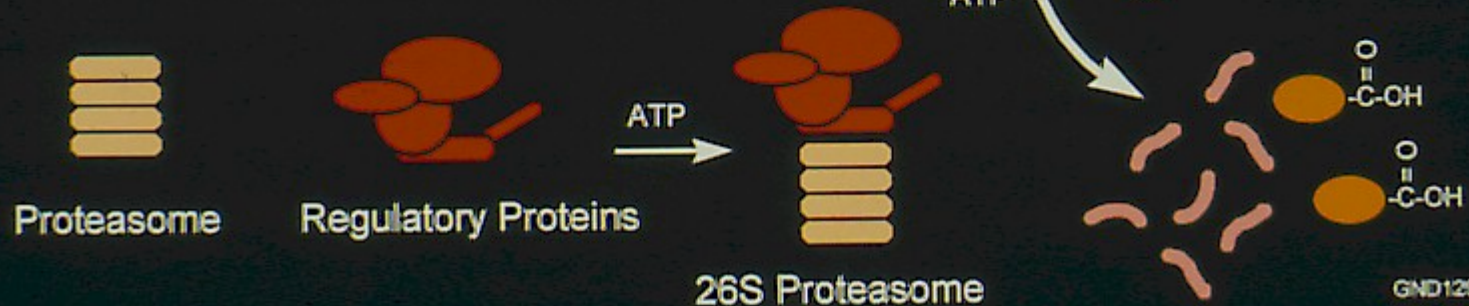


# The ubiquitin-proteasome pathway of intracellular protein degradation

## Modification of Proteins with Ubiquitin



## Degradation of Ubiquitinated Proteins

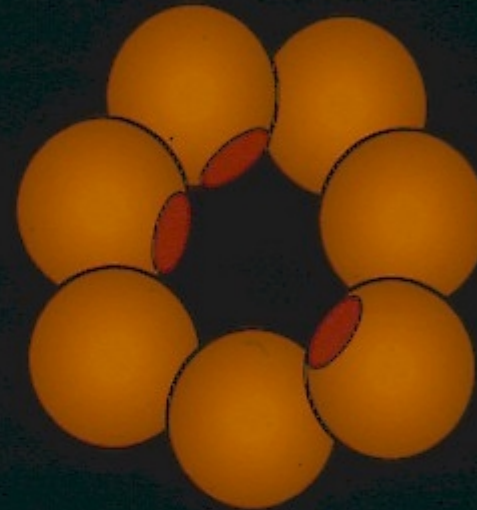
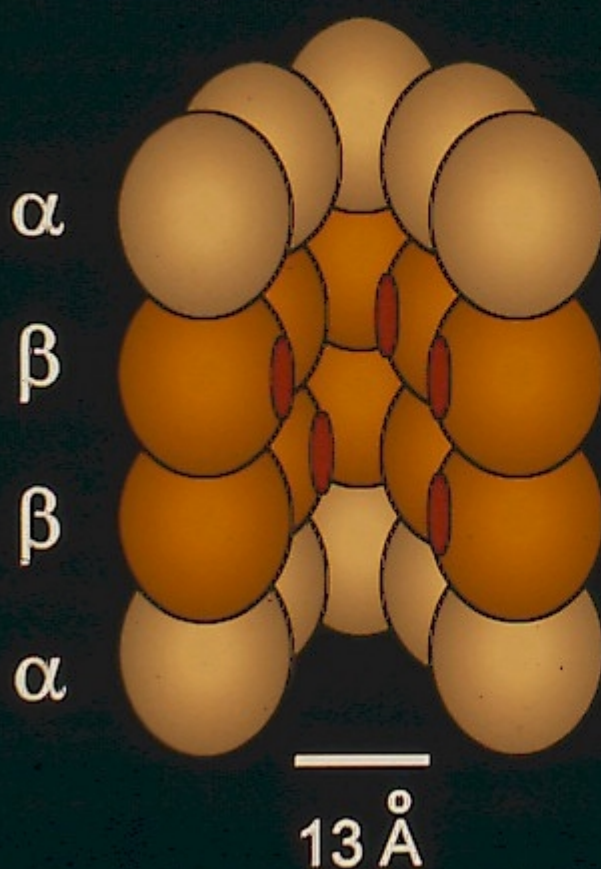


# The Proteasome

- ▶ **Distribution**
  - archeobacteria to humans
  - all cells and tissues
  - cytoplasm and nucleus
- ▶ **Structural Properties**
  - $M_r = 700,000$
  - 24-28 subunits ( $M_{rs} = 20,000-35,000$ )
  - cylinder-shaped particle
  - 13 novel, homologous gene products
- ▶ **Catalytic Properties**
  - degrades proteins and peptides
  - broad specificity
  - multicatalytic (3-5 distinct sites)
  - channeling
  - latent and active forms



