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Amitosis in human adrenal cells

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Summary. Adrenal pieces obtained from 3 female and 2 male patients showed morphological figures of amitosis in adrenal zona reticularis cells. Such aspects were observed in both normal and hyperactive adrenals.

Nuclei appeared constricted, heavily stained, with coarse chromatin, sometimes scattered among cytoplasmic organelles, but never marginating in crescentic caps.

Cleavage of the cells originated two halves with a nucleolus in each pole. Binucleated cells were also seen in zona reticularis.

The meaning of amitosis in human adrenal is discussed.

Key words: Human adrenal, Amitosis, Ultrastructure

Introduction

In recent years little attention has been paid to the biological meaning of adrenal cell division.

Lately, we have had the opportunity to study the adrenal cortex from a woman who underwent adrenalectomy as therapy for virilization syndrome. We have found a special type of nuclear morphology which seemed to be figures of amitosis (Prenant et al., 1904; Bucher, 1947, 1962, 1963; Obré et al., 1966; Pessacq, 1969; Tippit and Pickett-Heaps, 1976; Ferguson and Palm, 1976; Bargmann, 1977; Tucker et al., 1980; Chen and Wan, 1986). Such peculiar aspects were further seen in human adrenal of two male patients who underwent unilateral adrenalectomy as therapy for Buerger's disease and in two female patients with acrocyanosis. Some authors deny the occurrence of amitosis in mammals (Grynfeltt, 1932; Oberling and Bernhard, 1961; Bucher, 1962, 1963), whereas others consider that it is the only type of nuclear division in adult normal liver (cryptomitosis) (Elias and Hyde, 1982). Yet, for other authors, amitosis is only observed in lower invertebrates or under pathological conditions (Yiquan and Binkung, 1986). This may be the reason why there has been a decrease in amitosis research (Yinquan and Binkung, 1986).

The purpose of the present report is to describe morphological adrenal nuclear aspects, not yet observed in human adrenal, and to discuss their possible functional significance.

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Materials and methods

Human adrenal tissue was obtained from two male patients aged 23 and 48 and three female patients aged 18, 19 and 21. The male patients suffered from Buerger's disease, the two younger women from acrocyanosis and the older one from a virilization syndrome. The patients received thiopentone (Pentothal), succinylcholine (Scoline), dially-nor-toxiferine (Alloferine), and pethidine as anaesthetic drugs. Patients underwent unilateral adrenalectomy as therapy for the disease. Immediately after removal, adrenals were cut into small pieces and immersed in Bouin's solution for light microscopy and 2.5% glutaraldehyde in 0.1 M cacodylate buffer for electron microscopy; the fragments fixed in glutaraldehyde were postfixed in 1% osmium tetroxide in veronal-acetate buffer. After dehydration in a graded series of ethanols, the specimens were embedded, respectively, in paraffin and Epon 812.

Semithin sections, 1 or 2 μm thick were stained with methyleneblue-azur II (Richardson et al., 1960).

Ultrathin sections were double stained with uranyl acetate and lead citrate and the specimens were observed in a Jeol 100 B electron microscope.

Results

By light microscopy, the adrenal cortex looked normal in both male patients and in the two youngest women suffering from acrocyanosis (Fig. 1). In the

