that the species represented by AM F.57844, like those of *Python* and *Varanus*, arrived in Australasia from southeast Asia. This is consistent with the occurrence of the oldest ziphodont material of the Australasian plate in the most northerly region.

Also of interest is the persistence of a ziphodont lineage with a laterally compressed skull on a continent lacking large placental carnivores, and with only one (possible) large marsupial carnivore (*Thylacoleo*). This accords with the persistence in South America of sebecosuchians until the arrival there of large placental carnivores and the apparent disappearance of pristichampsines in the Northern Hemisphere approximately at the time of the appearance of the order Carnivora. Although more work is necessary to substantiate such a suggestion, it suggests that the ziphodont crocodilians with laterally compressed skulls may have been in competition with large placental carnivores and usually did not survive when such forms became abundant.

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Angiotensin Converting Enzyme: Induction by Steroids in Rabbit Alveolar Macrophages in Culture

Abstract. *Dexamethasone and prednisone in physiologic range increased angiotensin converting enzyme 7- to 16-fold in comparison to control in 3 days at maximal stimulation (4 nM steroid) in rabbit alveolar macrophages in culture. The increase was inhibited by actinomycin D (0.1 μg/ml) and 1 μM cycloheximide, suggesting that de novo transcription and enzyme synthesis are responsible for the increased enzyme activity. This result is evidence for a regulatory mechanism for angiotensin converting enzyme, which is important in blood pressure control.*

Angiotensin converting enzyme (ACE) is a dipeptidyl carboxypeptidase (E.C. 3.4.15.1, peptidyl dipeptidase), which converts the decapeptide angiotensin I, formed from the plasma protein angiotensinogen by the catalytic activity of renin, to the potent vasopressor octapeptide angiotensin II and L-histidyl-L-leucine. ACE also inactivates the vasodepressor nonapeptide bradykinin (*1*) and has been demonstrated, by immunofluorescent and immunocytochemical techniques, to be localized at the luminal surface of the vascular endothelium and in the brush border of renal proximal tubules (*2*). Angiotensin I and bradykinin are cleaved by ACE catalysis in passage through the pulmonary vasculature (*3*). Action of ACE on these substrates has a vasopressor effect that can be counteracted by inhibition of its catalytic activity by certain peptides and antibody to ACE (*4*).

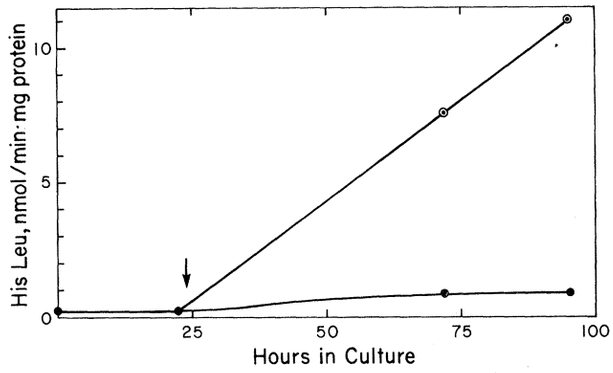
ACE is elevated in the pathologic lesions and circulation in the macrophage-related diseases sarcoidosis (*5, 6*) and Gaucher's disease (*7*), although barely detectable in endotoxin- or thioglycholate-activated or unactivated rodent macrophages and human leukocytes, and not elevated in the granulomatous lesions of tuberculosis and rat granuloma induced by Freund's adjuvant (*5, 8, 9*).

In order to elucidate the possible mechanisms of control of this enzyme and to explore the mechanism by which ACE may be regulated in the macrophage, the effect of corticosteroids on the ACE of macrophages in culture was investigated (*9, 10*). The results give the first evidence of a control mechanism for ACE, an enzyme important in blood pressure regulation.

Alveolar macrophages obtained from white New Zealand female rabbits in RPMI 1640 medium containing 100 units of penicillin G and 100 μg of streptomycin per millimeter by lung lavage (*11*) and centrifugation at 560*g* for 10 minutes at 5°C were either plated directly (35 by 10 mm plastic petri dishes; 6×10^6 cells in 1 ml) in RPMI 1640 medium containing 10 percent fetal calf serum (FC) which had been previously heated at 56°C for 30 minutes, 100 units of penicillin G and 100 μg of streptomycin per milliliter (RPMI containing 10 percent FC), or after first washing the cells three times in RPMI containing 10 percent FC. The cultures were incubated at 37°C in 10 percent CO_2. Cells were counted in a hemocytometer. Cells staining with eosin were considered nonviable. Steroids, colchicine, cycloheximide, and actinomycin D were dissolved in 95 percent ethanol and diluted in RPMI 1640 containing 10 percent FC, and 0.1 ml of the resulting solution was added to the cultures to give a final ethanol concentration of 0.043 to 0.15 percent in both experimental and control cultures.

Cells were harvested at intervals of up to 6 days by centrifugation as above. The media were saved for ACE assay. The cells were washed in 3 ml of 0.9 percent NaCl, centrifuged, suspended in 0.2 ml of 0.05*M* potassium phosphate buffer at *p*H 8.3, frozen at −85°C, sonicated at 0°C in four separated 10-second intervals (Biosonik III, setting of 30), and assayed immediately for ACE (*12*). The assay mixture was fortified with 0.28 percent (for cells) or 0.14 percent (for media) bovine serum albumin to stabilize the enzyme. The samples were also assayed for histidylleucine peptidase activity (*12*), and the ACE assay was corrected for any cleavage of the product during the assay. Protein was determined by the method of Lowry *et al.* (*13*).

