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CELLULAR LOCALIZATION OF CYTOCHROME(S) P-450 METABOLIZING POLYCYCLIC AROMATIC HYDROCARBONS IN THE RAT ADRENAL CORTEX

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Abstract—Cells were dispersed from the capsular, as well as the inner portion of female rat adrenal glands and subsequently separated on discontinuous Percoll gradients. The adrenal cells were distributed within a density interval ranging from 1.016 to 1.075 g/cm^3 and different subpopulations showed distinct morphological appearances in suspension, as well as in culture. The total cells from the inner portion of the adrenals metabolized [^{14}C]7,12-dimethylbenz(*a*)anthracene at a rate of 4.04 pmol/min10^6 cells and synthesized corticosterone in response to ACTH stimulation at a rate of 1.07 μg/hr/10^6 cells. These activities were 4- and 2.5-fold higher, respectively, than the corresponding activities in cells isolated from the capsular portion. 7,12-Dimethylbenz(*a*)anthracene monoxygenase activity and ACTH-stimulated steroidogenesis were enriched in two subpopulations of cells obtained on the Percoll gradient and were estimated to be 13.1 pmol/min/10^6 cells and 3.21 μg/hr/10^6 cells, respectively, in the most active fraction (at the 1.034/1.040 g/cm^3 interface). On the basis of cellular morphology, density and steroidogenic properties, it was concluded that adrenal 7,12-dimethylbenz(*a*)anthracene monoxygenase activity is localized mainly in the cells of the zona fasciculata.

The functional zonation of the mammalian adrenal cortex has been throughly investigated (for reviews see Refs 1 and 2). The outermost zone, the zona glomerulosa, accounts for the primary production of aldosterone for the various needs of the body. The middle layer of this gland, the zona fasciculata, has the highest capacity for the synthesis of glucocorticoids and also produces androgens to a certain extent. In the innermost part, the zona reticularis, the end-products of steroidogenesis are to a greater extent androgens.

The synthesis of steroids from cholesterol is achieved by a series of both mitochondrial and microsomal steroidogenic cytochrome P-450 enzymes [3, 4]. In addition, adrenal microsomes from, e.g., guinea pigs, rats, cows and human fetuses demonstrate cytochrome P-450-dependent xenobiotic metabolism [5–9], whereas adult human adrenal microsomes seem to be devoid of this activity [10]. Female rat adrenal microsomes convert both 7,12-dimethylbenz(a)anthracene (DMBA) and benzo(*a*)pyrene (BP), mainly to phenols and dihydrodiols, at a rate of 200 pmol/min/mg [6], which is about 2–4-fold higher than the rate observed in liver microsomes from control female animals [11]. This activity is maintained in cells isolated from rat adrenals [9] and in rat adrenal cell (RAC) cultures for at least 2 weeks [12, 13]. The identity of the cytochrome(s) P-450 responsible for adrenal DMBA monoxygenase activity has not yet been established. Western blot analysis of adrenal microsomal proteins revealed no components identical to the rat liver cytochrome P-450 isozymes a, b, c, d or PB/PCNE [14], suggesting that the adrenal xenobiotic-metabolizing isozyme(s) may represent a unique form.

A number of hydrophobic xenobiotics, e.g., PCB, DDT and toxaphene [15–17], some of which exert adrenotoxic effects, accumulate preferentially in the adrenal cortex. Lund *et al.* [18] reported that a DDT metabolite, 3-methylsulphonyl-DDE, accumulated in covalently bound form in the mouse adrenal cortex and caused selective necrosis of the zona fascicuta.

In addition, to their general carcinogenicity [19], certain methylated polycyclic aromatic hydrocarbons (PAH), e.g., DMBA and its liver metabolite 7-hydroxymethyl-12-methylbenz(*a*)anthracene (7-OHM-12-MBA), selectively cause a total destruction of the two inner zones of the rat adrenal cortex [20, 21], whereas unmethylated PAHs such as benz(*a*)anthracene and BP are inactive in this latter respect. *In vivo* [22, 23], as well as *in vitro*, using RAC [13], this DMBA-induced cytotoxicity can be partially prevented by inhibitors of adrenal DMBA monoxygenase activity, suggesting the involvement of cytochrome P-450-dependent metabolic activation of the hydrocarbon in its adrenotoxicity. The cytotoxicity caused by 7-OHM-12-MBA in RAC is, however, associated with a selective oxidation of mitochondrial glutathione [24, 25] and is not affected by inhibitors of the cytochrome P-450 system [13]. It should be pointed out in this connection that, although ACTH-induced steroidogenesis in RAC was totally abolished by 7-OHM-12-MBA, 50% of the cells remained intact, suggesting a heterogeneity in the sensitivity of the adrenal cells to this agent.

