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STEROIDS, LYSOSOMES AND SYSTEMIC LUPUS ERYTHEMATOSUS*

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RECENT studies on the effects of various agents upon the *in vivo* and *in vitro* release of enzymes from lysosomes have suggested that some of the varied clinical manifestations of connective tissue disease may result from an abnormality of these intracellular organelles. Lysosomes are a heterogeneous group of cytoplasmic granules first described by de Duve.[1] They contain a number of acid hydrolases such as acid phosphatase, DNase and RNase, beta glucuronidase, and acid protease(s). Following certain forms of injury to cells, these enzymes apparently are released into the cell sap or surrounding fluids where they are free to act upon their appropriate substrates, most of which are constituents of the organism's tissues (Figure 1). These functions have led to a view of the particles as potential "suicide bags".[1]

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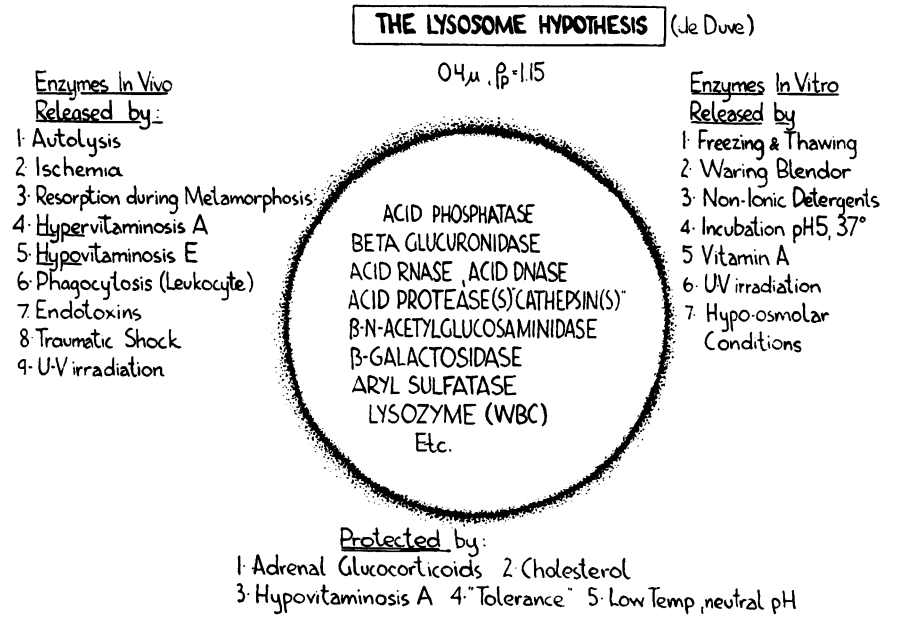


Fig. 1. Schematic representation of the lysosome hypothesis. The various agents known to effect this particle *in vivo* and *in vitro* are listed above. Modified from de Duve[1] by data from references 7, 8, 17, 18 and others.

The Effects of Endogenous and Exogenous Proteases on Connective Tissue

In 1956 it was demonstrated that the injection of an exogenous protease, papain, produced collapse of the ears of young rabbits, associated with a marked depletion of metachromatically-staining cartilage matrix.[2] Subsequently, this phenomenon was shown to result from degradation of the protein-polysaccharide complex of cartilage matrix, with release of a readily diffusible chondroitin sulfate into the circulation.[3] Fell and Mellanby[4] had found a remarkably similar depletion of matrix when cartilagenous avian bone rudiments were grown in organ culture and exposed to an excess of vitamin A. Further studies carried out at the Strangeways Research Laboratory in Cambridge, and in this department, demonstrated that hypervitaminosis A in rabbits produced changes in cartilage resembling those produced by papain,[5] and papain protease added to limb-bone rudiments *in vitro* produced a depletion of cartilage matrix very much like that effected by an excess of vitamin A.[6]