## *Topology of proteasome active sites*



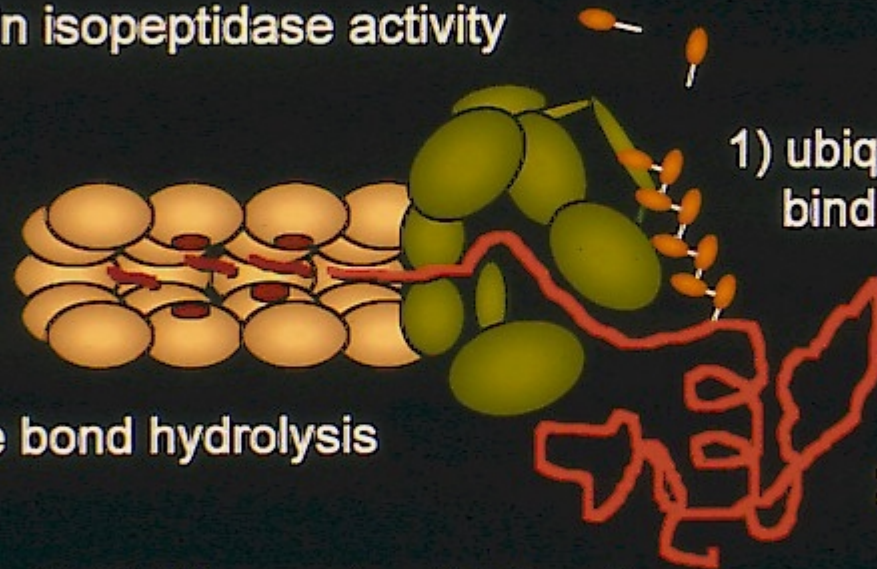
The proteasome's multiple catalytic sites are located on the  $\beta$  subunits and face the interior channel





## Functions of the proteasome-PA700 complex

6) ubiquitin isopeptidase activity



1) ubiquitin chain binding

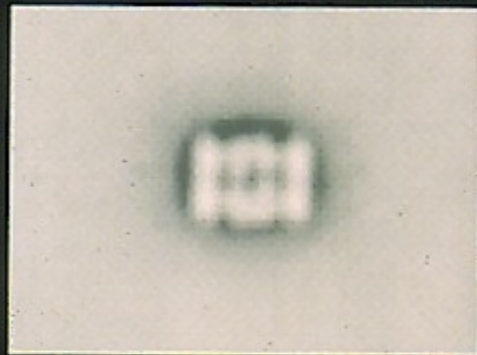
5) peptide bond hydrolysis

2) ATP hydrolysis

3) Folding and unfolding ?