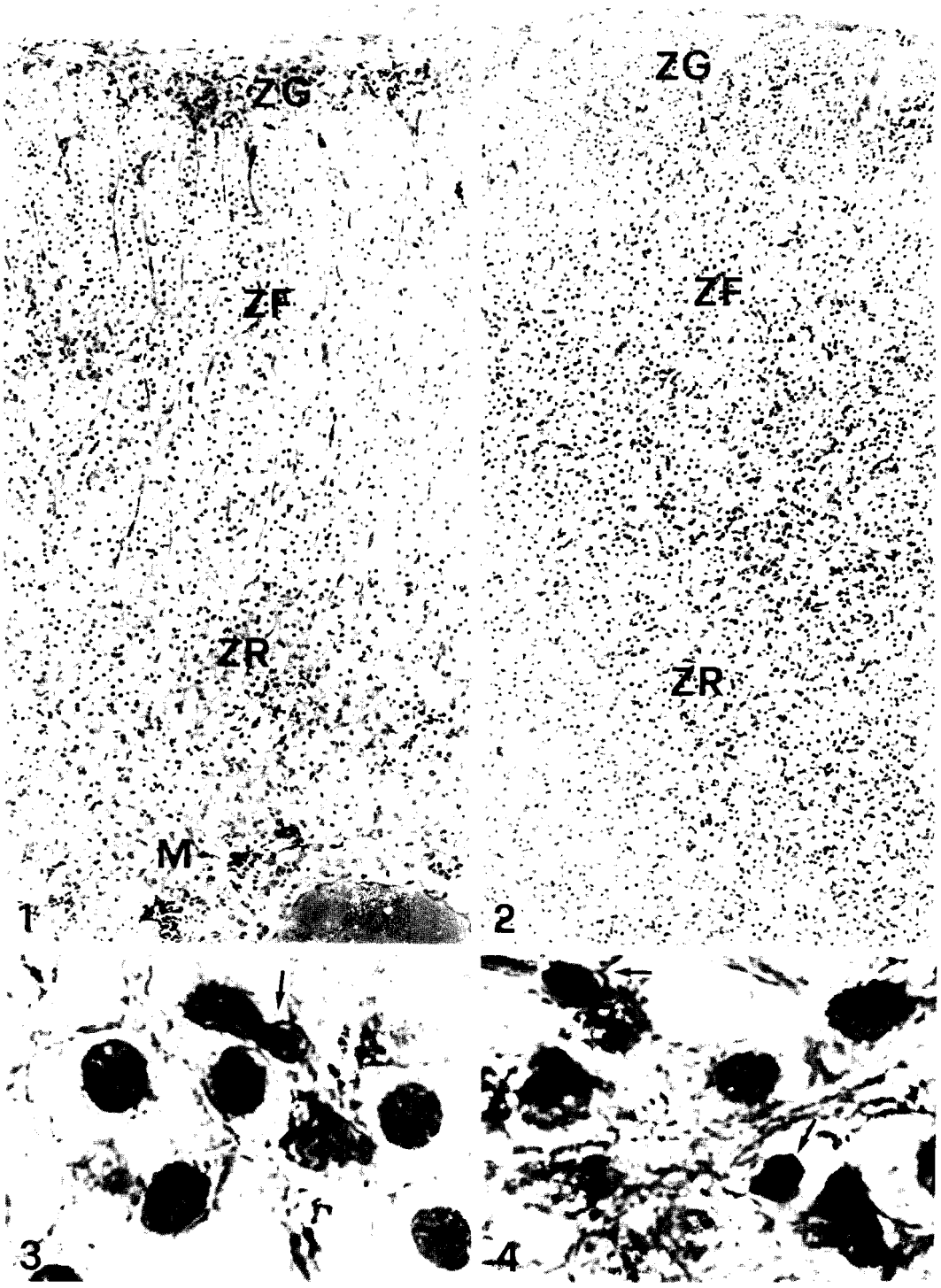

Fig. 1. Adrenal cortex from a woman aged 18. Adrenal cortex looks normal. A small area of adrenal medulla (M) is present. (H + E) × 360. ZG - zona glomerulosa; ZF - zona fasciculata; ZR - zona reticularis.

Fig. 2. Adrenal gland from a woman aged 21 with virilization syndrome. Hypertrophy of zona reticularis (ZR) is evident. (H + E) × 240

Fig. 3. Zona reticularis. Constricted nucleus (arrow). (H + E) × 1,470.

Fig. 4. Apoptosis. Note heavily stained nuclei (arrows). (H + E) × 1,470.

woman with virilization syndrome, the zona reticularis seemed larger (Fig. 2).

In all patients images of binucleated cells were observed in the adrenal cortex, most of them being located in the zone reticularis, and the zona fasciculata; sometimes nuclei were dumb-bell-shaped (Fig. 3). Though seldom seen, cells undergoing apoptosis and apoptotic bodies were observed in the zone reticularis (Fig. 4). Some apoptotic bodies appeared to pass into the sinusoids.

