Metabolism of 7,12-Dimethylbenz-[*a*]anthracene by Isolated and Cultured Cells from Rat Adrenals *

EINAR HALLBERG and
JAN RYDSTRÖM

Department of Biochemistry, Arrhenius Laboratory, University of Stockholm, S-106 91 Stockholm, Sweden

The adrenal constitutes the major organ for biosynthesis of mineralcorticoids and glucocorticoids which is regulated by the pituitary adrenocorticotropic hormone (ACTH). It also produces relatively small amounts of androgens and estrogens.[1] In addition to its normal steroidogenic functions this organ is also relatively active in metabolizing xenobiotics including carcinogens such as 7,12-dimethylbenz[*a*]anthracene (DMBA) and benzo[*a*]pyrene (BP).[2]

Due to these dual functions the adrenal has proven to be a valuable tool for the elucidation of the physiological role(s) of these xenobiotic-metabolizing systems, which apparently involves one or more cytochrome P-450 type of monooxygenase. Of particular interest is a possible functional relationship between xenobiotic-metabolizing systems and steroid-specific cytochrome P-450-dependent hydroxylases.[3,4] In an attempt to eliminate the contribution of other organs to the overall metabolism of DMBA by the intact adrenal, cells from rat adrenals were isolated and maintained in primary cultures. These cells were found to synthesize ketosteroids, mainly corticosterone from endogenous cholesterol in an ACTH-controlled manner, as well as metabolism of DMBA to a few hydrophilic but unidentified products.

Experimental. The present method for isolating adrenal cells is a slightly modified version of previously published procedures.[5,6] Adrenals were removal from 2–6 male Sprague-Dawley rats, decapsulated, minced and immediately transferred to a beaker with 5 ml culture medium (L15) without serum but containing 4 mg collagenase and 0.25 mg DNAase. Incubation was carried out for between 90 and 100 min at 37 °C and with gentle stirring; every 30 min the incubation mixture was drawn through a Pasteur pipette.

The cell suspension was then transferred to a 15 ml centrifuge tube and the cells were spun down at $100 \times g$ for 90 s, after which the cells were suspended in 1 ml culture medium; the cell-free supernatant was discarded. Viability of cells was determined in a Bürker chamber using the Trypan blue exclusion method, and varied between 60 and 85%. Typical values for the number of viable cells obtained were about 200 000 to 400 000 per adrenal. Isolated cells were then transferred to collagen-precoated multidishes and kept in culture at 37 °C under air and 95% humidity. Medium was changed every 48 h. No proliferative activity has been observed but the cells were responsive to ACTH and could be kept in culture for up to 2 weeks; ACTH response was measured according to Sayers *et al.*[5] HPLC analysis of metabolites of DMBA was carried out with a Waters instrument equipped with a Bondapak Porasil C18 column.

L15 culture medium was obtained from Flow Laboratories (Irvine, Scotland). Two types of synthetic ACTH were used both of which were of the Synacten Depot type obtained from Ciba-Geigy AB (Basel, Switzerland). However, one type was lyophilized and free of benzylalcohol and the other was a solution containing benzylalcohol (10 mg/ml). Other biochemicals were obtained from Sigma Chem. Co. (St. Louis, Miss., USA). [^{14}C]-DMBA and [^{3}H]-DMBA was purchased from NEN (Dreieichenhain, West Germany).

Results and discussion. The isolated adrenal cells attached to the collagen layer after 1–2 h, and were confluent after 1–2 days. With respect to composition the majority of the cells appeared to represent a mixture of fasciculata and reticularis cells but there were no glomerulosa cells. As shown in Fig. 1 the cultured cells synthesized

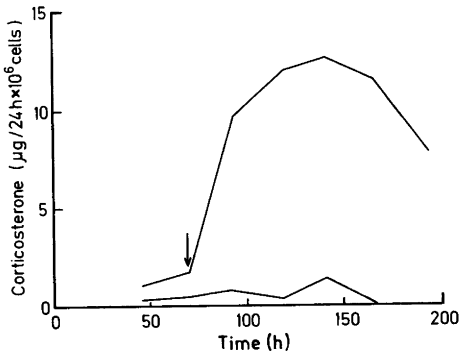

Fig. 1. Time course of steroid synthesis catalyzed by isolated rat adrenal cells. Approximately 9×10^5 cells were isolated, cultured and incubated as described in Experimental, in the absence (lower curve) and in the presence (upper curve) of 1 μg ACTH (benzylalcohol-containing) per ml medium. For each assay medium was removed and analyzed for ketosteroids, and replaced by fresh medium. The arrow indicates the first addition of ACTH.

* Communication at the Joint Meeting of the Swedish Biochemical and Swedish Biophysical Societies in Uppsala, 28–29th November, 1980.

