Intramitochondrial Bodies
in Bovine Adrenocortical Cells*

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Summary. Electron dense, homogeneous, mostly round intramitochondrial bodies were found in bovine adrenal glands, predominantly in the zona glomerulosa. The histochemical results obtained suggest that these bodies might contain a considerable amount of protein, although the possibility that substances other than proteins are contained in these bodies cannot be completely ruled out. The physiological significance of the intramitochondrial bodies remains unclear.

Key words: Adrenal cortex, bovine – Intramitochondrial bodies – Electron microscopy – Histochemistry.

Introduction

In light microscopical studies of bovine adrenal cortex, “cytoplasmic” granules were observed and termed “azocarmine granules” (Nicander, 1952), “giant granules” (Schultz and Meyer, 1958) and “eosinophil granules” (Yamauchi, 1965) by different authors. Later, electron microscopical studies revealed that these granules were identical to the inclusion bodies within mitochondria (Weber et al., 1962; Luthman, 1971).

Furthermore, numerous reports have appeared on intramitochondrial inclusions in many different animal cells which are generally classified as either granular, tubular or filamentous (see references in Munn, 1974). Very large single globules filling nearly the whole of the mitochondrial matrix have also been reported in various steroid hormone-secreting cells. However, relatively little

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information is available regarding their chemical composition. The nature of inclusion bodies found in various steroid hormone-secreting cells is generally believed to be lipoidal due to their appearance in osmium tetroxide-fixed material without histochemical analysis. Only Luthman (1971) suggested the existence of proteins in the intramitochondrial bodies. To our knowledge, no one has as yet definitely demonstrated the existence of proteins in the intramitochondrial bodies histochemically.

In the course of an investigation to clarify the general structure of the bovine adrenal gland, the authors have found that the intramitochondrial bodies do not possess lipids, as widely accepted, but rather proteins. The present study represents a re-examination of the ultrastructural morphology of the intramitochondrial bodies, in addition to a histochemical analysis.

**Materials and Methods**

Adrenal glands utilized in this study were collected from one bull, four heifers, six calves, 11 adult cows and 14 steers. Most material was obtained from slaughtered animals as soon as they reached the cutting floor at a local abattoir (30–40 minutes after death). 2–3 mm slices of the midportion of each gland were placed in Baker's formol-calcium, Bouin's, Regaud's, or Carnoy's fixatives. Paraffin embedded sections were stained with hematoxylin-eosin, Heidenhain's iron hematoxylin, Heidenhain's azan, Goldner's modification of Masson's trichrome, or Cleveland and Wolfe's method. Other histochemical tests carried out on paraffin or frozen sections are given in Table 1.

In addition, adrenal glands were processed for electron microscopical studies. Small pieces of the organs were fixed at 4°C in 4% paraformaldehyde-2.5% glutaraldehyde buffered with phosphate, pH 7.4, for two hours and then postfixed in 1% buffered osmium tetroxide solution for two hours at the same temperature. After dehydration in a series of graded ethanol, the specimens were embedded in Epon 812. Thick sections stained with toluidine blue were examined with the light microscope to ascertain the appropriate sites of the adrenal cortex. Thin sections were stained with uranyl acetate and a mixture of lead nitrate-lead acetate-lead citrate (Sato, 1968) and examined in a JEM T7S electron microscope.

**Results**

Light microscopically, “cytoplasmic” granules in adrenals of all animals studied were clearly demonstrated by the histological staining methods employed (Fig. 1). The granules were eosinophilic, carminophilic, fuchsinophilic, siderophilic and also selectively stained with orange G. The occurrence, amount and distribution of these granules varied in the different animals. When a large amount of the granules occurred in the gland, they could be found throughout the entire cortex, but tended to be much more numerous in the zona glomerulosa than in the zona fasciculata and zona reticularis (Figs. 2–4). If the total quantity of the granules was apparently decreased, the major site of occurrence was confined to the zona glomerulosa, but occasionally the granules were sporadically distributed in the zona fasciculata and zona reticularis. No correlation between the presence, location and quantity of the “cytoplasmic” granules to sex or age of the animals could be found, after thorough consideration of these relationships was made.

Electron microscopical studies revealed that mitochondria containing very electron dense, homogeneous, mostly round inclusion bodies were encountered throughout the entire cortex, although the zona glomerulosa generally showed the