In general, it has become increasingly obvious that individual proteins are not uniformly distributed between the cells in a tissue. For instance, the cells in the pericentral and the periportal areas of the liver demonstrate different phenotypes [26, 27]. In

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addition to immunostaining of tissue slices, fractionation of dispersed cells has proven to be a powerful tool for determining the distribution of various cellular components and functions in different types of cells [28–30], especially when specific antibodies are not available. For example, in the human ovary and rat testis, DMBA monoxygenase activity is highest in the granulosa cells and Leydig cells, respectively [31, 32]. Martin and Black [33] have recently shown that cytochrome P-450-dependent xenobiotic metabolism in the guinea pig adrenal is present in cells of the inner zona reticularis, rather than the outer fasciculata/glomerulosa zones.

In order to further characterize the involvement of adrenal metabolic activation in PAH-induced adrenotoxicity, it is important to determine the cellular localization of the DMBA monoxygenase activity in the rat adrenal. Access to homogeneous preparations of different types of cells would also be a useful tool in further studies on the peroxidative processes which may be involved in the selective oxidation of mitochondrial glutathione observed in RAC exposed to PAH [24, 25]. As reported in this communication, localization of the DMBA monoxygenase activity to the cells from the zona fasciculata has been performed by density-gradient separation and characterization of RAC. However, homogeneous preparations of each of the different cell types in the rat adrenal cortex have not yet been obtained.

MATERIALS AND METHODS

Isolation of cells. Adrenals from 5–20 200 g female Sprague–Dawley rats were bisected according to Tait *et al.* [29]. The inner and the capsular portions of these glands were incubated separately in 0.5 mL/adrenal of PBSGA (0.117 M NaCl, 3.35 mM KCl, 11.1 mM Na_2HPO_4, 1.8 mM KH_2PO_4, 5.6 mM glucose and 0.5% bovine serum albumine; pH 7.3) supplemented with collagenase (2.5 mg/mL) and DNase (0.05 mg/mL) at 37° for 90 and 60 min, respectively. At the end of this digestion period, the tissue fragments were agitated by a few gentle suctions into and expulsions from a 5 mL automatic pipette. After such agitation, very little of the decapsulated material remained, whereas the capsules resisted dispersion. The cell suspensions were filtered through a 105 μm and, subsequently, a 69 μm nylon mesh. The cells from the filtered suspension were collected by a 4 min centrifugation at 100 *g*. The resulting pellets were resuspended in 0.5–2 mL of PBS (0.117 M NaCl, 3.35 mM KCl, 11.1 mM Na_2HPO_4, 1.8 mM KH_2PO_4) containing DNase (0.05 mg/mL). An aliquot of these suspensions was applied on top of the Percoll gradient. Aliquots of the total unfractionated cell suspensions were stored on ice during the Percoll centrifugation.

Cell culture. Aliquots of the various populations of cells were diluted in minimal essential medium supplemented with 10% calf serum and cultured on 35-mm collagen-coated Petri dishes as described earlier [13]. The medium was changed once each day. The plating efficiency was about 75% for the unseparated cells and about 90% in all fractions from the Percoll gradient except for the 0/10% fraction.

Percoll gradient. Isoosmotic Percoll (designated as 100%) was prepared by mixing Percoll with 10-fold concentrated PBS, after which the different concentrations of Percoll were obtained by diluting the stock solution with PBS. All Percoll solutions were supplemented with DNase (0.5 mg/mL). The different layers of Percoll were gently applied on top of each other with the help of a bent needle.

The cells were separated on the gradients by centrifugation at 400 *g* for 20 min. The cells banding at the various interfaces were collected with a bent needle and washed free from Percoll by dilution in PBS and repetitive centrifugations at 100 *g* for 2 min. The unfractionated cells were also washed in the same manner, after which all cell preparations were stored on ice.

Microscopy. The cells were counted in a hemocytometer after appropriate dilution. Viability, as judged by trypan blue exclusion, exceeded 95% in all preparations, except in cells recovered at the 0/10% Percoll interface. Only viable cells (at least 200) were counted. The bright field micrographs of the cells in suspension, as well as the phase contrast photographs of the cultured cells, were made using an Olympus inverted microscope (IMT), with a 100 ASA Kodak film for black and white prints.

DMBA monoxygenase activity. Cells ($5–10 \times 10^4$) were incubated in 0.5 mL PBSGA containing 10 μM [^{3}H]DMBA (100 mCi/mmol) and 0.2% acetone at 37° for 4 hr, i.e., under optimal conditions as determined earlier [9, 12]. Cell-free incubations were used as blanks.