0302-4369/81/020145-02$02.50

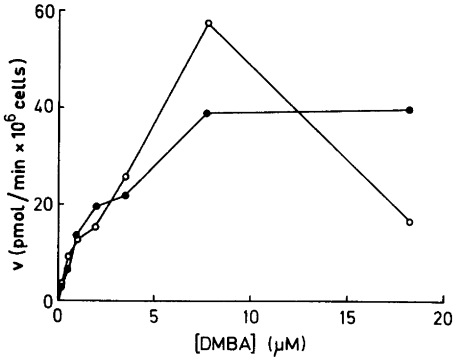

Fig. 2. Metabolism of DMBA catalyzed by isolated adrenal cells. Adrenal cells were cultured in the absence (○) and in the presence of ACTH (●). Conditions were as in Fig. 1 except that the cells were preincubated with 20 μg benzylalcohol-free ACTH per ml medium for 16 h. Fresh ACTH-containing medium was added at the time DMBA was added.

corticosterone, which is the main steroid produced by rat adrenals,[1] from endogenous cholesterol in the absence of added ACTH. In the presence of ACTH the rate of steroid synthesis was increased between 10- and 70-fold, indicating that the isolated adrenal cells have maintained their normal regulatory properties typical of the intact adrenal. In spite of the fact that fresh ACTH was added after every measurement of the amount of corticosterone synthesized, the response tended to be transient indicating a modification or destruction of the cells; exhaustion of the substrate cholesterol appeared less likely. The maximal steroid synthesizing activities of the cells in the absence and in the presence of ACTH are summarized in Fig. 1.

In the presence of [^{3}H]-DMBA the adrenal cells catalyzed the formation of more hydrophilic products, presumably by cytochrome P-450-dependent monooxygenases.[4] Incubation with various concentrations of DMBA ranging from 0,2 $-$ 18 μM indicated that the maximal activity was about 40 pmol/min × 10^6 cells and the apparent K_m 3 μM (Fig. 2). The major products were tentatively identified as phenols, mainly 5-OH-DMBA, and two unidentified compounds with R_f values of 0.58 and 0.68. At nonsaturating concentrations of the hydrocarbon ACTH did not have any significant effect on DMBA metabolism (Fig. 2) under conditions where steroid synthesis was stimulated by saturating concentrations of the benzylalcohol-free ACTH. At concentrations of DMBA higher than 5 μM, ACTH had a variable effect the significance of which is presently unknown. However, the benzylalcohol-containing ACTH solution was occasionally found to give a 2-fold stimulation and lead to a change in the distribution of metabolites (not shown). The reasons for the discrepancy between the effects of the two ACTH preparations are presently being investigated. In this context it should be pointed out that hypophysectomy and administration of ACTH to rats has been shown to decrease and increase, respectively, the rate of metabolism of benz(a)-pyrene.[7]

The present results show that rat adrenal cells isolated by collagenase treatment are highly responsive to ACTH with respect to steroid synthesis, and catalyze an apparently ACTH-insensitive metabolism of DMBA and presumably other carcinogens (*cf.* Ref. 4). It appears conceivable that these cells constitute an excellent model system for the characterization of detoxifying systems in steroidogenic organs.

Acknowledgements. This work was supported by grants from the Swedish Cancer Society and the Swedish Council for Planning and Coordination of Research.

1. Jenkins, J. A. *An Introduction to Biochemical Aspects of the Adrenal Cortex*, Edward Arnold (Publishers) Ltd., London 1968.
2. Huggins, C. B. *Experimental Leukemia and Mammary Cancer. Introduction, Prevention, Cure*, Univ. Chicago Press, Chicago and London 1979.
3. Montelius, J. and Rydström, J. In Coon, M. J., Conney, A. H., Estabrook, R. W., Gelboin, H. V., Gillette, J. R. and O'Brien, P. J., Eds., *Microsomes, Drug Oxidations and Chemical Carcinogenesis*, Academic, New York 1980, p. 1231.
4. Rydström, J., Montelius, J., Papadopoulos, D., Hallberg, E., Helfer, L. and Bengtsson, M. In Gustafsson, J.-Å., Carlstedt-Duke, J., Mode, A. and Rafter, J., Eds., *Biochemistry, Biophysics and Regulation of Cytochrome P-450*, Elsevier/North-Holland Biomedical Press, Amsterdam 1980, p. 491.
5. Sayers, G., Swallow, R. L. and Giordano, N. D. *Endocrinology 88* (1971) 1063.
6. Ramachandran, J. and Suyama, A. T. *Proc. Natl. Acad. Sci. U.S.A. 72* (1975) 113.
7. Guenthner, T. M., Nebert, D. W. and Menard, R. H. *Mol. Pharmacol. 15* (1979) 719.

Received December 9, 1980.