4) translocation of polypeptide chain

## *Electron microscopy of proteasome- PA700 complexes*



Proteasome

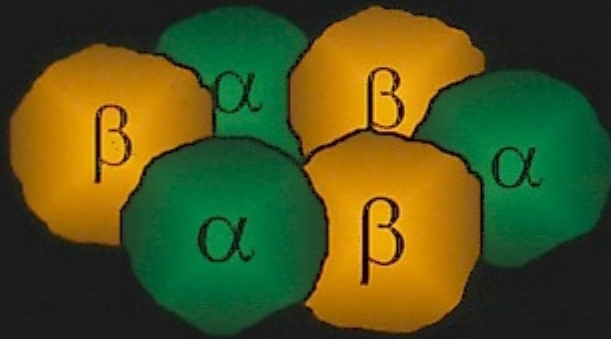


Proteasome -PA700

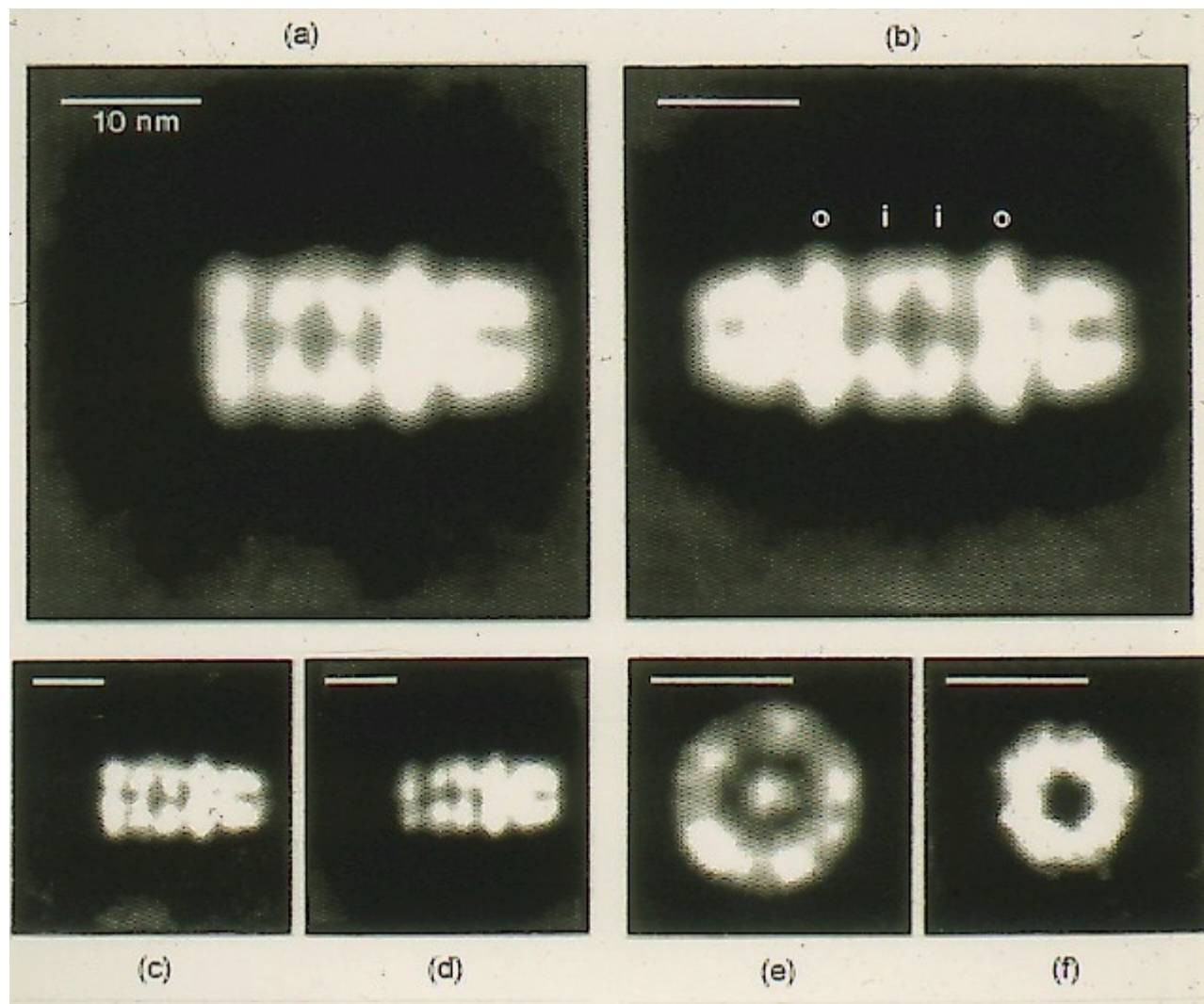




## *PA28 is a ring-shaped heterohexamer*

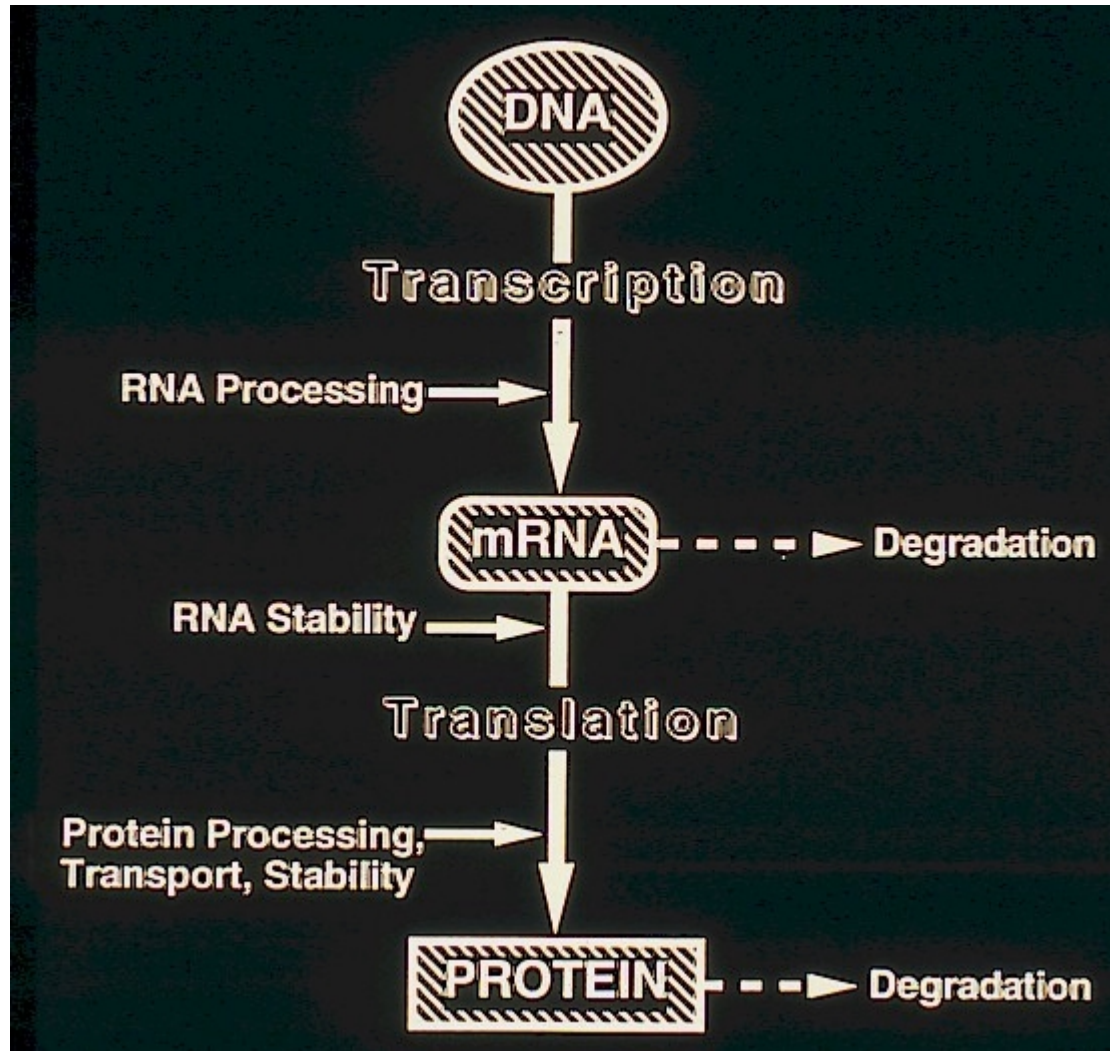


- $\alpha$  and  $\beta$  subunits are co-immunoprecipitated with subunit-specific antibodies
- $\alpha$  and  $\beta$  subunits are present in 1:1 molar ratios in purified PA28 and in cell extracts
- $\alpha$  and  $\beta$  subunits can be chemically cross-linked