By electron microscopy some nuclei exhibited coarse chromatin pattern and perinuclear chromatin appeared heavily stained. The double nuclear membrane was sometimes discontinuous, and dense chromatin masses lay among cytoplasmic organelles (Fig. 5). Only a few images of karyorrhexis were

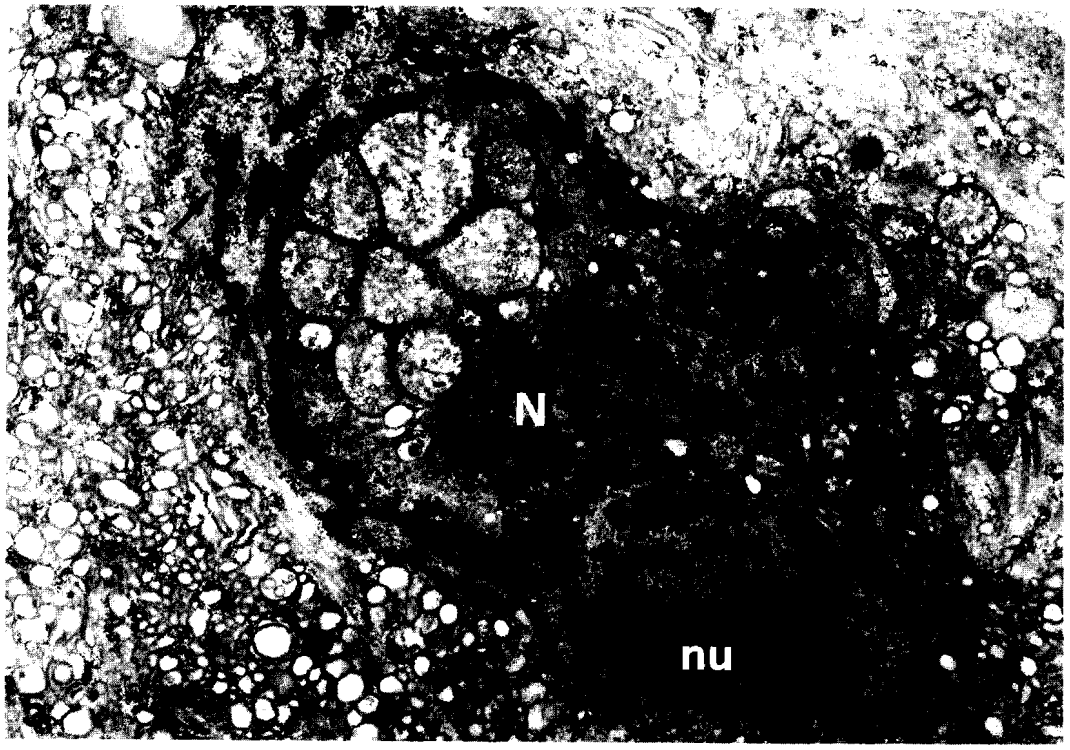

Fig. 5. Virilization syndrome. Complex structure of a cell nucleus (N). Dense chromatin masses lie among cytoplasm organelles (arrows). × 15,000. nu - nucleolus.

observed. Chromatin never marginated in crescentic caps, like the morphological feature of apoptosis in electron microscopy. Sometimes a constriction appeared in the nuclei and two daughter nuclei appeared to be formed (Figs. 6-9). The cells showed a cleavage furrow, the nucleus being perpendicular to it (Figs. 6-9): the cleavage deepened and widened, the neck decreased in diameter becoming «stretched» along the longitudinal axis of the cell (Figs. 6, 7). Such a cleavage may lead to the formation of two sometimes unequal halves with one nucleolus in each pole of the nucleus (Fig. 6) and, in the end, nuclear fission would seem to occur. No chromosomes were detectable within the nucleus at any stage. The nucleolus in cells with a bilobate appearance of amitotic division was well preserved, and contained fibrilar centres (Fig. 6). Nucleolar material was never scattered through the cytoplasm except in karyopyknosis.

Cytoplasmic organelles were well preserved and bundles of parallel microtubules were never visible near the nucleus.

