carcinogenic product. We postulate from previous[6] as well as from the present study that α-BHC induces microsomal enzymes that participate in the inhibition of 3′ me-DAB, DL-ethionine and AFB_1 carcinogenesis in a similar hepatotoxic pathway. This antagonistic effect of α-BHC on the carcinogenic activity of several chemical agents is of interest, and further investigation is needed to clarify the mechanisms involved.

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Ultrastructural differences in mitochondria of skeletal muscle in the prerigor and rigor states[1]

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Summary. Loss of cristae and matrix occur in the mitochondria of skeletal muscles prior to any observable changes in myofibrillar proteins during the development of rigor mortis. Care must be observed because ultrastructural changes in mitochondria in some studies may be attributed to a specific trauma, whereas the changes may be due to the lower pH in postmortem muscle.

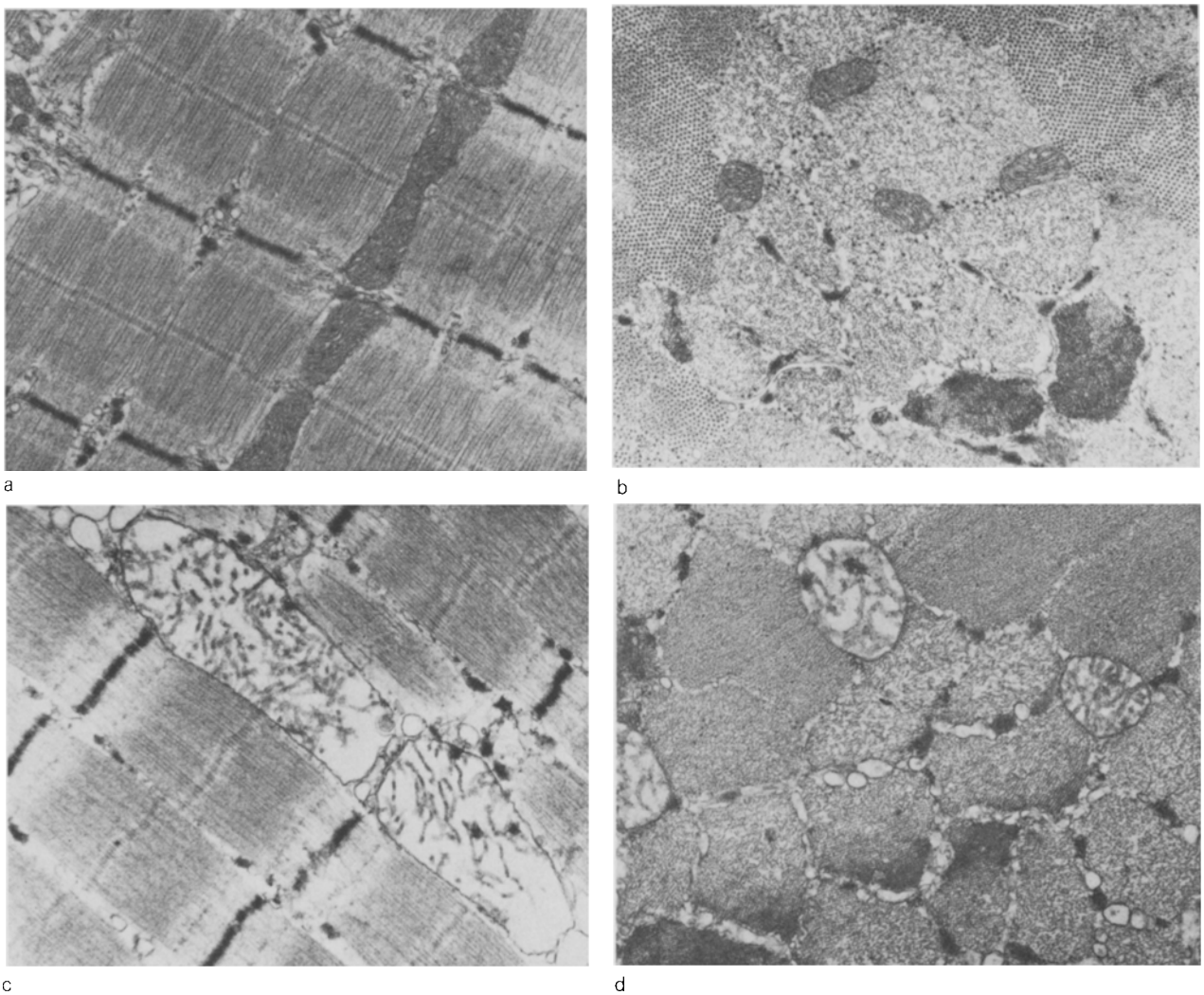
Changes in the size and ultrastructural components of mitochondria have been observed in a variety of metabolic, developmental and pathological states. Of particular interest is the effect of various stresses that produce atrophy or degeneration of the mitochondria. These stresses would include: nutritional[2-4], tenotomy[5] and denervation[6] of skeletal muscles, malignancy[7], ecdysis[8], aging[9,10] and in ischaemic[11,12], degenerating[13] and autolysing[14] cardiac muscle cells. The tissues in the above studies, though undergoing atrophy, do not present a uniform pattern of changes in mitochondrial ultrastructure. For example, damage to the myofibrillar components, especially the I-band and Z-line, occurred before mitochondrial damage[11,12] in ischaemic dog myocardium. However, in degenerating cardiac muscle cells from humans with cardiac hypertrophy, some severely degenerated cells had few or no myofibrils, but these cells were virtually filled with intact mitochondria[13]. Proposed mechanisms for the degradation of the mitochondria vary. Mitochondrial disintegration in sperm after fertilization has been described as a process of self-autolysis[15]. An explanation for the increased membrane junctions in mitochondria from heart tissue that were stored after isolation was attributed to a lowering in pH[16]. Lower pH-values would be expected for stored tissue because the cells become anaerobic with time, and glycolysis would produce hydrogen ions. The effect of lowering pH on mitochondrial ultrastructure has been demonstrated recently in autolysing dog heart muscle[14]. pH-values 6.0–6.2 at 3 h postmortem produced marked mitochondrial swelling, loss of matrix density and disorganization of the cristae[14]. Some skeletal muscles may attain the ultimate pH (approximately 5.6) very rapidly, depending on the extent of physical activity of the muscle before death[17]. Therefore, it is of practical significance to determine the effect of postmortem changes on the ultrastructure of mitochondria from skeletal muscles lest ultrastructural changes in some studies are attributed to effects other than the postmortem effect produced by the lower pH-values.

