Microtubule-associated protein \( \tau \) was characterized in 5 Alzheimer and 5 control brains using two monoclonal antibodies, Alz 50 and Tau-1. Quantitative analysis of immunoblots with the antibodies showed that both homogenate and supernatant fractions (12,000 \( \times \) g) from Alzheimer brains contained 38–65% less \( \tau \) immunoreactivity compared to normal brains. The reduction was found in all brain regions studied (frontal and temporal lobes and thalamus) and in both gray and white matter. In partially purified \( \tau \) preparations, the yield of protein was lower in Alzheimer (by 35%) than in control brain. Incubation of brain proteins, transferred onto nitrocellulose paper, with alkaline phosphatase had either no effect or slightly increased the antibody binding to \( \tau \) proteins from both brain tissues. Immunoblots of \( \tau \)-enriched preparations subjected to two-dimensional gel electrophoresis showed no major changes in the staining pattern of \( \tau \) isoforms in Alzheimer samples except for a weaker reactivity of the basic isovariants compared to non-Alzheimer samples. The elution volume of \( \tau \) from Alzheimer brain supernatant on a Sepharose CL-6B column was similar to that from non-Alzheimer brain and equal to that of aldolase (\( M_r \approx 158,000 \)). Our data suggest that most of \( \tau \) proteins from both types of brain have similar biochemical properties. The reduction in \( \tau \) reactivity in Alzheimer tissue may be due to a reduction in neuronal cell population or incorporation of soluble \( \tau \) into stable structures such as neurofibrillary tangles, since the tangles have been shown to react with anti-\( \tau \) antibodies.

Alzheimer’s disease is characterized histopathologically by NFT\(^*\) and senile plaques in brains of affected individuals (1). NFT and many neurites in senile plaques contain bundles of paired helical filaments (2). These pathological structures are not found in normal young subjects and are detected only in a small number of neurons in some nondemented elderly persons. In previous studies, normal cytoskeletal proteins have been suggested to play a role in the formation of NFT (3–6). In this regard, heat-stable microtubule-associated proteins, including \( \tau \) proteins, have recently gained considerable attention (7–10). This is primarily due to the fact that all \( \tau \) antibodies tested so far react with NFT, and that many NFT-reactive antibodies (11–13) or antibodies raised against NFT cross-react with \( \tau \) (7–9, 14). The binding of a monoclonal anti-\( \tau \) antibody, Tau-1, to NFT was enhanced by pretreatment of tissue sections with phosphatase (9, 15). This observation has formed the basis for the theory that \( \tau \) may be abnormally phosphorylated in Alzheimer’s disease. In addition, comparison of immunoblots of homogenates from a single case of Alzheimer’s disease and a normal individual for their reactivity with Tau-1 suggested that Alzheimer’s disease brains may contain more \( \tau \) proteins than normal (9). Whether this is a common feature in all Alzheimer cases and whether it involves specific regions of the brain has not been examined.

In the following studies, two monoclonal antibodies reactive with human \( \tau \) (13) were used to probe both quantitative and qualitative alterations of \( \tau \) in Alzheimer’s disease. We compared brain areas that are known to express more pathological changes, frontal and temporal lobes, with the regions where the Alzheimer pathology is usually absent, cerebellum and thalamus. Five Alzheimer and five non-Alzheimer brains were studied.

**EXPERIMENTAL PROCEDURES**

**Material and Monoclonal Antibodies**—Human brain tissue was obtained at autopsy performed 4–20 h postmortem. All five Alzheimer cases were histopathologically confirmed using the diagnostic criteria of Khachaturian (16). Control cases were brains from five individuals with either minimal or no senile changes. Frontal lobe, temporal lobe, thalamus, and cerebellum were dissected. The tissue was stored at \(-70 \) °C, or used immediately after dissection. In some studies, white and gray matter were separated. Bovine brains were obtained from a local slaughterhouse and used fresh.

Monoclonal antibodies, Tau-1 and Alz 50, were produced as described earlier (13) and used at 1:2000 and 1:5 dilution, respectively. Heat-stable Microtubule-associated Proteins—Bovine brain microtubules were purified as before (13). Microtubules were boiled under conditions described previously (17) to obtain a heat-stable fraction containing microtubule-associated protein 2 and \( \tau \).