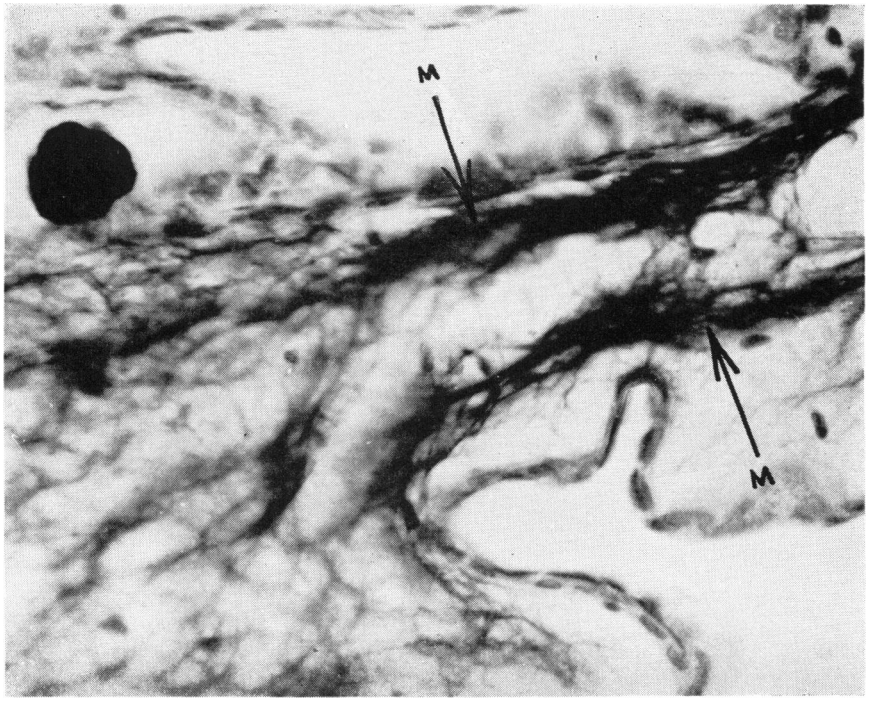


Fig. 2. Tail of larva of *Xenopus laevis*. "M" represents strands of ground substance, which stains metachromatically. Dark spot is melanophore. Toluidine blue. (Reduced 35% from an original magnification of 780.)

Lucy, Dingle and Fell[7] were able to show that an excess of vitamin A caused the release of an endogenous protease, most active at an acid pH, from avian cartilage *in vitro*. Dingle[8] speculated that since the acid proteases of most cells were contained within lysosomes, the vitamin might act directly upon these cytoplasmic organelles to release proteolytic activity. Consequently, he isolated subcellular fractions rich in lysosomes from homogenates of rat liver prepared in 0.25M sucrose, and demonstrated a temperature and pH-dependent release of acid protease activity from such suspensions when exposed to vitamin A alcohol or acid.

Additional *in vivo* confirmation of an effect of hypervitaminosis A obtained on lysosomes was in another species.[9] Prior to metamorphosis, the larvae of *Xenopus laevis* have a remarkable increase in the catheptic activity of their resorbing tails. It was postulated that if these acid pro-