Methods. 3 adult turkeys were killed by exsanguination. The semitendinosus muscle was removed immediately postmortem and processed for electron microscopy by a procedure outlined elsewhere[18]. The semitendinosus muscle on the other limb was allowed to enter rigor mortis at 20 °C. Rigor mortis, based on ATP, pH and response to electrical stimulation, occurs after 6–8 h postmortem. The semitendinosus muscle of the turkey was chosen because it is a red muscle[19], and therefore, high in mitochondrial density.

Results. Normal mitochondria in prerigor muscle can be observed in longitudinal and transverse sections (figure, a, b). After rigor development, the matrix has disappeared, and intramitochondrial dense inclusions are observed (figure, c, d). These dense inclusions develop after relatively small changes in pH, and before lactate ions are present in sufficient quantity to exert their swelling effect on the mitochondria[14]. The pH of the skeletal muscles in rigor mortis in this study was 5.5–5.7. The observed effect in the figure, a–d, was uniform in all sections examined. Therefore, the effect of low pH may be a factor in gross alteration in mitochondrial ultrastructure. However, a perusal of electron micrographs in our laboratory indicated that similar ultrastructural changes occurred in the biceps brachii muscle of the mouse[20]. The ultimate pH of this muscle in rigor mortis is about 6.4[21]. It would be wrong to infer from these statements that pH decrease, per se, is the sole factor responsible for the ultrastructural changes observed in the muscles. Many biochemical changes occur in postmortem skeletal muscles[17]. An interesting mechanism of muscle necrosis in various muscle diseases has been proposed recently, and similar events may occur in postmortem muscle. This involves an increased net influx of calcium into cells which triggers a 'vicious cycle' of mitochondrial calcium overloading, energy depletion and structural damage to the mitochondria.

It should be noted that the weakest component in the myofibrillar ultrastructure is at the Z-line-I-band junction[22]. The Z-line is the first myofibrillar component to show signs of destruction in the postmortem degradation of skeletal muscle. The electron micrographs in the figure, c–d, indicate that the mitochondrial changes occurred prior to any significant myofibrillar degradation.

It is apparent, therefore, that structural changes occur in the mitochondria of postmortem skeletal muscles prior to any observable changes in the myofibrillar proteins. Concomitant with these structural changes is a decrease in muscle



a Longitudinal (× 15,900) and *b* transverse (× 22,200) sections of semitendinosus muscle of turkey immediately postmortem. Note the intact mitochondria. *c* Longitudinal (× 18,750) and *d* transverse (× 27,400) sections of the same muscle in rigor mortis. Note the lack of cristae and matrix and the occurrence of intramitochondrial dense inclusions.

pH. If there is a direct relationship between pH-values and mitochondrial damage, then this observation is of significance in morphological studies because a pH-decline has been observed in a great variety of cells as a result of injury[23]. Therefore, muscle samples must be fixed immediately postmortem, and the sample size of the muscle and penetration rate of the fixative should allow all mitochondria to be fixed before the pH of the muscle falls significantly. Otherwise, the abnormal appearance of the mitochondria may be due to an artefact caused by the decline in pH of the muscle.

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