**Human Brain Fractions**—Brain tissue, stripped of leptomeninges, was homogenized in a Teflon glass homogenizer (or blender) at 4 °C in a buffer containing 0.1 M MES-Na, pH 6.8, 2 mM EGTA, 1 mM MgCl\(_2\), 5 mM NaF, and 1 mM phenylethylsulfonfyl fluoride. The ratio of tissue to buffer was in the range of 1:1.5 to 1:5 (w/v). A portion of the homogenate was mixed with SDS to a final concentration of 2%. The remaining homogenate was centrifuged for 20 min at 12,000 \( \times \) g, and the supernatant (regarded as brain extract hereafter) was collected. In some experiments, the brain extract was used to produce a heat-stable fraction (17) or \( \tau \)-enriched preparation (13).

**Gel Chromatography of Human Brain Extract**—One ml of brain extract (equivalent to approximately 0.7 g of tissue) was subjected to gel filtration on a Sepharose CL-6B (Pharmacia) column (0.7 \( \times \) 30 cm) as described by Cleveland et al. (18). The fractions were analyzed on SDS-polyacrylamide gels and then tested for reactivity with anti-\( \tau \) antibodies by immunoblotting.

**Gel Electrophoresis and Immunoblotting**—One-dimensional gel electrophoresis in 10% polyacrylamide was carried out according to the method of Laemmli (19). Some human \( \tau \)-enriched samples were
Proteins in Normal and Alzheimer's Disease Brains

Quantitative Analysis of \( \tau \) Immunoreactivity on Blots—The binding of Tau-1 and Alz 50 to proteins on immunoblots was quantified with a densitometric scanner (Dual-Wavelength TLC Scanner; Shimadzu, model CS-910) linked to a data processor (C-RIB Chromatopack; Shimadzu). A bovine brain heat-stable microtubule fraction served as the standard. The amount of bovine \( \tau \) protein on blots was determined by comparing the final green staining of \( \tau \) with that of protein standard (bovine serum albumin, fraction V). The immunoreactivity per unit of \( \tau \) protein was calculated by dividing the data obtained from the scans of immunoblots by that from final green staining.

RESULTS

Quantitation of Tau-1 and Alz 50 Binding to Bovine \( \tau \)—Tau-1 and Alz 50 both stained four to five bands in a molecular weight range consistent with \( \tau \) proteins on immunoblots of heat-stable microtubule proteins from bovine brain. The results were similar to those presented in the accompanying paper (13). Quantitative analysis showed that binding of antibodies was proportional to the amount of bovine \( \tau \) proteins (in all four to five bands) blotted onto nitrocellulose paper in a range of 0.25-0.5 \( \mu \)g for Alz 50 and up to 0.25 \( \mu \)g for Tau-1. The results also showed that Tau-1 was more sensitive than Alz 50 in detecting \( \tau \) proteins.

Immunoreactive \( \tau \) in Alzheimer and Control Brains—The age, sex, and postmortem delays and the histopathological findings of the 10 cases are listed in Table 1. Clinical features are sparse on all but two of the Alzheimer cases. Patient 6 had chronic progressive dementia while patient 9 had a rapidly progressive disease over the last year. Patients 8 and 10 had concomitant Parkinson’s disease.

Homogenates and extracts from frontal and temporal lobes, thalamus, and cerebellum were immunoblotted with Tau-1 and Alz 50. Except for the cerebellum, which contained a very small amount of \( \tau \), undetectable by our methods, other regions of the brain contained immunoreactive \( \tau \) proteins (62-50 kDa) that reacted with both antibodies (Fig. 1). Thalami from all cases showed either similar or moderately less immunoreactivity than frontal and temporal lobes. In addition to bands in the 50- to 62-kDa region, several proteins smaller than 50 kDa were also stained. These latter proteins were considered to be degraded \( \tau \) proteins (13). When equivalent amounts of samples, per g of wet weight of brain tissue, were compared, \( \tau \) proteins in homogenate and extract from Alzheimer tissues were stained less intensely by both antibodies than the respective control samples (Fig. 1).

In one Alzheimer case (patient 9; Fig. 1, lane T) a band in the 68-kDa region was immunostained by Alz 50, but not Tau-1. A similar 68-kDa band was not detected by Alz 50 in the samples prepared from control cases and other Alzheimer cases. In homogenates from all cases, two bands in the 70- to 75-kDa region and one band at 120 kDa were also stained. However, unlike the staining of \( \tau \) or smaller \( \tau \) fragments, the staining of these three bands was nonspecific, probably due to biotin-containing proteins (13, 24). In extracts, only one nonspecific band (70 kDa) remained.