Steroidogenesis. ACTH-induced synthesis of steroids from endogeneous cholesterol was determined by incubating $5–10 \times 10^4$ cells in 0.5 mL PBSGA containing ACTH (0.1 I.U./mL) at 37° for 4 hr, after which the incubations were extracted and analysed as described by Silber *et al.* [34].

The conversation of [^{14}C]progesterone was estimated by preincubating 50×10^3–100×10^3 cells with 20 μM cyanoketone for 1 hr at 37° as described by Hornsby [35], thus inhibiting the 3β-hydroxysteroid dehydrogenase and emptying the endogenous pool of steroid precursors. The treated cells were then incubated in 0.5 mL PBSGA containing 0.3 μM [^{14}C]progesterone (3.0 mCi/mmol) for 1 hr at 37°. The incubations were extracted and analysed after separation on TLC according to Ref. 35.

Chemicals. Percoll was purchased from Pharmacia Fine Chemicals AB (Uppsala, Sweden); MEM, dialysed calf serum (cat. No. 063-6440), penicillin and streptomycin from Grand Island Biological Company (Grand Island, NY, U.S.A.); Petri dishes from Falcon Products (LaborA, Stockholm, Sweden); [^{3}H]DMBA from NEN (6072 Dreieich, F.R.G.). ACTH and cyanoketone were kindly donated by Ciba-Geigy (Basal, Switzerland) and Sterling-Winthrop Research Institute (Renssealer, New York).

RESULTS

In order to localize adrenal DMBA monoxygenase activity, isolated cells from the inner part as well as from the capsular portion of rat adrenal glands were separated on discontinuous Percoll gradients. Table

Table 1. Distribution and yield of rat adrenal cells separated on Percoll gradients. Isolated cells from the inner portion were layered on top of a discontinuous Percoll gradient after which they were separated at 400 *g* for 20 min. The fractions were washed free of Percoll and counted in a haemocytometer

Percoll interface (%/% [vol/vol])	Density ($g/cm^3/g/cm^3$)	N*	Distribution† (%)	Yield‡ (%)
0/10	1.004/1.016	3	11.3 ± 3.8	8.0 ± 2.7
10/20	1.016/1.028	3	29.4 ± 9.3	21.1 ± 7.7
20/25	1.028/1.034	3	19.8 ± 7.8	13.9 ± 5.4
25/30	1.034/1.040	3	8.1 ± 4.2	5.7 ± 2.5
30/40	1.040/1.052	3	14.3 ± 1.6	10.1 ± 1.2
40/50	1.052/1.064	3	8.7 ± 2.2	6.3 ± 1.9
50/60	1.064/1.075	3	8.3 ± 2.4	5.9 ± 1.3
60/80	1.075/1.099	3	0 ± 0	0 ± 0
Σ		3		71.0 ± 4.4

* Number of experiments.
† Percentage of the cells detected, mean ± SD.
‡ Percentage of the cells applied on the gradient, mean ± SD.

1 summarizes the distributions and yields of viable cells at the various density interfaces. 548 ± 111 × 10^3 and 166 ± 60 × 10^3 cells per gland were obtained from the inner and capsular portions of the adrenals, respectively. The cells dispersed by collagenase digestion distributed at various densities, from the 0/10% to the 50/60% Percoll interfaces. The average yield of cells was 71.0 ± 4.4% of the cells layered on the gradient. In order to avoid cross contamination between the various cell fractions, some material was left behind during aspiration of each band from the Percoll gradient. This procedure probably accounted for much of the loss of cells. The same distribution of cells was obtained after prolonged periods of centrifugation or when the cells were mixed with Percoll (final concentration 80%) and layered at the bottom of the tubes (not shown).

At least four different types of cells could be distinguished by microscopic examination of the total cell population obtained by digestion of the inner portion of the adrenals (Fig. 1): cells with a granulated morphology, presumably due to the presence of lipid droplets, and a diameter of 10–20 μm (type I) (Fig. 1A); "clear" cells with a diameter of 10–20 μm (type II) (Fig. 1B); clusters of clear cells with a diameter around 10 μm (type III) (Fig. 1C); and single clear cells of the same diameter, i.e., around 10 μm (type IV) (Fig. 1D). The cells from the capsular portion of the gland were generally smaller. The appearance in suspension of the majority of these cells did not differ from the type III and IV cells, although a significant number of type I cells were also observed in this case.

The 0/10% fraction contained mostly dead and partially damaged cells (not shown). The large lipid rich type I cells were predominating in the 0/10 to the 30/40% fractions (the 1.004/1.016–1.040/1.052 g/cm^3 density interfaces) (Table 2). The clear type II cells were distributed among the fractions from the 25/30 to the 40/50% interfaces. The small type III cells appeared at the 30/40%, and predominated at the 40/50 and 50/60% interfaces. The type IV cells were distributed among the same fractions as type III cells although they were less numerous. The 60/80% interface contained solely erythrocytes (not shown).