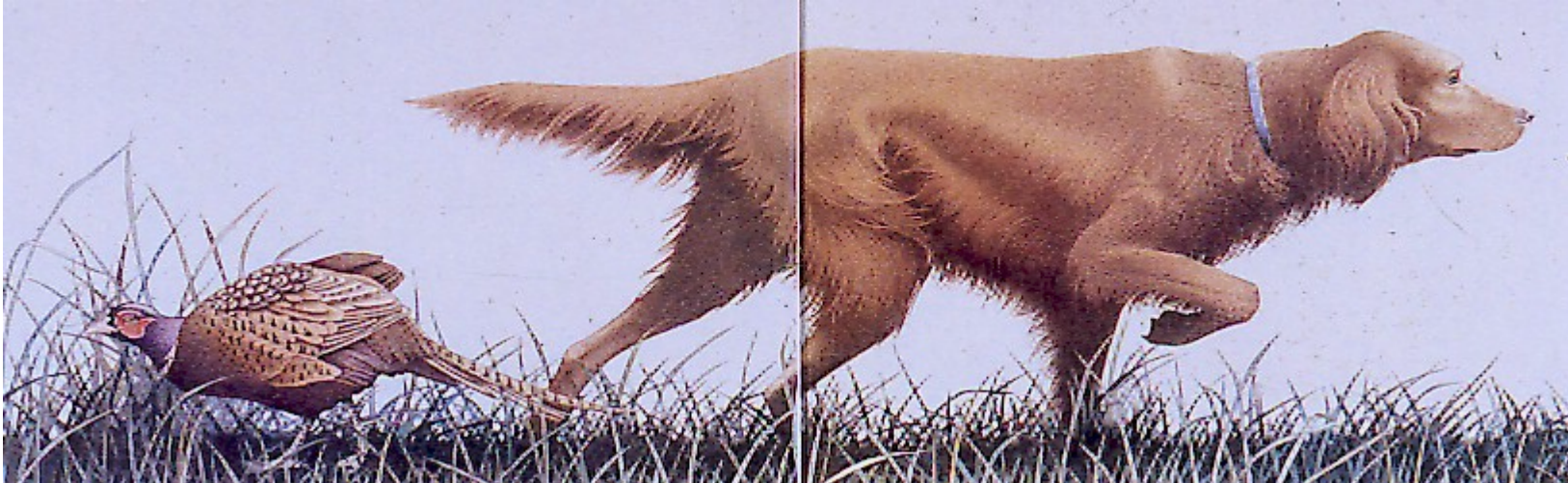


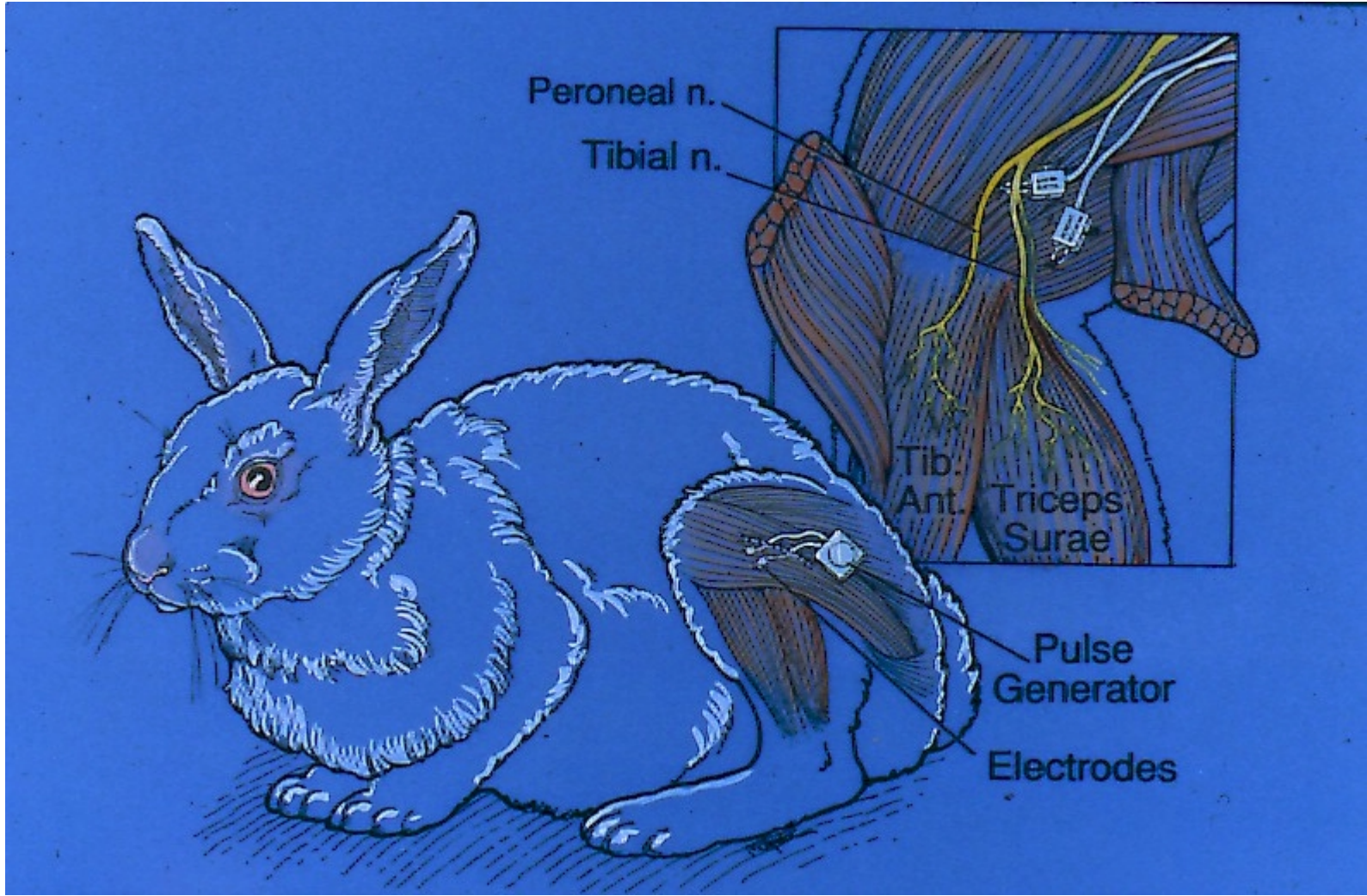




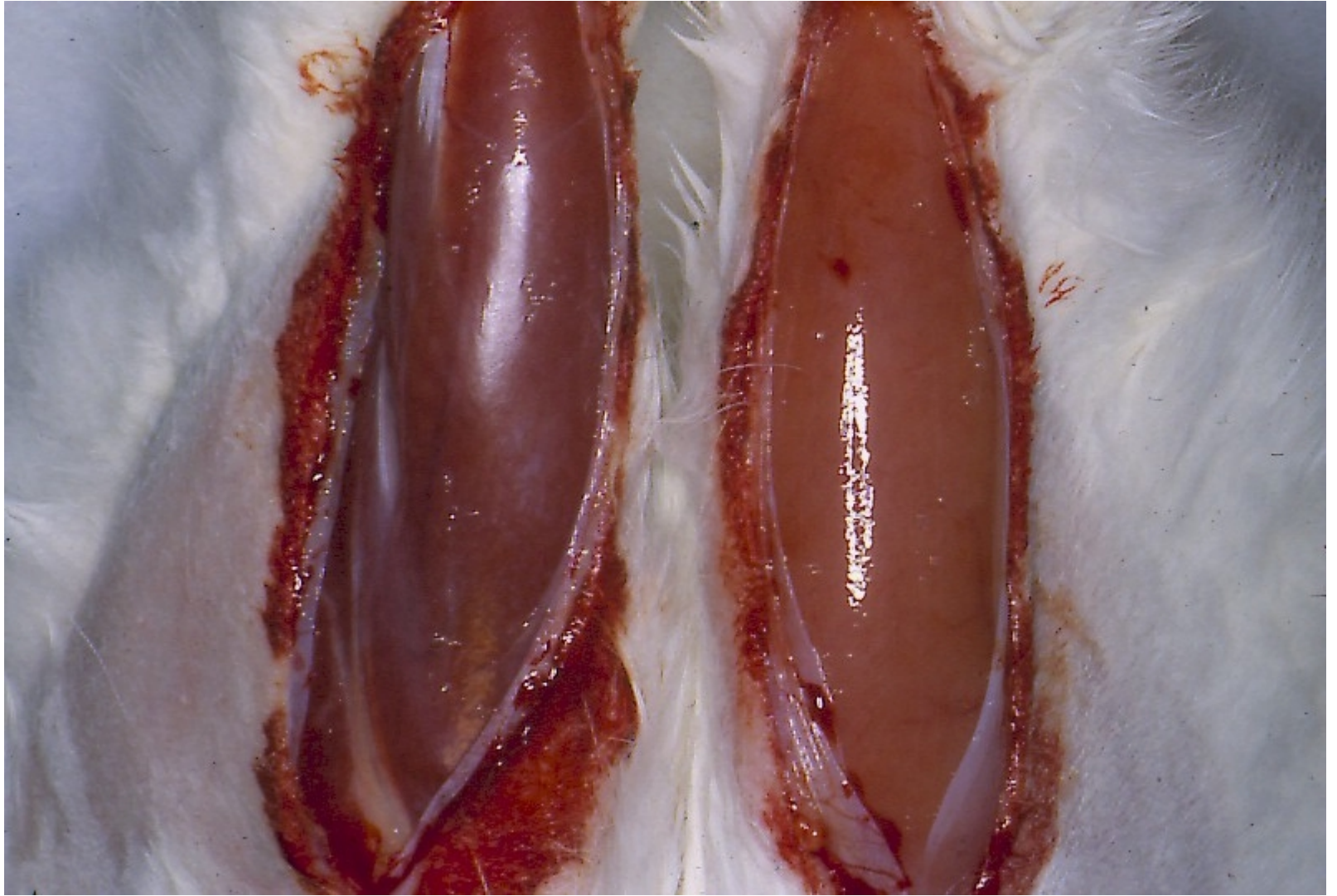












Control

1d Stim

3d Stim

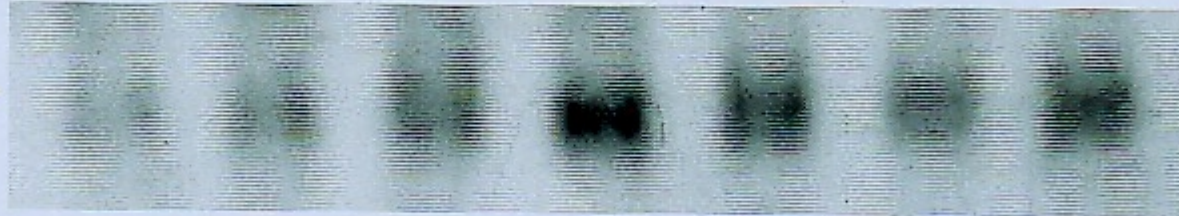