Quantitative analysis of antibody binding to \( \tau \) proteins and presumed \( \tau \) degradation products in the region below 50 kDa (Tables II and III) clearly demonstrated that, regardless of which antibody was used, Alzheimer brain tissues contained less \( \tau \) immunoreactivity than control brains. In Alzheimer samples from frontal and temporal cortex, and from thalamus, the amount of \( \tau \) was 35 to 65% of control values. These differences were statistically significant and they were apparent not only in samples of tissues that included both white and gray matter, but also in white and gray matter separately.

Our analysis also showed that gray and white matter contain similar amounts of \( \tau \) immunoreactivity, corresponding to 55-64 \( \mu \)g of protein/g of normal tissue and 33 \( \mu \)g of protein/g of Alzheimer tissue. The difference between control and Alzheimer brains in \( \tau \) immunoreactivity was also detected in thalami, which usually shows very little Alzheimer pathology (NFT or senile plaques). In control thalamus, the amount of \( \tau \) was nearly the same as in control cortex (47 \( \mu \)g of protein/g of tissue).

Using bovine \( \tau \) as the standard of immunoreactivity with Alz 50, we estimated that the amount of the 68-kDa protein found in case 9 was 3.8 \( \mu \)g/g of tissue. Compared with the level of \( \tau \), the 68-kD protein was much less abundant. In Alzheimer brain, there was five times more \( \tau \) than the 68-kDa protein (Table III).

Effect of Phosphatase Treatment—To determine if Alzheimer brain contains abnormally phosphorylated \( \tau \), brain homogenates and extracts from control (patients 1-3) and Alzheimer (patients 5-7) cases were electrophoblotted onto nitrocellulose paper and incubated with alkaline phosphatase from either E. coli or calf intestine. The enzyme treatment did not preferentially affect the binding of Tau-1 and Alz 50 to \( \tau \) proteins from Alzheimer samples (Fig. 2). As analyzed quantitatively, Tau-1 immunoreactivity remained in a range...
τ Proteins in Normal and Alzheimer’s Disease Brains

**Table I**

| Case          | Gray matter | White matter | Thalamus |
|---------------|-------------|--------------|----------|
| Non-Alzheimer |             |              |          |
| 1             | 47 (8)      | 48 (3)       | 43 (2)   |
| 2             | 70 (4)      | 60 (2)       | 40 (2)   |
| 3             | 60 (2)      | 56 (2)       | 59       |
| 4*            | 39 (2)      | 38 (2)       | 38       |
| 5*            | 61 (2)      |              |          |
| Mean ± S.E. (N) | 55 ± 6 (5) | 54 ± 4 (3)   | 47 ± 6 (3) |
| Alzheimer     |             |              |          |
| 6             | 37 (8)      | 24 (3)       | 25 (2)   |
| 7             | 41 (4)      | 40 (4)       | 29 (2)   |
| 8             | 21 (2)      | 35 (2)       | 34       |
| 9*            | 34 (2)      |              |          |
| 10*           | 34 (2)      |              |          |
| Mean ± S.E. (N) | 33 ± 3* (5) | 33 ± 5* (3) | 29 ± 3* (3) |
| % of non-Alzheimer | 60       | 61          | 62       |

*Temporal lobe was used.

b P < 0.01 (Student's t test, two-tailed).

c P < 0.05.

**Table II**

| Tau-1 reactive proteins in different regions of Alzheimer and non-Alzheimer brain tissue
| Case          | Frontal lobe | Thalamus |
|---------------|-------------|----------|
|               | Gray matter | White matter |
| Non-Alzheimer |             |            |
| 1             | 98 (7)      | 46        | 19       |
| 2             | 63 (3)      | 108       | 132      |
| 3             | 52          |            |          |
| 4*            | 68 (2)      |            |          |
| 5*            | 42 (2)      |            |          |
| Mean ± S.E. (N) | 64 ± 10 (5) | 77 (2)    | 76 (2)   |
| Alzheimer     |             |            |
| 6             | 47 (7)      | 8         | 7        |
| 7             | 36 (3)      | 65 (2)    | 58       |
| 8             | 21 (2)      | 42        | 15       |
| 9*            | 22 (2)      |            |          |
| 10*           | 32 ± 6* (4) | 27 ± 10 (3) | 27 ± 16 (3) |
| % of non-Alzheimer | 49       | 35         | 35       |

*Temporal lobe was used.

b P < 0.05.

