Figure 13  Preparations enriched for the scrapie infectivity were resistant to inactivation by procedures that selectively altered nucleic acids but susceptible to those that modified proteins. Enzymes called nucleases degrade DNA and RNA, slicing them up into their constituent bases; some, called DNases, chew up only DNA, while others, called RNases, degrade RNA, and yet others degrade both. Six procedures that modified only proteins inactivated the purified scrapie agent. When diethylpyrocarbonate (DEPC) was added to our purified preparations, the scrapie agent was inactivated. DEPC modifies both proteins and nucleic acids, so this experiment, by itself, was unhelpful; however, DEPC could be removed from proteins—but not from nucleic acids—by another chemical, hydroxylamine. Equally important, in the absence of DEPC modification, hydroxylamine reacts with nucleic acids but not with proteins.
Still another questioner argued, “Why do you use such odd procedures to purify a virus? You should use a salt gradient composed of cesium chloride.”

“I’ve tried many approaches, including those commonly used to purify viruses, but they simply do not work,” I said.

“No one who knows anything about viruses would try isolating a virus by the methods you’re employing.”

“But I’m not studying a virus. My results clearly support this conclusion.”

A few weeks later, David Perlman, the science writer for the *San Francisco Chronicle*, interviewed me about my work. Today, at ninety-three, David Perlman looks the same as he did when I first met him—a man of modest height and medium build. His full head of gray hair crowning his penetrating blue eyes adorned by glasses remains unchanged, as does his warm, kind, inquiring personality. David had a feeling there was a good story hiding somewhere in all these scrapie studies. I explained the scientific findings but continued to keep my new term to
Figure 25  With George Carlson (top left), we mated ILnJ mice that had long prion incubation times with short-incubation-time NZW mice. All the \((NZW \times ILnJ)\) F1 offspring of this cross had long incubation times similar to those of the ILnJ parent. To determine whether the long incubation times in F1 hybrid mice were due to the effects of a single prion incubation time gene, we mated the F1 hybrids to short-incubation-time NZW mice. Approximately half of the offspring of this backcross had short incubation times and the other half had long ones, compatible with the major effect of a single gene. Strikingly, fifteen mice that inherited the NZW allele of the PrP gene \((a/a)\) from their F1 parent had short incubation times (upper graph), while fifty-one mice that inherited the ILnJ allele \((a/b)\) had long incubation times (lower graph). Later, David Westaway (top right) found that the PrP genes of NZW and ILnJ mice encoded proteins that differed by two amino acids.
“Well, that’s one way to look at it, but you still needed to do all the protein chemistry, molecular biology, and genetics you did,” said Setlow. “Without those studies, no one would have believed that prions exist.”

Interlude: The Key to Interpreting the Trypsin Inactivation Curve

Proteins absorb ultraviolet light maximally at 280 nanometers, due to their aromatic amino acids, such as tyrosine and tryptophan, which absorb light at 280 nm. But the absorption spectrum doesn’t always correspond to the inactivation profile. When a protein has cysteine residues, the absorption and
beit at a much lower frequency, with nonmutant precursor proteins. For example, everyone harboring a mutant PrP gene will develop familial CJD if he or she lives long enough, but only one in a million with wild-type PrP will develop sporadic CJD. Importantly, many of the mutant proteins...
brain. Braak also reported the spread of Lewy bodies containing the α-synuclein prion beginning in the gut and spreading backwards up into the brain. Long before Braak’s seminal studies, Bill Hadlow and others described the migration of the scrapie agent along neuronal pathways in sheep and goats, beginning in the gut and traveling up to the brain. Studies with Tg mice have likewise demonstrated the spreading of tau aggregates along neural pathways.

Fungal prions provide an important perspective from which to better understand Aβ, tau, and α-synuclein prions. Reed Wickner, a thoughtful, intense, and prudent man working at the NIH, discovered fungal prions through his studies in yeast; his insights have been invaluable in defining the widening spectrum of mammalian prions. The yeast prions are not infectious in the sense of being released into the culture medium and entering other yeast cells, but they are transmissible from mother to daughter cells and thus readily multiply. A segment of most yeast prion proteins is rich in the amino acids glutamine and asparagine,

Figure 34  Spread of Aβ plaques and neurofibrillary tangles in the brains of patients with Alzheimer’s disease.