Figure 2 shows phase contrast micrographs of cells obtained in the various fractions, but after 3 days in culture. The plating efficiency in the various fractions were similar, indicating that the different cell populations were equally viable. There was a clear difference in the morphology of the cell cultures obtained from the capsular portion and the inner portion of the adrenals. The majority of the cells from the capsular portion (Fig. 2A) had a round, "mosaic"-like appearance and scanty cytoplasm, whereas a significant proportion of the cells appeared similar to type I cells (Fig. 2B). Three main types of cells could be distinguished in cultures from the inner portion of the adrenals: one type containing abundant visible lipid droplets, often obscuring the nucleus, (type 1) (Fig. 2B), a second with fewer or no lipid droplets (type 2) (Fig. 2C) and a third without visible lipid droplets, but with dark nuclei (type 3) (Fig. 2D). No cells with the characteristic spindle-shaped appearance of fibroblasts could be detected in the cultures, suggesting minimal contamination by cells from the capsule or other connective tissues.

In the cultures from the Percoll gradients the lipid rich (type 1) cells predominated in the 10/20 to the 25/30 layers, i.e., layers having a density of less than 1.040 g/cm^3 (Table 2). The proportion of type 1 cells gradually decreased at increased densities. The opposite was true for type 2 cells, which dominated the 40/50 and 50/60 fractions. In culture cells from the 30/40 fraction there were equal amounts of type 1 and type 2 cells. The type 3 cells were also more abundant in the cultures of cells recovered at higher densities.

The distribution of DMBA monoxygenase activity is shown in Fig. 3. The total cell preparation from the inner portion of the adrenals metabolized DMBA at a rate (4.04 ± 1.00 pmol/min/10^6 cells) which was 4-fold higher than that of the capsular cells (1.04 ± 0.15 pmol/min/10^6 cells). Enrichment of this activity to 13.05 ± 1.81 pmol/min/10^6 cells and 6.36 ± 0.37 pmol/min/10^6 cells was observed in the