**Table III**

| Alz 50 reactive proteins in different regions of Alzheimer and non-Alzheimer brain tissue
| Case          | Frontal lobe | Thalamus |
|---------------|-------------|----------|
|               | Gray matter | White matter |
| Non-Alzheimer |             |            |
| 1             | 98 (7)      | 46        | 19       |
| 2             | 63 (3)      | 108       | 132      |
| 3             | 52          |            |          |
| 4*            | 68 (2)      |            |          |
| 5*            | 42 (2)      |            |          |
| Mean ± S.E. (N) | 64 ± 10 (5) | 77 (2)    | 76 (2)   |
| Alzheimer     |             |            |
| 6             | 47 (7)      | 8         | 7        |
| 7             | 36 (3)      | 65 (2)    | 58       |
| 8             | 21 (2)      | 42        | 15       |
| 9*            | 22 (2)      |            |          |
| 10*           | 32 ± 6* (4) | 27 ± 10 (3) | 27 ± 16 (3) |
| % of non-Alzheimer | 49       | 35         | 35       |

a Percentage of untreated samples (i.e., within experimental error), while Alz 50 immunoreactivity increased in both Alzheimer and control samples by 27–34%. In addition, this treatment did not reveal new immunoreactive bands, either in control or Alzheimer cases.

**Alz 50 reactive proteins in different regions of Alzheimer and non-Alzheimer brain tissue**

Results of quantitative analysis of Alz 50 binding on immunoblots of brain extracts. Alz 50 reactivity with bovine τ served as standard. In addition to τ bands, a 68-kDa band was present in Alzheimer case 9, and it was quantified separately.

**Preparation of Human τ-enriched Samples**—The procedure used previously to prepare τ proteins from normal human brain (13) was applied to Alzheimer brain. In normal cases, τ-enriched fractions obtained from 1 g of frontal lobe or from both control (Fig. 3A) and Alzheimer extracts (Fig. 3B) eluted in fractions corresponding to the elution volume of aldolase (M, = 158,000). Alzheimer fractions, however, contained less τ1-immunoreactivity than controls.

Initial extracts, fractions from the void volume, and the peak for τ1 reactivity were also tested for Alz 50 (not shown). The immunoblots with Alz 50 were similar to those with Alz 1, and the 68-kDa protein was not detected. No τ1 or Alz 50 reactivity was found in the void volume of the samples tested.

**Gel Chromatography of Brain Extracts**—Extracts from normal and Alzheimer brains were subjected to gel chromatography on a Sepharose CL-6B column. Proteins from the fractions were separated on SDS-polyacrylamide gels and tested for immunoreactivity on immunoblots with Tau-1. τ binding was determined on immunoblots of extracts from frontal lobe; Th, thalamus; C, cerebellum; T, temporal cortex. Except for homogenate of thalamus with Tau-1, all samples are from extracts. Each lane was loaded with extract from approximately 4 mg of tissue. In respective (left to right) lanes, the control cases were: 2, 1 (or 2 in lower panel), 1, 1. The Alzheimer cases were, respectively: T, 7, 6, 7. Arrowheads mark the location of a 68-kDa band recognized by Alz 50 but not by Tau-1. Nonspecific staining of one to three bands in the 60- to 70-kDa range for gray and white matter, Thalamus; and it was quantified separately.
thalamus contained 0.24–0.28 mg of protein (Table IV). Alzheimer preparations contained 32–37% less protein compared to controls. These values are consistent with the data from quantitative analysis of immunoblots (Tables II and III), which showed a 38–65% decrease in \( \tau \) immunoreactivity in Alzheimer compared to control samples. Judging from the immunoblots with anti-\( \tau \) antibodies and silver-stained gels, the purity of \( \tau \) preparations from normal and Alzheimer brains was similar. Human \( \tau \) preparations described here were 5–6-fold enriched in \( \tau \) as compared to the initial brain extracts (Tables II and III versus Table IV) and were suitable for two-dimensional gel analysis.