Discussion

Amitosis or direct division (Prenant et al., 1904; Obré et al., 1966) has been postulated as a form of cell division, and in many cases it appears to be a kind of physiologically-induced tissue proliferation (Yiquan and Binkung, 1986). However many questions exist with respect to the significance of amitosis, and even today there is no truly satisfactory research concerning this type of cell division (Bucher, 1963). In fact, large numbers of observations described as amitosis were really not, often merely being artifacts of fixation, mechanical influences, etc. (Bucher, 1963; Puza, 1969).

On the other hand, for many authors, amitosis only occurred in lower invertebrates, aged cells or in pathological conditions (Prenant et al., 1904; Bucher, 1963); for others, it was even considered as the only type of nuclear division in normal adult liver (Elias and Hyde, 1982). In addition, amitosis has been studied in living cells cultivated in vitro (Bucher, 1963), and Bargmann (1977) referred to the fact that it was often seen in highly differentiated tissues, such as the liver, heart and kidney. Accordingly, amitosis, in the classical sense, does seem to play a role in the multiplication of cancer cells; a desdifferentiated cell (Oberling and Bernhard, 1961). Concerning the adrenal, amitosis has been observed in interrenal tissue of *Rana temporaria* (Pehlemann, 1968) and in rat adrenal zona glomerulosa after a prolonged low-sodium diet (Palacios et al., 1976), but it has never been reported in human adrenal cortex, as far as our literature suggests.

In this manuscript, morphological aspects of nuclei, which might be considered suggestive of amitosis, were observed in human adrenal zona reticularis, a differentiated zone of low mitotic activity (Ford and Young, 1963; Wright, 1971), but which in some circumstances can be stimulated and submitted to an increased metabolic activity.

Curiously enough, the zona reticularis is also the cortical zone which exhibits more apoptotic bodies; a sign of programmed cellular death (Wyllie et al., 1973, 1980). However, apoptosis has peculiar morphological aspects which are easily distinguished from those of amitosis, such as margination of chromatin in crescentic caps and cytoplasmic densification.

Molecular mechanisms of amitosis in mammals are

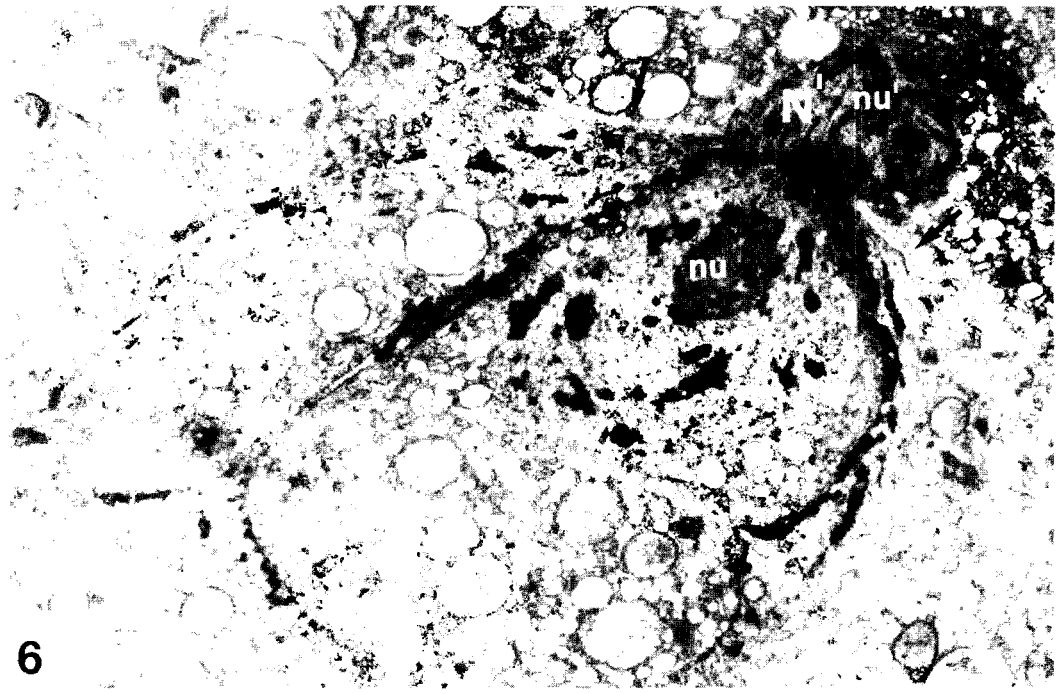

Fig. 6. Zona reticularis from the adrenal cortex of the woman with virilization syndrome. A constriction (arrows) provokes the appearance of two unequal halves of the nuclei (N, N') with one nucleolus (nu, nu') in each pole. × 10,800