7d Stim

14d Stim

21d Stim

28d Stim

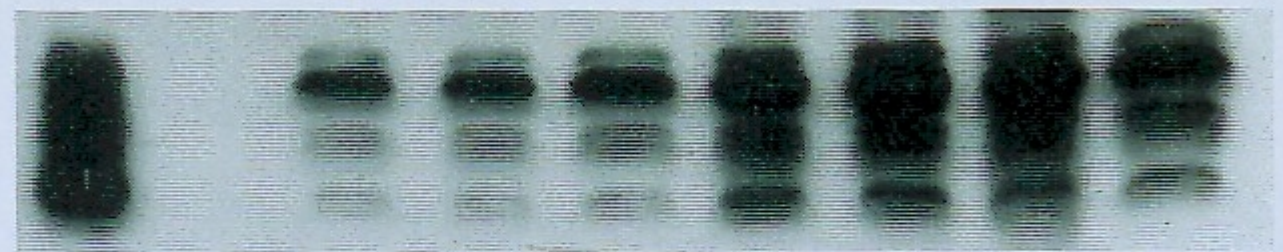
1.35 kb —





Proteasome

Control 1d Stim 3d Stim 7d Stim 14 d Stim 21d Stim 28d Stim



P31



PA28



20S Proteasome +

Control

1d Stim

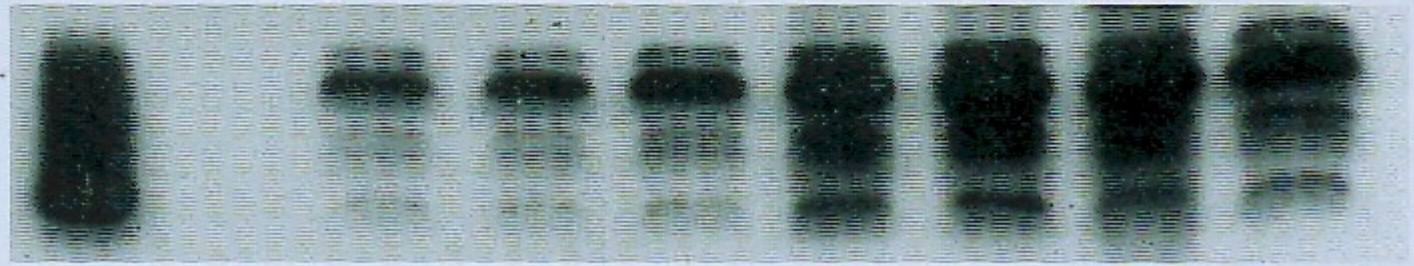
3d Stim

7d Stim

14d Stim

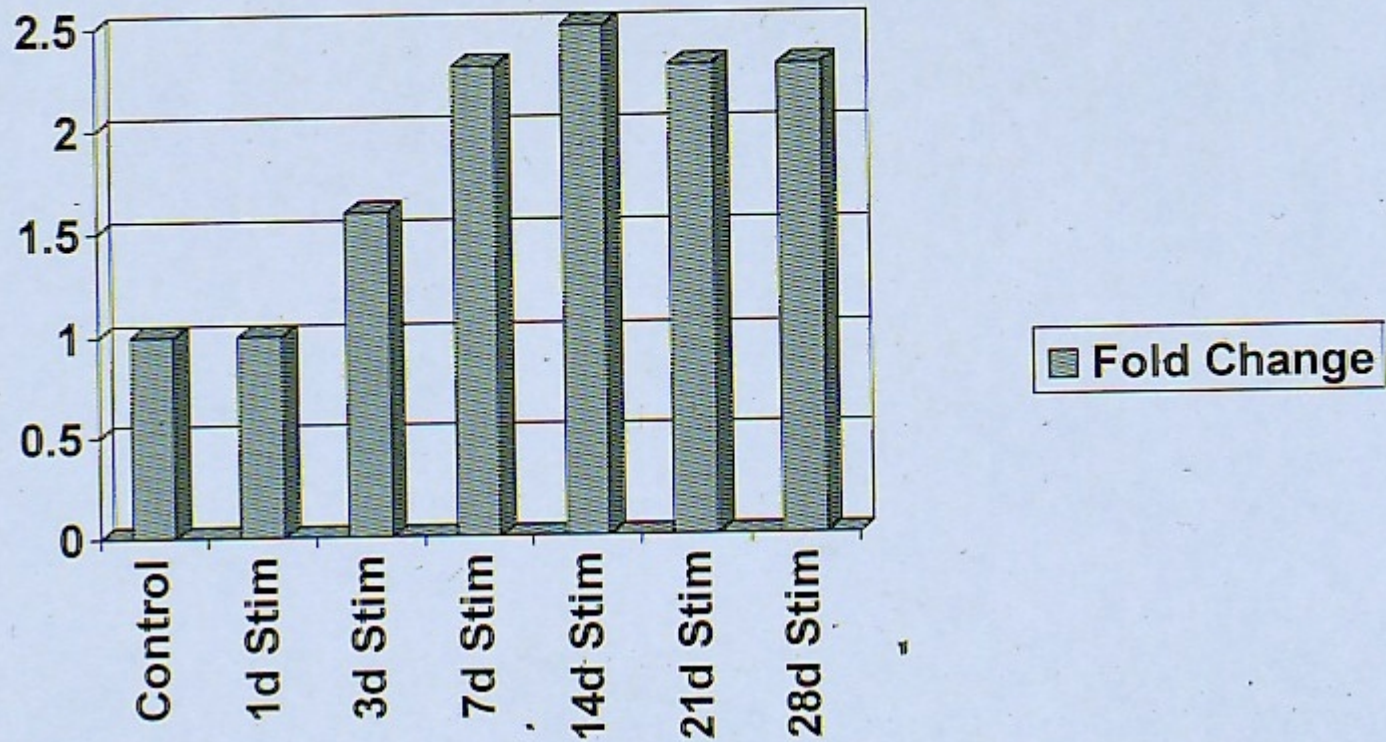
21d Stim

28d Stim