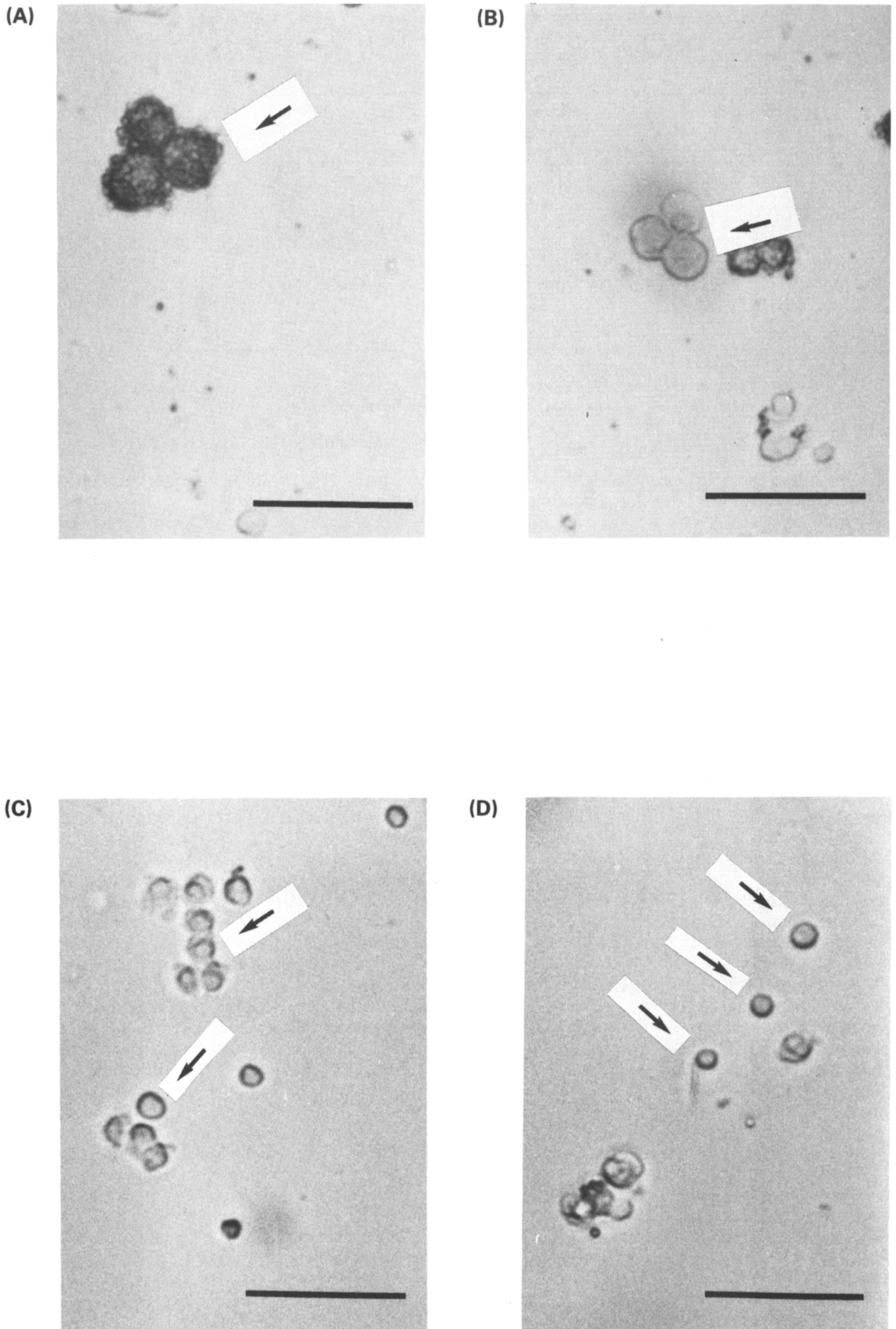

Fig. 1. Light micrographs of different types of cells dispersed from rat adrenals. The micrographs show: A, type I cells; B, type II cells; C, type III cells and D, type IV cells (bar = 50 μm).

Table 2. Relative distribution of various cell types in suspensions and cultures of adrenal cells recovered at different interfaces after Percoll fractionation

	Suspension				Culture		
	Type I	Type II	Type III	Type IV	Type 1	Type 2	Type 3
Diameter (μm)	10–20	10–20	≈10	≈10			
Lipid droplets*	Abundant				Abundant	No	No
Appearance†	Granulated	Clear	Clear clustered	Clear single			Dark nuclei
Interfaces: 0/10	+						
10/20	+++				+++		
20/25	+++				+++		+
25/30	++	++			++	+	+
30/40	++	++	+	+	++	++	+
40/50		+	++	+		++	++
50/60			++	+		++	
60/80							

* Visible in the light microscope.
† Appearance in the light microscope.

cells recovered from the 20/25% and the 25/30% interfaces, respectively. The overall recovery of DMBA monoxygenase was 78% of the total activity applied to the gradients.

The ability to produce corticosterone from endogeneous cholesterol in response to ACTH stimulation was also investigated. As shown in Fig. 4, the rate of ACTH-induced steroidogenesis was 1.07 ± 0.07 μg corticosterone/hr/10^6 cells and 0.40 ± 0.16 μg corticosterone/hr/10^6 cells in the total dispersed cells from the inner and capsular portions of the adrenals, respectively. The distribution of ACTH-induced steroid production between the Percoll interfaces correlated almost perfectly with the distribution of DMBA monoxygenase activity. The overall recovery of the steroid-synthesizing activity was 81% of the total applied to the gradients.

The ability of cyanoketone-treated cells to convert [^{14}C]progesterone to aldosterone was investigated. Incubations, extractions and chromatography was performed as described in Materials and Methods. Radioactive spots comigrating with aldosterone were only detected in samples from incubations with dispersed cells from the outer portion of the rat adrenals (not shown), suggesting that cells from the zona glomerulosa were mainly recovered in this fraction. A low contamination of these cells in fractions obtained before and after Percoll separations of cells from the inner portion of the gland is indicated by the absence of detectable aldosterone synthesis (not shown) (c.f. Ref. 29).

DISCUSSION

The present investigation describes for the first time the separation of rat adrenal cells in combination with cellular distribution of cytochrome P-450 metabolizing polycyclic aromatic hydrocarbons. After collagenase dispersion, the capsular and the decapsulated portions of rat adrenals gave rise to different types of cells as judged by light microscopy. The majority of the cells from the inner portion of the gland had a diameter ranging from 10–20 μm and a granulated appearance. From light micrographs it was impossible to distinguish the suspended capsular cells from cell types obtained from the inner portion of the gland, although the former were generally smaller.