**Two-dimensional Gel Analysis of \( \tau \)-enriched Preparations**—Immunoblotting of partially purified \( \tau \) preparations from cortex or thalamus demonstrated that both antibodies bound to multiple variants of \( \tau \), with pI ranging from approximately 6.5 to pI 8.5, present in both normal and Alzheimer samples. The staining pattern with Tau-1 was similar to that with Alz 50 in all pI regions (Fig. 4, A and B). Larger forms (62 kDa versus 50 kDa) contained variants with more acidic pI and these variants appeared to have comparable immunoreactivity in samples from Alzheimer and control brains. However, \( \tau \) variants in the basic region (pI > 7.4) were stained less intensely in Alzheimer samples as compared to controls, and the same effect was seen with both antibodies (Fig. 4, C and D). The reduction in the staining pattern of basic \( \tau \) variants was present in \( \tau \) preparations either from frontal cortex or thalamus (Fig. 4, E and F).

**DISCUSSION**

The results of the present studies demonstrate that Alzheimer brains contain less immunoreactive \( \tau \) proteins than control brains. Not only brain regions, such as temporal and frontal lobes, where numerous NFT and plaques are typically observed, showed a reduction in \( \tau \), but also areas such as thalamus, with few NFT and plaques. The reduction was not confined to homogenates or extracts from gray matter, where most of the histopathological changes occur, but was also found in white matter. The changes appeared independent of the age of the subjects and postmortem intervals ranging from 4–20 h and age of the subjects. Our observations differ from those of Wood et al. (9) since five cases of Alzheimer and control were examined in our studies, the changes we found are likely a common feature of Alzheimer disease. However, it is possible that hippocampal tissue (9) exhibits Alzheimer changes different from cortex and thalamus in the present study.

**FIG. 3.** Gel filtration on a Sepharose CL-6B (0.7 × 30 cm) column of extract from frontal cortex of the control case (patient 1) (A) or Alzheimer case (patient 6) (B). Proteins in extracts from 0.7 g of fresh tissue were eluted at 1 ml/8 min with a buffer containing: 0.1 M MES-Na, pH 6.8, 2 mM EGTA, and 1 mM MgCl₂. One-ml fractions were monitored at 280 nm (○—○) and analyzed for \( \tau \) reactivity with Tau-1 (■—■). Arrowheads mark the elution volume of (from left) blue dextran (void volume) and the following protein standards: thyroglobulin (\( M_r = 669,000 \)), catalase (\( M_r = 232,000 \)), and aldolase (\( M_r = 158,000 \)).

**FIG. 4.** Immunoblots of two-dimensional gel of \( \tau \)-enriched preparations from different human brain regions with Alz 50 (left column) or Tau-1 (right column). A and B, frontal cortex from control case (patient 1); C and D, frontal cortex from Alzheimer case (patient 6); and E and F, thalamus from Alzheimer case (patient 6). One-dimensional analysis of the samples is shown at the left or right side of respective two-dimensional gels. Arrowheads indicate the location of internal pH standard (phosphoglycerate kinase, pI 7.4), which was visualized by fast green staining of electrophrobes prior to staining with antibodies (in the photographs, fast green staining is compensated by filters). The acidic side is shown to the right. Mass standards (in kDa) are indicated.

**TABLE IV**

| Case                 | Frontal lobe mg protein/g tissue | Thalamus mg protein/g tissue |
|----------------------|---------------------------------|-----------------------------|
| Non-Alzheimer        | 0.28 ± 0.05 (4)                 | 0.24                        |
| Alzheimer            | 0.19 ± 0.04 (3)                 | 0.15                        |
| % of non-Alzheimer   | 68                              | 63                          |
It is uncertain what mechanisms are involved in the seeming-ly general reduction of \( \tau \). One possibility is that it reflects a loss of neurons in Alzheimer's disease, if not just a shrinkage of neurons and of cell processes as suggested (25). Terry et al. (26) have reported a reduction of about 40–46% of neurons, especially prominent in frontal and temporal cortices in a population of large neurons. Others have observed a loss of 50% or more of neurons in temporal cortex III and V (27), basal forebrain nuclei (27, 28), hippocampus (29), and changes in other areas, including dilation of the third ventricle and atrophy of thalamus (30).