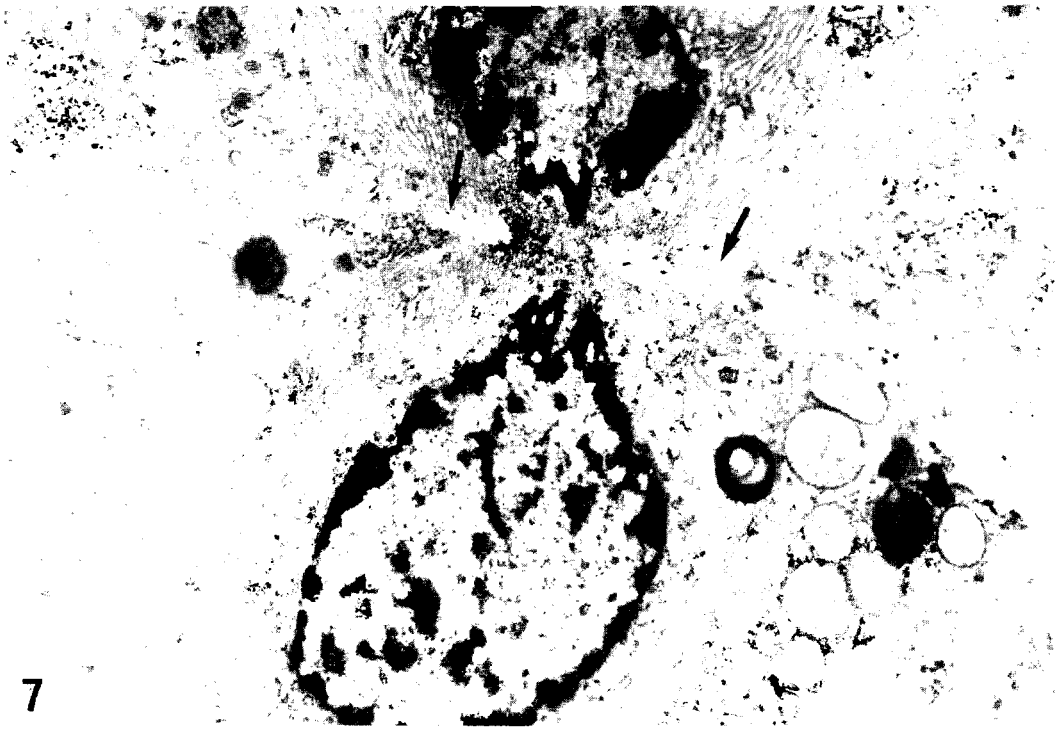

Fig. 7. Buerger's disease. Man aged 23. A cleavage furrow is observed (arrows), the nuclei (N and N') being perpendicular to it. × 15,000

unknown; in fact, we do not know what kind of inductive signal can cause initiation of amitosis; neither do we know if amitotic nucleus is euploid or suffers subsequent degeneration. In the present study amitosis was observed both in normal and in hyperactive adrenal, and in both males and females. Hence, amitosis seems to be a form of nuclear division in human adrenal, being perhaps the fastest form of cell division, but not the usual form since figures that might be interpreted as amitosis were scarce.

We can speculate that amitosis, like apoptosis, occurs in adrenal reticular cells as part of their normal development and is probably correlated with an activation process or one leading to cell death, in relation to the maturation state of the glandular cell. If this glandular cell is immature, death will occur, while amitosis seems to occur in mature cells. Hence, the type of cellular responses will depend on the