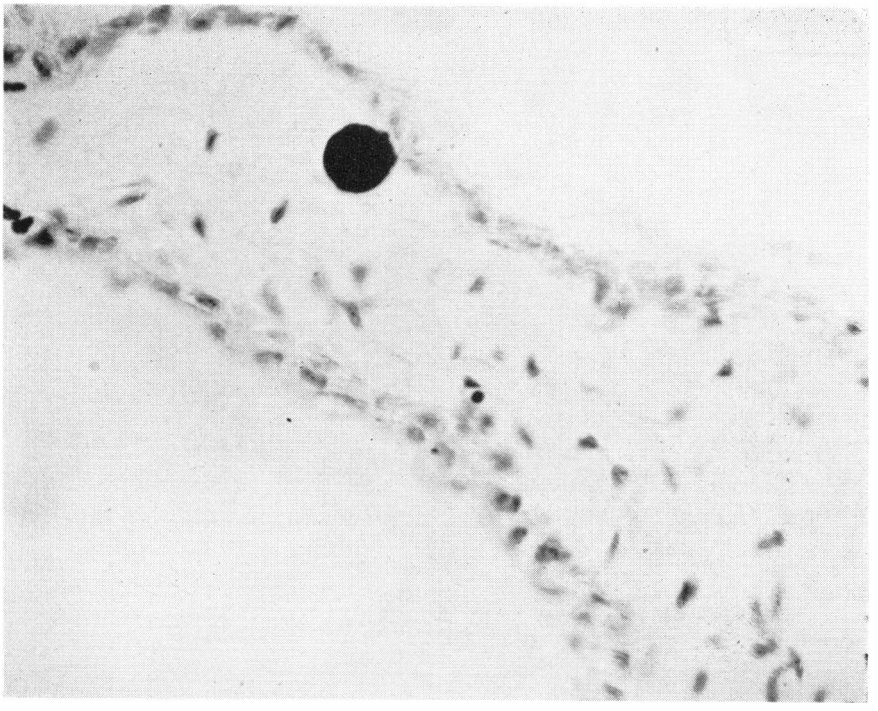
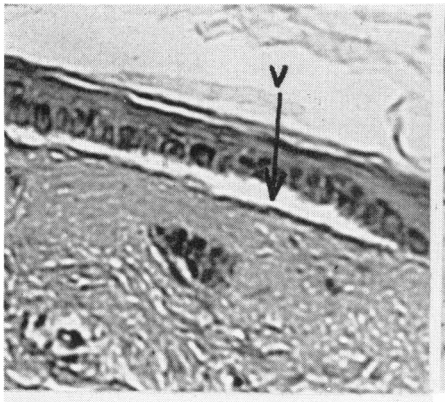
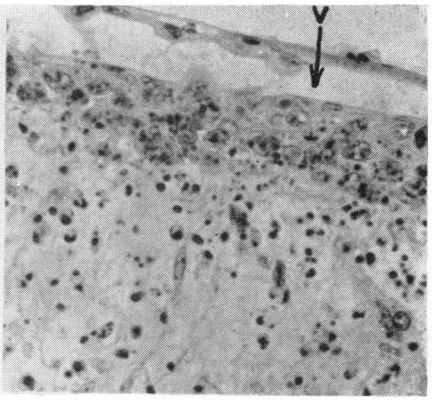


Fig. 3. Tail of larva of *Xenopus laevis* exposed for 4 weeks to an excess of vitamin A alcohol. No metachromatic material is left in the ground substance of the tail. Toluidine blue (reduced 35% from an original magnification of 780).

teases were indeed to be localized within lysosomes prior to metamorphosis, then the induction of hypervitaminosis A (with lysosomal rupture) should result in the resorption of tails *before* metamorphosis. Such an effect was readily demonstrable, and lent support to the idea that vitamin A in excess activated lysosomal enzymes not only *in vitro* but *in vivo*. One of the more dramatic consequences of tail resorption following hypervitaminosis A may be seen in Figures 2 and 3. Figure 2 shows the densely metachromatic ground substance of the amphibian tail. Figure 3 shows a similar area from an animal given vitamin A alcohol for four weeks; the ground substance was depleted of metachromasia and by this time resorption of tail substance had begun before the onset of metamorphosis.

Since damage to components of connective tissue had been associated with the liberation of an enzyme from the lysosomes, it appeared





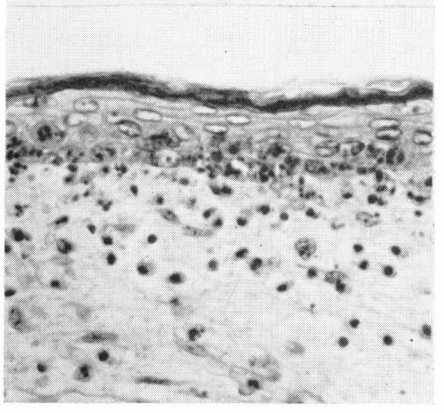


Fig. 4. (TOP LEFT) Biopsy of bulla in skin of patient with Systemic Lupus Erythematosus. Subepidermal detachment is evident. "V" represents vesication. H & E (x 250).