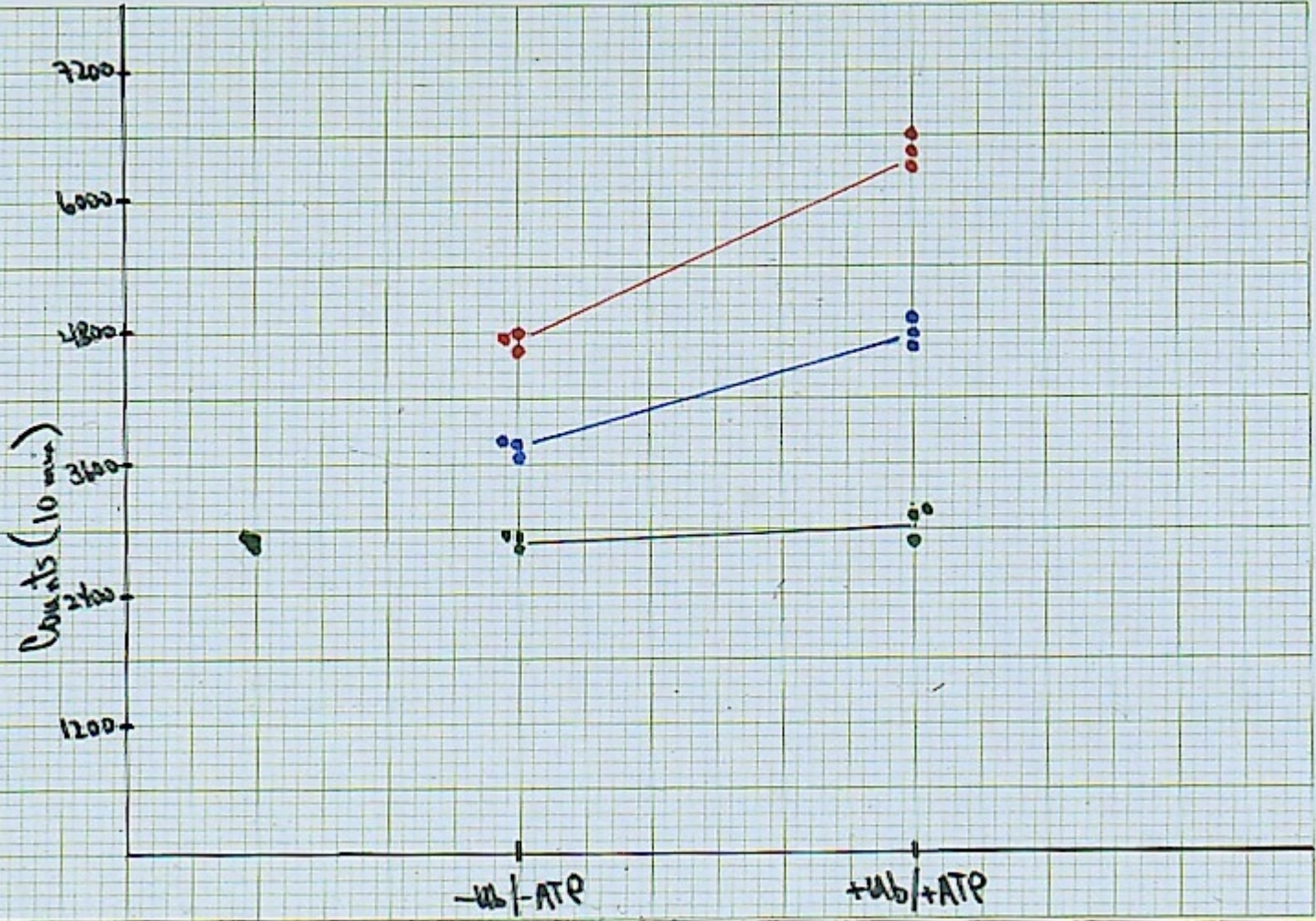


# Proteasome Specific Activity



<sup>14</sup>C-Casein Assays 1-8-99

45 min incubation







*"His final wish was that all his medical bills be paid promptly."*