Another explanation for the reduction in \( \tau \) is that the synthesis or turnover of \( \tau \) and possibly other proteins, e.g. tubulin, is depressed by the disease (25). Positron emission tomography studies of 28 Alzheimer patients by Bustany et al. (31) showed that the in vivo incorporation of L-[\( ^{14} \)C] methionine into cerebral proteins was markedly decreased, indicating a general reduction in protein synthesis. In vitro protein synthesis with mRNA isolated from human brains showed that the synthesis of tubulin and other proteins was less than control, whereas the synthesis of glial filament proteins appeared unaffected (32). The synthesis of \( \tau \) was not addressed in these studies. Quantitative biochemical analysis has also shown a reduction of tubulin in Alzheimer brains (33, 34), especially in cases with neuronal loss. Moreover, morphological studies have demonstrated that many neurons in Alzheimer brains, especially those with NFT, often are depleted of microtubules (35, 36), and dendritic microtubules of frontal cortex—layers II and III—are abnormally oriented and greatly reduced in number (37).

Decreased immunoreactivity of Alzheimer samples with Tau-1 and Alz 50 is not likely due to a masking or alteration of antigenic sites of \( \tau \) by phosphorylation, since treatment of control and Alzheimer samples with alkaline phosphatase altered their immunoblotting reaction to the same extent. The results suggest that most of the \( \tau \) in Alzheimer brains is not abnormally phosphorylated. However, one cannot rule out the possibility that a small amount of \( \tau \), below the sensitivity of the methods used in the present studies, and \( \tau \) epitopes present in NFT are abnormally phosphorylated. Grundke-Iqbal et al. (15, 38) have prepared microtubules from an Alzheimer brain and found the immunoblotting pattern of the \( \tau \) region with Tau-1 altered by pretreatment of blots with alkaline phosphatase. If \( \tau \) is abnormally phosphorylated, it remains to be determined whether this has an effect on the ability of \( \tau \) to promote microtubule assembly, as in the case of normally phosphorylated \( \tau \) (39), and if so what the minimum concentration required is as compared to normal \( \tau \).

Two-dimensional gel analysis demonstrated that all of the \( \tau \) variants found in Alzheimer brains are present in normal control brains. No new variants were detected. Instead, Alzheimer \( \tau \) appeared to contain fewer variants with basic PI than the control. The significance of such a change in PI of Alzheimer \( \tau \) is not known. It has been suggested (40) that the charge of proteins may be altered by phosphorylation, acetylation, addition of carbohydrate group, or glutamine/asparagine deamidation. It remains to be determined whether the reduction of \( \tau \) variants with PI > 7.4 observed in Alzheimer tissues is due to one of these modifications (21, 29). Deamidation of \( \tau \) by glutaminase or asparaginase (41) and abnormal phosphorylation (15) have been implied to play a role in the formation of NFT.

Only quantitative differences were noted in comparisons of normal and Alzheimer \( \tau \) from brain extracts by gel filtration (Sepharose CL-6B). Both proteins eluted at the same volume that corresponded to the elution volume of a protein standard with \( M_r = 158,000 \) (aldolase). Since during gel filtration \( \tau \) proteins behave as a larger molecule than estimated by other techniques (e.g. sedimentation in sucrose gradient or amino acid composition), native \( \tau \) has been considered to be a highly asymmetric monomer (axial ratio 20:1), rather than a complex or aggregate (13, 42). The absence of any \( \tau \) reactivity in the void volume of Sepharose CL-6B column suggests that in Alzheimer extracts, as in controls, \( \tau \) proteins are present as monomers.

Alz 50 has been demonstrated to recognize a novel 68-kDa antigen in Alzheimer brains (43). The 68-kDa protein was extractable with a buffer and excluded in the void volume during filtration on Sepharose CL-6B. In the present study of Alzheimer brain homogenates and extracts, only one Alzheimer case (patient 9) contained the 68-kDa protein recognized by Alz 50. In contrast, \( \tau \) proteins were detectable in all Alzheimer samples. The amount of 68-kDa protein was very low, at most 3.5 \( \mu \)g/g tissue. In comparison to \( \tau \) proteins, it was only 6% of normal \( \tau \) and 18% of Alzheimer \( \tau \).