Fig. 5. (TOP RIGHT) Fetal rat skin grown for 2 days on normal plasma clot, then irradiated with ultraviolet light. Section taken 24 hrs. after irradiation. Subepidermal detachment is evident, "V" represents area left by vesication. Delafield's hematoxylin and chromotrope (x 300). (Experiment with Dr. H. B. Fell.)

Fig. 6. (LOWER LEFT) Fetal rat skin grown for 2 days on plasma clot containing 7.5 μg of hydrocortisone before being irradiated by ultraviolet light. Section taken 24 hrs. after irradiation. No vesication is seen, cell death is less than in Figure 4 and epidermis is left relatively intact. Delafield's hematoxylin and chromotrope (x 300). (Experiment with Dr. H. B. Fell)

possible that other manifestations of connective tissue disease could be explained by effects upon these particles. Two clinical features of systemic lupus erythematosus seemed readily amenable to experimental exploration:

1) the activation, by sunlight, of active lupus erythematosus. In such patients, giant bullae sometimes appear after exposure to sunlight.[10] Figure 4 shows such a lesion, with subepidermal detachment, some cellular necrosis, and the bulla space itself.

2) the dramatic response of systemic lupus erythematosus and related syndromes to adrenal glucocorticoids, and the equally dramatic "rebound" following withdrawal of these agents.

The Effect of Ultraviolet Irradiation on Lysosomes

In view of the activation of lupus erythematosus by sunlight, large granule fractions containing lysosomes of rat, rabbit and guinea-pig liver were exposed to the mixed beam of a mercury vapor lamp. Release not only of cathepsin[11] but of acid phosphatase, acid DNase, and beta glucuronidase have been demonstrated. This release of enzymes was shown to be temperature dependent; was negligible at 4°C, was quite low at 25°C and maximal at 37°C, suggesting that the effect was mediated through an enzyme system or systems. If such lysosomal suspensions had been frozen and thawed before irradiation, release of enzymes was no longer increased by irradiation, suggesting that the U-V effect (localized to wave lengths below 3,000 Å[12]) was on membranous organelles rather than upon soluble enzymes or their activators. But the lysosomes of liver, except for a unique case reported in the Greek literature,[13] are not usually exposed to sunlight. Therefore, in collaboration with Dr. Fell, fetal rat skin was grown on plasma clots in organ culture, and after two days, one segment was irradiated by means of a mercury arc lamp. The histologic consequences of irradiation may be seen in Figure 5. There was subepidermal detachment with some keratolysis. Much cellular necrosis was evident, and a vesicle formed which was reminiscent of the lesion seen in the skin of a patient with lupus erythematosus (Figure 4). On this and on subsequent days, when skin was maintained in culture after irradiation, the finding of cellular necrosis was associated with evident digestion of intracellular fibers and ground substance. A generally deranged supporting architecture of the skin resulted from irradiation; healing and re-epithelialization were retarded.

The Protection, by Hydrocortisone, of Lysosomes in Vivo and in Vitro

When fetal rat skin was grown in the presence of hydrocortisone in organ culture (7.5 μg./ml. of plasma clot) for two days before irradiation, it was partially protected from the effects of the mercury beam. In Figure 6 it may be seen that vesication was inhibited, less cellular necrosis was evident, and less digestion of extracellular fibers was found. Subsequent repair after radiation damage was more rapid, and prompt epithelialization was observed. Similarly, avian limb-bone rudiments were