Isolated rat adrenal cells were separated on a discontinuous Percoll gradient. The cells did not, however, appear in specific bands characteristic for a certain zone of the gland. Although certain cell types with differential morphologies were enriched at certain density interfaces, adrenal cells were recovered at all Percoll interfaces, from the 0/10% to the 50/60% interfaces. During separation of the zona fasciculata cells (large diameter, low density) from the zona reticularis and zona glomerulosa cells (small diameter, high density), there is a certain risk that the larger zona fasciculata cells form an impenetrable layer ahead of the other cells, preventing them from reaching equilibrium. This possibility was tested and eliminated, since prolonged times of centrifugation or application of the cells at the bottom of the tubes had no effect on the distribution of cells, DMBA monoxygenase activity or ACTH-induced steroidogenesis.

Tait and co-workers [28–30] have pointed out the large variability in the physical properties, i.e. density and diameter of isolated rat adrenal cells. This variability is probably due in part to the presence of intermediary cells involved in the adrenal centripetal mode of evolution of cell types. Another factor influencing the density is the cellular content of cholesterol-containing lipid droplets. The cholesterol content of the cells is a function of steroidogenesis, uptake from the plasma and neosynthesis, factors which are all affected by the hormonal status of the animal due to, for instance, stress or diet [2, 36].

Tait and co-workers [28–30] have also performed an extensive functional, morphological and ultrastructural characterization of dispersed rat adrenal cells purified by unit gravity sedimentation, cells which retained most of their *in vivo* characteristics

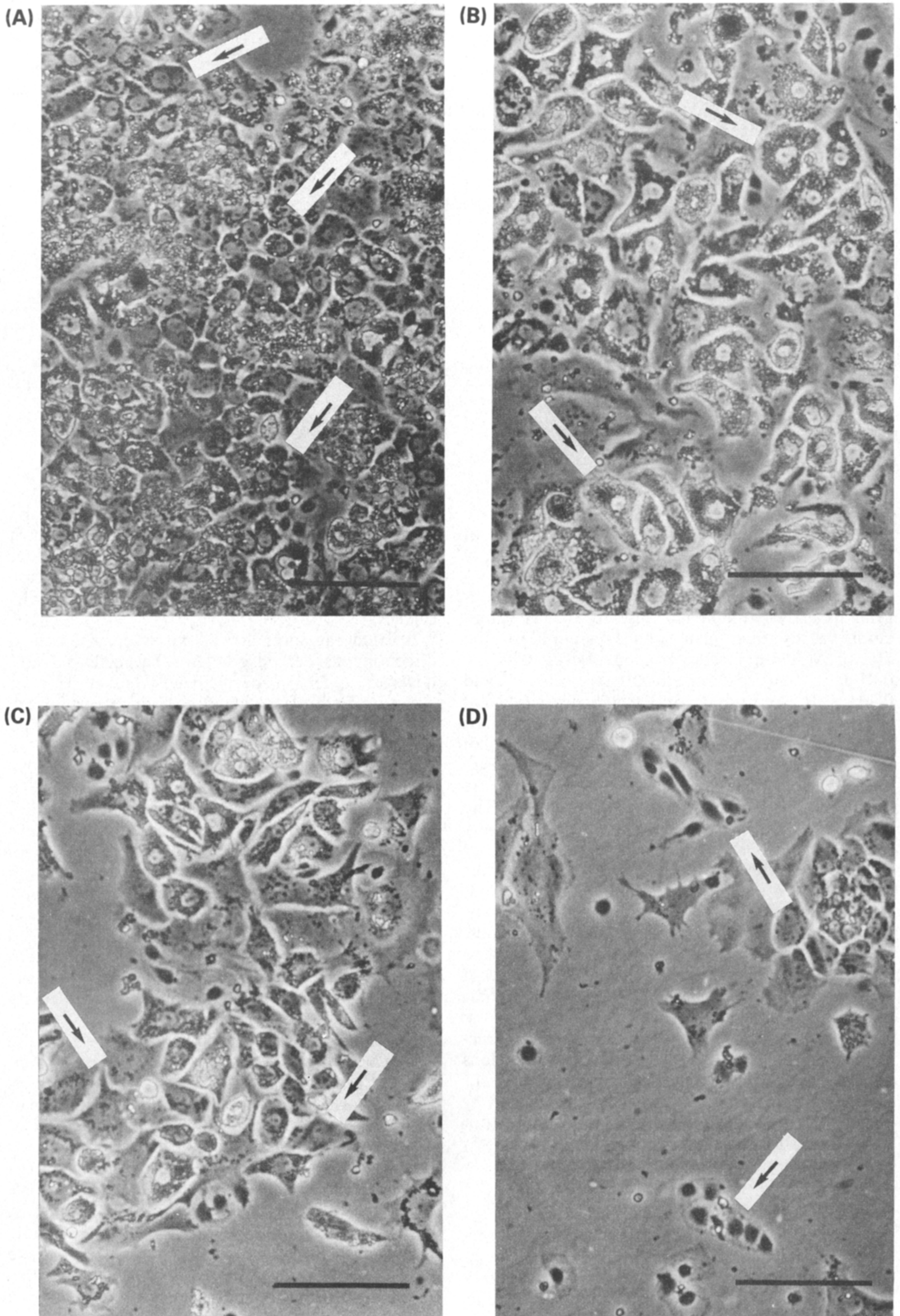

Fig. 2. Phase contrast micrographs of cultures of different types of cells dispersed from rat adrenals. The micrographs show 3-day-old cultures of cells. A, cells from the capsular portion; B, type 1 cells; C, type 2 cells and D, type 3 cells (bar = 50 μm).