Although immunochemical analysis of animal and human brains showed that Alz 50 behaved similarly to Tau-1 with respect to recognition of \( \tau \) proteins, the 68-kDa protein recognized by Alz 50 was not detected by Tau-1. Lack of Tau-1 reactivity with the 68-kDa protein can be explained by protease digestion studies (13) which revealed that the Tau-1 and Alz 50 epitopes are not the same. Another difference between \( \tau \) and the 68-kDa protein is that \( \tau \) proteins are not detected in the void volume from Sepharose CL-6B. Thus, the 68-kDa protein and \( \tau \) share antigenic sites, but have distinct physicochemical properties, such as native molecular mass.

In addition to \( \tau \) and 68-kDa protein, isolated NFT2 and NFT3 in tissue sections (43) are also recognized by Alz 50. We do not know if there is any relationship among these Alz 50-reactive proteins other than sharing of an antigenic site. There is a possibility that the 68-kDa protein represents an aberrant form of \( \tau \). In affected neurons, this abnormal \( \tau \) could form large aggregates (>600 kDa) and participate in the formation of a soluble pool of NFT. Since the amount of the soluble pool of NFT is small (44) and differs between cases, this may explain why the 68-kDa protein can be detected on immunoblots in limited amounts and in certain brains only. Further studies need to determine if the 68-kDa protein detected in the present study is similar to the one reported by Wolozin et al. (43) and if the 68-kDa protein is an aberrant form of \( \tau \) serving as a precursor of NFT.

Acknowledgments—We thank Dr. Peter Davies for providing human material and the antibody Alz 50, Dr. Dennis W. Dickson for the assessment of clinical and histopathological reports and valuable comments on the manuscript, and C.-H. Chien for technical assistance.

REFERENCES

1. Wianiewski, H. M., and Terry, R. D. (1976) in Neurobiology of Aging (Terry, R. D., and Gernhon, S., eds) pp. 265–280, Raven Press, New York
2. Kidd, M. (1964) Brain 87, 307–320
3. Grundke-Iqbal, I., Johnson, A. B., Wianiewski, H. M., Terry, R. D., and Iqbal, K. (1979) Lancet 1, 578–580
4. Yen, S.-H., Gaskin, F., and Terry, R. D. (1981) Am. J. Pathol. 104, 77–89
5. Anderton, B. H., Erienbarg, D., Downes, M. J., Green, P. J., Tomlinson, B. E., Ulrich, J., Wood, J. N., and Kahn, J. (1982) Nature 296, 84–86
6. Perry, G., Ruzutto, N., Auttilio-Gambetti, L., and Gambetti, P. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 3916–3920

2 H. Ksiezek-Reding, L. I. Binder, and S.-H. Yen, unpublished observation.
7. Grundke-Iqbal, I., Iqbal, K., Quinlan, M., Tung, Y.-C., Zaidi, M. S., and Wisniewski, H. M. (1986) *J. Biol. Chem.* 261, 6084–6089.

8. Kosik, K. S., Joachim, C. L., and Selkoe, D. J. (1986) *Proc. Natl. Acad. Sci. U. S. A.* 83, 4044–4048.

9. Wood, J. G., Mirra, S. S., Pollock, N. J., and Binder, L. I. (1986) *Proc. Natl. Acad. Sci. U. S. A.* 83, 4040–4043.

10. Nukina, N., and Ihara, Y. (1986) *J. Biochem.* (Tokyo) 99, 1541–1544.

11. Ksieczak-Reding, H., Dickson, D. W., and Yen, S.-H. (1987) *Proc. Natl. Acad. Sci. U. S. A.* 84, 3410–3414.

12. Nukina, N., Kosik, K. S., and Selkoe, D. J. (1987) *Proc. Natl. Acad. Sci. U. S. A.* 84, 3415–3419.

13. Ksieczak-Reding, H., Davies, P., and Yen, S. H. (1988) *J. Biol. Chem.* 263, 7943–7947.

14. Yen, S.-H., Dickson, D. W., Crowe, A., Butler, M., and Shelanski, M. L. (1987) *Am. J. Pathol.* 126, 81–91.

15. Grundke-Iqbal, I., Iqbal, K., Tung, Y.-C., Quinlan, M., Wisniewski, H. M., and Binder, L. I. (1986) *Proc. Natl. Acad. Sci. U. S. A.* 83, 4913–4917.

