# Lewis Thomas and droopy rabbit ears

**In 1956, Lewis Thomas discovered that injecting rabbits with a plant protease called papain caused their ears to collapse. This experiment led to the identification of the endogenous proteases that cause the tissue destruction seen in diseases such as rheumatoid arthritis.**

Lewis Thomas

Tissue injury can occur when the immune system gets over-activated. The first clues to the cause of this immune-mediated damage came in the early 1950s from studies of rheumatic fever—a complication of *Streptococcal* infections that results in tissue destruction in the heart, skin, and joints.

Heart lesions typical of rheumatic fever could be induced in experimental animals injected with bacterial proteases (1). The results, however, were difficult to reproduce. One scientist who had run into this problem was Lewis Thomas. Thomas had begun studying rheumatic fever as he awaited discharge from a naval research unit on Guam after the Second World War. He attributed the inconsistencies to varying concentrations of bacterial products in each preparation. In 1954, while working in his own lab at New York University, Thomas came up with another way to find out whether proteases really caused tissue injury.

## An amusing discovery

Thomas reasoned that if bacterial proteases could cause tissue destruction, injecting animals with any known protease should recapitulate the symptoms of rheumatic fever.

He injected rabbits with readily available proteases such as trypsin, ficin, and papain—an enzyme derived from the papaya plant. To his disappointment, the proteases did not produce lesions.

Papain, however, caught his attention when it induced "a bizarre cosmetic change" (2). Within a few hours of papain injection, the rabbits' normally stiff and upright ears drooped.

When he examined the papain-injected ears, Thomas realized that much of the cartilage matrix had disappeared. The cartilage reappeared after three or four days and straightened out the ears, which wilted again if reinjected with papain.

Thomas published these results in a 1956 *Journal of Experimental Medicine* paper that was an instant hit (3). The droopy-eared bunnies earned the attention of the popular press and were featured in *The New York Times* and *Life* magazine as an amusing side-effect of a research project. Thomas, however, was more excited by the implications for human disease. He had found that papain also attacked cartilage in bones and joints in the rabbits.

## The cortisone connection

Thomas had been interested in the newly available antiinflammatory drug cortisone, which he believed would cure the joint inflammation and destruction seen in rheumatoid arthritis. His early experiments with the drug, however, produced confusing results. Although cortisone stopped inflammation as expected, it worsened the disease in animal models of collagenase-induced arthritis.

As papain-induced cartilage destruction resembled the damage seen in arthritis patients, Thomas tested the effect of cortisone in the papain-injected rabbits. Cortisone prolonged ear-wilting, suggesting that it prevented the repair of the cartilage matrix (3). Thomas's next set of experiments provided the clue to how cortisone worked.

## Rabbits to rheumatic disease

Having proven that a protease can induce cartilage damage, Thomas reasoned that the body normally protects itself from its own proteases by keeping them sequestered until an internal stimulus releases them. An earlier study hinted at the identity of this stimulus when it was shown that vitamin A induced cartilage loss from fetal mouse and chick bones. Noting the similarities to his own findings with papain, Thomas showed that vitamin A also wilted rabbit ears (4).

Thomas and his team speculated that vitamin A triggered the release of an endogenous protease from cartilage cells that then broke down the matrix. They later showed that proteases are normally sequestered in lysosomes, which are ruptured by vitamin A but stabilized by cortisone (5). How cortisone prolongs tissue injury yet prevents protease release was resolved when it was later shown that cortisone also prevents cartilage synthesis (6).

The endogenous proteases were later identified as metalloproteinases. These matrix-degrading enzymes are released by macrophages that have been activated by vitamin A–specific receptors or by inflammatory cytokines that are overactive in rheumatoid arthritis and emphysema (7). Who could have guessed that limp-eared rabbits would one day blaze a trail to the root cause of these debilitating diseases?

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## REFERENCES

1. Unny, S.K., and B.L. Middlebrooks. 1983. *Microbiol Rev.* 47:97–120.
2. Thomas, L. 1983. The Youngest Science. Viking Press, New York. 270 pp.
3. Thomas, L. 1956. *J. Exp. Med.* 104:245–252.
4. Thomas, L., et al. 1960. *J. Exp. Med.* 111:705–718.
5. Weissmann, G., and L. Thomas. 1963. *J. Clin. Invest.* 42:661.
6. Kyriazis, A.P., and T.T. Tsaltas. 1971. *Am. J. Pathol.* 63:149–160.
7. Weissmann, G. 2004. *Biogr. Mem. Natl. Acad. Sci.* 85:314–347.