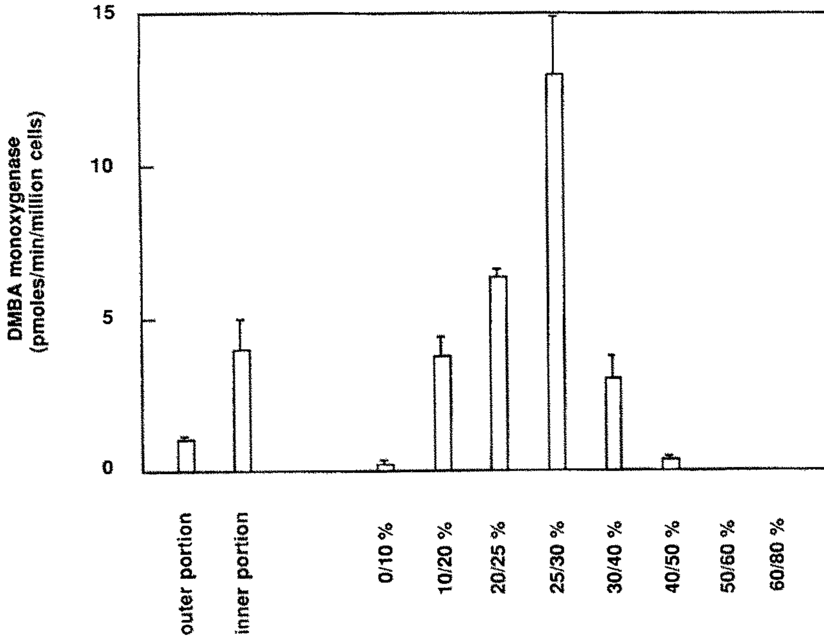

Fig. 3. Distribution of DMBA monoxygenase activity in different rat adrenal cell populations. The DMBA monoxygenase activity was determined in the cell preparations indicated. The staples represent the means of four samples from two different experiments ± SD (the bars).

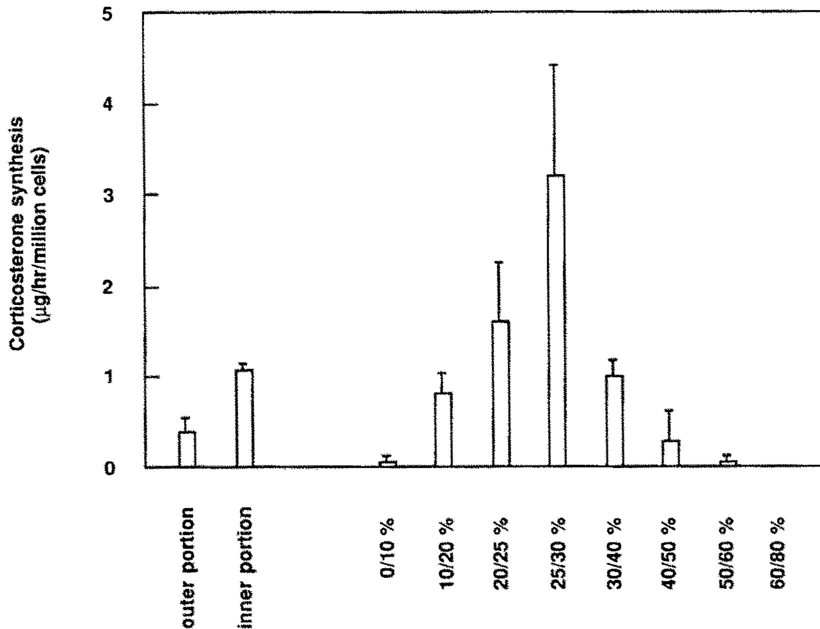

Fig. 4. Distribution of ACTH-induced endogeneous corticosterone synthesis in different rat adrenal cell populations. The ACTH-induced corticosterone production was determined for the cell preparations indicated. The staples represent the means of four samples from two different experiments ± SD (the bars).

after dispersion. The dispersed zona fasciculata cells had a mean density of $1.040\,g/cm^3$ and were described as large cells (modal diameter of $19\,\mu m$), containing numerous large lipid droplets (which "usually obscured the nucleus") and exhibiting a small nuclear to cytoplasmic ratio. These cells had by far the greatest capacity to synthesize corticosterone in response to ACTH stimulation. The zona

glomerulosa and reticularis cells had a mean density of 1.072 g/cm^3 and 1.067 g/cm^3, respectively. Both celltypes had a modal diameter of 9 μm and considerably fewer and smaller lipid droplets and a high nuclear to cytoplasmic ratio. The characteristic lipid droplets of the cells from the zona fasciculata were reported to be maintained in culture [37, 38].