protected against the depletion of cartilage matrix caused by an excess of vitamin A when grown in the presence of hydrocortisone.[14] It was also possible to protect articular and ear cartilage of rabbits from the depletion of matrix induced by hypervitaminosis A.[15] Another *in vivo* action of hydrocortisone could be demonstrated in *Xenopus* larvae. When hydrocortisone was given to larvae exposed to high doses of vitamin A *alcohol*, release of vitamin A from liver stores was held responsible for acceleration of damage caused by hypervitaminosis.[16] Vitamin A *acid*, however, is *not* stored in the liver. When larvae were fed vitamin A acid in excess, connective tissue damage even more drastic than that caused by the alcohol was produced, and simultaneous administration of hydrocortisone inhibited these changes significantly. After four weeks of hypervitaminosis A, all animals were dead; whereas 60 per cent of larvae given hydrocortisone concurrently with the vitamin were still alive. The densely metachromatic structure of ground substance still present in their tails was indistinguishable from controls; and cartilage also remained unaffected.

Such effects on whole tissue suggested the possibility that hydrocortisone protected lysosomes against damage by a variety of agents. Therefore hydrocortisone (50 mg./kg. for four days) was given to rats before they were killed, their livers homogenized, and the fraction richest in lysosomes was prepared. These fractions showed diminished release of acid protease *in vitro* when exposed to the mercury beam, compared to fractions prepared from control rats, indicating a protective effect of steroid pretreatment.[11] DOCA pretreatment was not effective in retarding release of acid protease from lysosomes. Preliminary studies have shown that hydrocortisone *in vitro* (1×10^{-3}M) added to normal suspensions will decrease the release of enzymes from lysosomes caused by mercury arc irradiation, and that pretreatment of animals with cortisone inhibited the release of enzymes from lysosomes by vitamin A given *in vivo*.[17] All of these findings have been interpreted to indicate that lysosomes were stabilized by glucocorticoids against injury by many toxic substances. The recent demonstration that an early effect of bacterial endotoxins was a release of acid hydrolases from granules isolated from the livers of affected animals (and that pretreatment with cortisone diminished this response) seemed to support this hypothesis.[18]

The stabilization of intracellular organelles by steroids would retard the release of potentially harmful enzymes into the cell sap or circula-

tion following several sorts of injury. Evidence for an increased lysozyme activity in the sera of patients with rheumatoid arthritis having positive tests for anti-nuclear factor was presented by Potter, Alexander and Duthie.[19, 20] These authors have shown that patients with active rheumatoid disease have a decreased ability to bind *exogenous* protease, such as papain, to those fractions of serum where normal rabbits and humans seem to bind added protease.[21] These results suggest that either such patients lack the normal binding protein, or that another, perhaps *endogenous* enzyme is already bound. The acute crises of lupus might thus arise when, due to a variety of injuries, the capacity of the carrier proteins was exceeded, and injurious enzymes could freely act upon the extracellular components of tissue.

It seems not unlikely that somewhere in the pathogenesis of systemic lupus erythematosus (and related syndromes) involvement of the lysosomes, which ordinarily contain endogenous proteases and lysozyme, may be postulated. Whether the defect will prove to be a primary one, or to be secondary to the many "auto-immune" phenomena of lupus erythematosus, cannot be discussed at present. The numerous studies which have documented immunologic abnormalities in patients with systemic lupus have unfortunately not clarified whether such features are the cause or the consequence of the disease. Inquiry into the role of lysosomes in the preparation of antigens within the reticuloendothelial cell should help to decide what part is played in some of the connective tissue diseases by these "suicide bags". Indeed, the preparation of abnormal antigens following the ante-mortem liberation of lysosomal enzymes may be a stimulus to auto-immunity.

The studies we have described would suggest a tentative explanation for the activation, by sunlight, of systemic lupus erythematosus. They also indicate that one, and perhaps a major pharmacologic effect of cortisone and its analogues in diseases of connective tissue, may be the protection of lysosomes against injury. Such an action would retard the release of potentially harmful enzymes from cells into tissue spaces where the inflammatory cycle is begun.

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