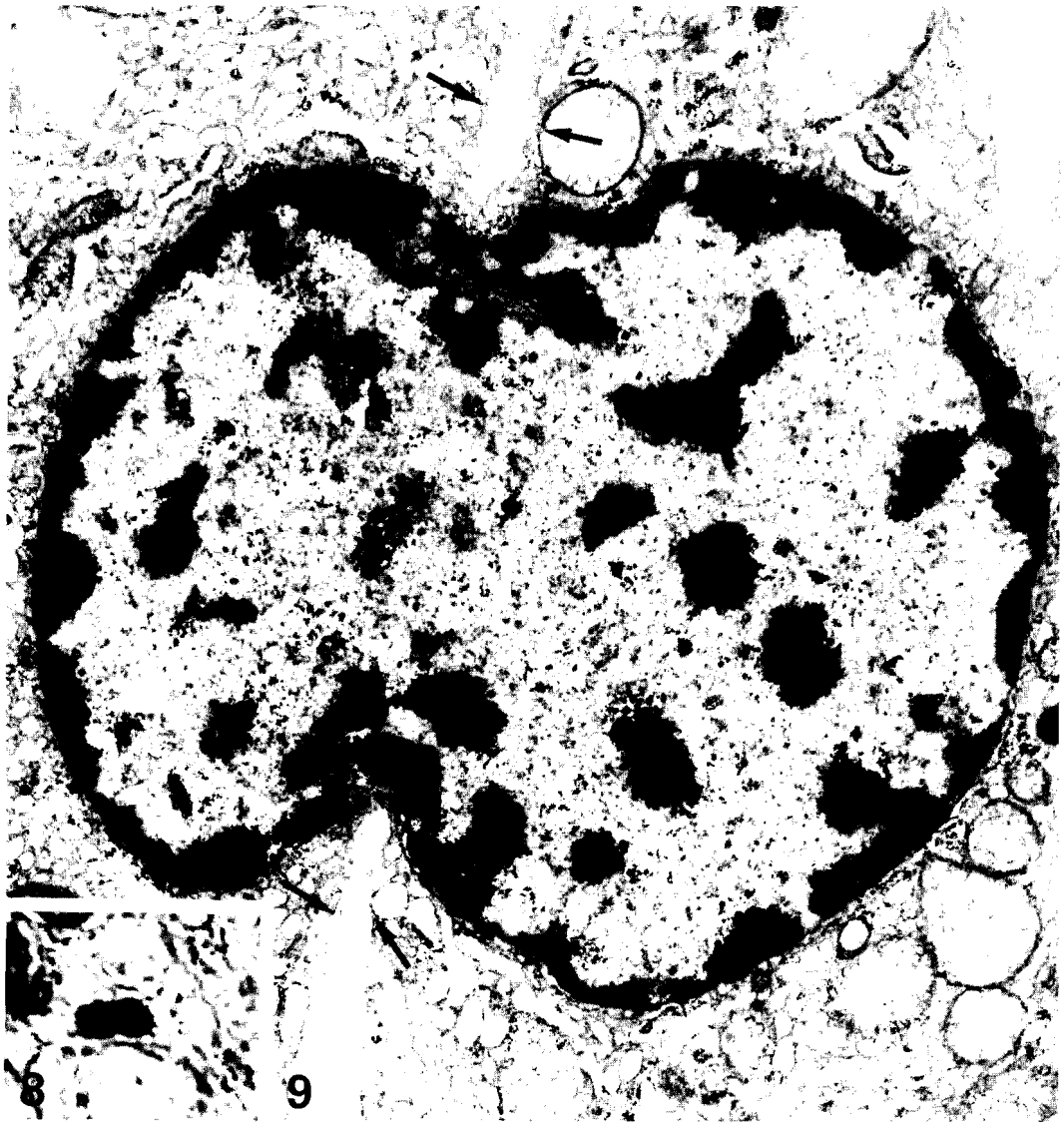

Fig. 8. Adrenal cortex from a woman aged 19. Zona reticularis. Constricted nucleus. × 1,470

Fig. 9. The same as for Fig. 8. Cleavage furrow (arrows); neck with a large diameter. No chromosomes (N) are detectable in the nucleus (N). No microtubules are observed near the nucleus. × 20,700

developmental state of the cell. A hypothesis such as this was proposed by Golstein (1989) for cellular death in immune system. Further studies are necessary in order to search for the nuclear morphologies as described in this manuscript in other adrenal cortex zones, since it is not unusual to observe binucleated cells, possibly caused by amitosis (Clubb and Bishop, 1984; Kriesten, 1984), in zona fasciculata.

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