvement clinically and on psychological test performance with little or no quantitative or qualitative change in CT scans. In both these patients the first scan was done after a period of recovery of mental function; it is possible that changes detectable by CT scans had occurred earlier. Two patients who continued drinking but were available for follow-up showed no change in either CT scan or clinical state. Neither of these patients was markedly impaired on admission. In our sample we had no patients who were markedly impaired on admission, continued to drink, and were subsequently retested.

These initial results raise many questions. Apparent reversible cerebral atrophy could be due to CT artifact, systematic variation in locus of cuts, water and electrolyte imbalance, derangements in cerebrospinal fluid (CSF) pressure, altered cerebral blood flow, or CNS regeneration and plasticity.

The changes of atrophy on our CT scans are well described, typical, and not in a pattern heretofore recognized as artifact. By including a minimum of four contiguous cuts in the measurements, we examined a combined thickness of 52 mm of tissue on each scan. The two scans on each patient included most or all of the same tissue section. "Reversible" atrophy was described by Heinz *et al.* (*11*) in children with anorexia nervosa and other protein-losing states. They suggest that this phenomenon results from changes in distribution of brain water and electrolytes. If brain "dehydration" caused apparent cerebral atrophy in our patients, subsequent decreased atrophy could have resulted from rehydration while patients received a hospital diet. However, all patients in this study had their first scans at least 2.5 weeks after their last drink, longer than normally expected for rehydration of body tissues. Similarly, any shifts in osmotic pressure and electrolyte concentrations between the extracellular and intracellular space in the CNS would be expected within days, rather than weeks, of the last drink. The electrolyte abnormalities of the alcohol withdrawal syndrome usually disappear within 1 week of start of abstinence (*7*); none of these patients demonstrated significant electrolyte abnormalities after 1 week. No information is available on dynamics of CSF pressure in chronic alcoholics. Changes in cerebral blood flow can also contribute to alterations of brain volume. Chronic alcoholics usually demonstrate decreased cerebral blood flow (*12*), but conclusive data are not available concerning changes in blood flow associated with abstinence and concomitant clinical recovery.

It is generally thought that neuronal regeneration does not occur in adults. Certainly, no one has demonstrated mitoses of normal neurons in adult mammalian brain. However, there is considerable evidence of functional plasticity in the mammalian and human CNS (*13*). Morphological plasticity has also been demonstrated in vertebrates by anatomical (*14*) and electrophysiological (*15*) techniques. These studies indicate a time course of weeks to months for reinnervation of partially deafferented neurons. Collateral sprouting of intact axons is the mechanism usually suggested or demonstrated. Dendritic degeneration has been noted after transynaptic deafferentation (*16*) or after retrograde neuronal degeneration (axon reaction) (*17*); in both cases the dendritic changes are remarkably similar (*18*). However, little attention has been paid to the possibility of reversible dendritic changes. Progressive cortical dendritic damage with loss of spines has been noted in aged rats (*19*) and humans (*20*); human psychomotor impairment was correlated more with the degree of dendritic damage than with age.

Noble and Tewari (*21*) reported that chronic ethanol ingestion in mice produced a 50 percent inhibition of brain protein synthesis, and that when ethanol

was withdrawn for 2 weeks protein content increased significantly. This inhibition of protein synthesis may be the mechanism for ethanol's neurotoxic effect. The cellular morphological changes that account for cerebral atrophy of chronic alcoholics are not known. However, recent Golgi studies of rats chronically fed ethanol compared to pair-fed controls have shown decreased dendritic spines in cortical and hippocampal pyramidal cells (*22*), and in hippocampal dentate granule cells and cerebellar Purkinje cells (*23*).

We propose the following hypothesis to explain the partially reversible cerebral atrophy in recently abstinent chronic alcoholics. Chronic ethanol abuse can lead to neuronal damage by some unknown mechanism which results in functional mental impairment and cerebral atrophy. Many neurons disappear along with supporting glia and vasculature; this accounts for the "irreversible" aspect of the observed atrophy, even when some improvement is shown. Some neurons are only partially damaged; these show decreased dendritic and axonal arborization and diminished innervation as evidenced by a decreased number of dendritic spines, possibly with concomitant decrease in supporting glia and vasculature. Conversely, the primary insult of chronic ethanol abuse could be to the supporting tissues with secondary neuronal damage. When the chronic neurotoxin (ethanol) is removed, regrowth of the damaged neurons (that is, the axonal and dendritic neuropil) and the supporting glial and vascular tissues occurs over weeks to months; this accounts for the reversible component of the atrophy seen on the CT scan and the concomitant prolonged psychological improvement. The presence of more pronounced changes in the cerebral cortex than in the ventricles suggests preservation of axons with supporting cell bodies, but loss of one or more of the following: (i) dendritic and axonal arborization, (ii) supporting glia, (iii) supporting vasculature, and (iv) interneurons. Any decrease in ventricular size with maintained abstinence could be caused by remyelination rather than regrowth of axons.

A chronic encephalopathy similar to that seen with ethanol and partly or completely reversible with drug abstinence has been described in subjects treated with diphenylhydantoin (*24*) and in users of multiple drugs, particularly sedative-hypnotic drugs (*25*). The data and hypotheses presented here should encourage further investigations of CNS plastic changes resulting from chronic encephalopathies of various etiologies. Use of CT scanning offers an important tool in the evaluation of these patients.

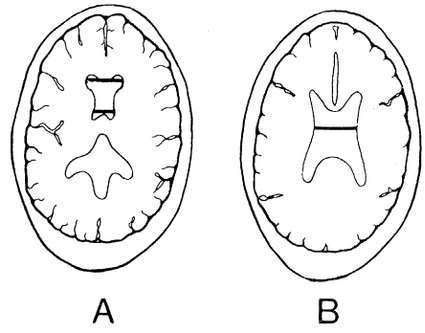

Fig. 2. Measurements of cerebral atrophy. The location of the measurements of ventricular size, from standard Polaroid photographs used by Huckman *et al.* (*10*), is shown in (A). The sum (in millimeters) of the distance between the most lateral portion of each of the frontal horns and the width of the lateral ventricles in the region of the caudate nuclei constituted the patient's score. We added the width of the waist of the lateral ventricles (B) to this score, subtracting any clearly visible intervening cerebral tissue, if present. Cortical sulcal atrophy was estimated by summing the widths of the eight largest cortical sulci from all the CT scan cuts. The mean width of visible sulci was slightly more than 1 mm. The measurements were accurate only to ± 0.25 mm, so some measurement error was inevitable. The measurements were made without knowledge of the date of the CT scan and confirmed the clinical assessment. These measurements reflect but do not quantify the cross-sectional area of atrophy.

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