The different cell types observed in the present study can not be identified with certainty, but based on the physical and morphological properties of adrenal cells, described elsewhere (c.f. Refs 28–30 and 37–39), it is reasonable to assume that the lipid-rich type I and type 1 cells are identical and resemble zona fasciculata cells. These cells were also in the majority in fractions with densities (<1.040 g/cm^3) close to what has been previously reported for rat adrenal zona fasciculata cells [29] and had the greatest capacity to synthesize steroids from endogeneous cholesterol. Although the other cell types observed in this study showed characteristics similar to those described in the literature (c.f. Refs 28–30 and 37–39) a definite identification require ultrastructural information.

These results also show that the DMBA monoxygenase activity and the ACTH-induced steroidogenic capacity were 4- and 2.5-fold higher in the cells from the inner portion of the adrenals, as compared to the outer portion. It is possible that most of the activity in the capsular fraction was derived from contaminating zona fasciculata cells. These results are thus in agreement with the findings of Guenthner *et al.* [7], who observed a 3-fold higher rate of formation of fluorescent products from BP in microsomes isolated from the fasciculata/reticularis region than the corresponding rate in microsomes from the glomerulosa region. Based on cellular morphology, density and steroidogenic properties, it is concluded that the DMBA monoxygenase activity is mainly localized in cells from the zona fasciculata region of the rat adrenal cortex. In fact, more than 80% of the DMBA monoxygenase activity and the steroidogenic capacity (data derived from Table 1, Figs 1 and 2) were localized in the 10/20%–25/30% fractions, i.e., in lipid rich cells with a density less than 1.040 g/cm^3. These results support the conclusions from a preliminary study [40], where the highest xenobiotic-metabolizing activity, as well as ACTH-induced production of fluorescent steroids, was found in the same fraction of rat adrenal cells separated on BSA-gradients.

Studies on microsomes from inner and outer adrenal cortices of two different strains of guinea-pig [41], have shown that activities of BP monoxygenase, ethylmorphine demethylase and steroid 21-hydroxylase were several-fold higher in the reticularis region, whereas steroid 17-hydroxylating activity was higher in the glomerulosa/fasciculata region. Cortisol was almost exclusively produced by cells dispersed from the outer guinea-pig adrenal cortex [5]. These results were supported by Martin and Black [33], who reported considerably higher activities of ethylmorphine demethylase and 21-hydroxylase in microsomes from the inner than from the outer regions of adrenals from mature and immature guinea pigs. Comparisons of these results with those reported in the present study suggest interspecies variations in the distribution of adrenal xenobiotic-metabolizing enzymes.

An interesting question is whether these results support the involvement of adrenal bioactivation of PAH in the adrenocorticolysis. The localization of xenobiotic-metabolizing activity in the zona fasciculata cells is not necessarily contradictory to the fact that DMBA destroys both the zona fasciculata and the zona reticularis of treated animals [20, 22], as well as destroying all cultured RAC [13]. Toxic metabolites, generated by the zona fasciculata cells, are released into the culture medium *in vitro* and *in vivo* and might be transmitted to the reticularis region by the adrenal centripetal directional blood flow [42]. A similar mechanism has been proposed for the DMBA-induced destruction of spermatogenesis in the rat testis [43]. In order to further elucidate this question, it will be necessary to determine whether the separated adrenal cells show differential, DMBA monoxygenase-dependent sensitivity towards PAH and to investigate whether the selective oxidation of mitochondrial glutathione observed after exposure to 7-OHM-12-MBA [24, 25] is restricted to a specific cell type.

In conclusion, DMBA monoxygenase activity and ACTH-induced steroidogenesis were highest in a fraction of adrenal cells separated on a discontinuous Percoll gradient and identified as cells from the zona fasciculata. Further purification of these adrenal cells, as well as additional biochemical and electron microscopic characterization, has to be performed in order to further investigate the role of adrenal DMBA monoxygenase activity in adrenocytotoxicity and to study cell-type specific regulation of gene expression.

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