16. Khachaturian, Z. S. (1985) *Arch. Neurol.* 42, 1097–1105.

17. Herzog, W., and Weber, K. (1978) *Eur. J. Biochem.* 92, 1–8.

18. Cleveland, D. W., Hwo, S.-Y., and Kirschner, M. W. (1977) *J. Mol. Biol.* 116, 207–225.

19. Laemmli, U. K. (1970) *Nature* 227, 680–685.

20. O’Farrell, P. Z., Goodman, H. M., and O’Farrell, P. H. (1977) *Cell* 12, 1133–1142.

21. Butler, M., and Shelanski, M. L. (1986) *J. Neurochem.* 47, 1517–1522.

22. Towbin, H., Staehelin, T., and Gordon, J. (1979) *Proc. Natl. Acad. Sci. U. S. A.* 76, 4350–4354.

23. Brunk, A., and Englund, E. (1986) *Ann. Neurol.* 19, 253–262.

24. Chandler, C. S., and Ballard, F. J. (1985) *Biochem. J.* 232, 385–393.

25. Bowen, D. M., and Davison, A. N. (1986) *Br. Med. Bull.* 42, 75–80.

26. Terry, R. D., Peck, A., DeTeresa, R., Schechter, R., and Horoupian, D. S. (1981) *Ann. Neurol.* 10, 184–192.

27. Mann, D. M. A., Yates, P. O., and Marcyniuk, B. (1985) *Neurosci. Lett.* 56, 51–55.

28. Whitehouse, P. J., Price, D. L., Struble, R. G., Clark, A. W., Coyle, J. T., and DeLong, M. R. (1982) *Science* 215, 1237–1239.

29. Doebler, J. A., Markesbery, W. R., Anthony, A., and Rhoads, R. E. (1987) *J. Neuropathol. Exp. Neurol.* 46, 28–39.

30. De Leon, M. J., Ferris, S. H., George, A. E., Reisberg, B., Kricheff, I. I., and Gershon, S. (1980) *Neurobiol. Aging* 1, 69–79.

31. Bustany, P., Henry, J. P., Sargent, T., Zarifian, E., Cebanis, E., Collard, P., and Comar, D. (1983) in *Positron Emission Tomography of the Brain* (Heiss, W.-D., and Phelps, M. E., eds) pp. 206–211, Springer-Verlag, Berlin.

32. Sajdel-Sulkowska, E. M., Coughlin, J. F., Staton, D., and Marotta, C. A. (1985) in *Banbury Report: Biological Aspects of Alzheimer’s Disease* (Katzman, R., ed) Vol. 15, pp. 193–200, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

33. Borthwick, N. M., Yates, C. M., and Gordon, A. (1985) *J. Neurochem.* 44, 1436–1441.

34. Kosik, K. S., Gilbert, J. M., Selkoe, D. J., and Strocchi, P. (1982) *J. Neurochem.* 29, 1529–1538.

35. Terry, R. D., and Wisniewski, H. (1970) in *Alzheimer’s Disease and Related Conditions* (Wolstenholme, G. E. W., and O’Connor, M., eds) pp. 145–165, Churchill-Livingstone, London.

36. Gray, E. G., Paula-Barbosa, M., and Roher, A. (1987) *Neuropathol. Appl. Neurobiol.* 13, 91–110.

37. Paula-Barbosa, M., Tavares, M. A., and Cadete-Leite, A. (1987) *Brain Res.* 416, 139–142.

38. Iqbal, K., Grundke-Iqbal, I., Zaidi, T., Merz, P. A., Wen, G. Y., Shaikh, S. S., and Wisniewski, H. M. (1986) *Lancet* 2, 421–426.

39. Lindwall, G., and Cole, R. D. (1984) *J. Biol. Chem.* 259, 5301–5305.

40. O’Farrell, P. H. (1975) *J. Biol. Chem.* 250, 4007–4021.

41. DeGarcini, E. M., Serrano, L., and Avila, J. (1986) *Biochem. Biophys. Res. Commun.* 141, 790–796.

42. Cleveland, D. W., Hwo, S.-Y., and Kirschner, M. W. (1977) *J. Mol. Biol.* 116, 227–247.

43. Wolozin, B. L., Pruchnicki, A., Dickson, D. W., and Davies, P. (1986) *Science* 232, 648–650.

44. Wisniewski, H. M., Iqbal, K., Grundke-Iqbal, I., and Rubenstein, R. (1987) *Neurochem. Res.* 12, 93–95.

**τ Proteins in Normal and Alzheimer’s Disease Brains**

7953