The specific activity of ACE in rabbit alveolar macrophages increased several-fold in comparison to the control within 24 hours of exposure to 0.45 μ*M* dexamethasone or prednisone, and 7- to 16-fold after 3 days of exposure (Fig. 1). In the absence of glucocorticoid, only a modest increase occurred. After 4 days in culture and 3 days of corticosteroid stimulation, the largest increase in ACE from the level prior to culture was 53-fold in specific activity of cells and 39-fold in total activity recovered from cells and medium. There was little change in ACE in the medium 24 hours after corticosteroid induction and a significant increase after 3 days, coincident with a decrease in the number of cells, suggesting that the increased ACE in the medium may have been mainly due to release



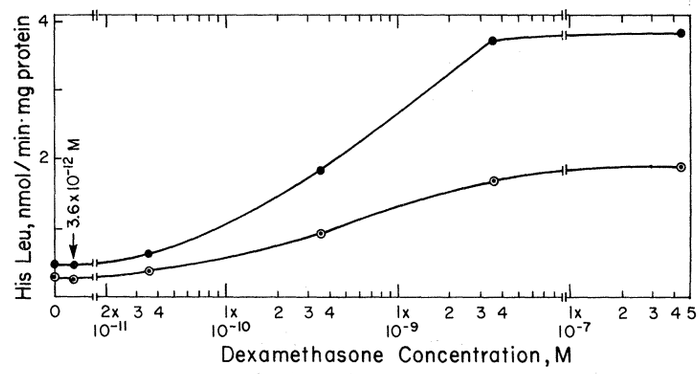


Fig. 1 (left). Induction of angiotensin converting enzyme in rabbit alveolar macrophages by dexamethasone. All cultures contained 0.043 percent ethanol after 24 hours of incubation (arrow), at which time dexamethasone was added. (○) 0.45 μM dexamethasone; (●) control. Fig. 2 (right). Effect of dexamethasone concentration on the induction of angiotensin converting enzyme in rabbit alveolar macrophages. Dexamethasone was added after 22 hours of cell culture along with ethanol at a final concentration of 0.043 percent. (○) At 46 hours of cell culture; (●) at 70 hours of cell culture.

from disintegrating cells. Both the glucocorticoid stimulated increase and the modest increase in the absence of steroid were somewhat greater when unwashed cells were used, suggesting that a factor enhancing the ACE increase is removed in the wash. Dexamethasone (1.4 μM) had no direct effect on the enzyme activity when added to the assay mixture. The properties of the greatly increased ACE activity in macrophages were entirely similar to those of rabbit lung ACE with respect to EDTA inhibition and $CoCl_2$ activation (*14*). Maximal, half-maximal, and slight ACE induction were obtained at approximately 4 nM, 0.6 nM, and 30 pM, respectively (Fig. 2), suggesting that ACE induction is mediated by binding of corticosteroid at physiologic concentration to a high affinity receptor site.

A 3.5-hour treatment with actinomycin D (0.1 μg/ml), introduced together with 0.45 μM dexamethasone after 22 hours of incubation, reduced by 47 percent the stimulatory increment in ACE observed in cells treated with 0.45 μM dexamethasone alone when the macrophages were harvested 24 hours after initiation of the dexamethasone treatment. The actinomycin D had no apparent effect on the viability of these cells. This result suggests that transcription of new messenger RNA stimulated by corticosteroid action is required for the corticosteroid induction of ACE (*15*). Simultaneous addition of cycloheximide (1 μM) and 0.45 μM dexamethasone resulted in a 77 percent reduction in the dexamethasone-stimulated increase in ACE after 47 hours of culture when about a 50 percent cell viability was observed, suggesting that the ACE is synthesized in response to corticosteroid stimulation (*16*).

Colchicine has a stimulatory action on the secretion of neutral proteinases by macrophages (*17*). In rabbit alveolar macrophages, 5 μM colchicine had no effect on steroid induction of ACE, which is active at neutral pH, and resulted in a slight decrease in ACE activity in the cells and medium when acting alone or in the presence of steroids.

Neither uninduced nor steroid-induced ACE activity was present in HeLa G, L-929, or LLC-MK2 cell lines grown in modified Dulbecco's medium supplemented with 5 percent FC and observed for 5 days in culture and 4 days after addition of 0.45 μM dexamethasone, although rabbit macrophage ACE increased 4.8-fold in relation to the control 24 hours after steroid addition under the same conditions. Thus, ACE activity or its induction by corticosteroids is not a general property of eukaryotic cells in culture.

Our results indicate that ACE is a corticosteroid inducible enzyme in rabbit alveolar macrophages, cells that normally have a low level of the enzyme in common with other macrophages (*9*). ACE was not induced in endotoxin- or thioglycholate-activated macrophages (*9*), although certain other enzymes are increased (*18*). These observations lend further support for the selectivity and diversity of action in macrophages (*18*).

Our investigation provides evidence for a regulatory mechanism for the synthesis of ACE, an important enzyme in blood pressure control. It is of interest that there is a molecular relationship between corticosteroids and ACE since both result in a vasopressor effect, although there is no evidence at present that the steroid hypertensive effect is significantly mediated by an ACE induction mechanism. The relation between macrophages and endothelial cells, which normally actively synthesize ACE, and the nature of ACE regulation in endothelial cells is under investigation. Our observations also suggest the possibility that an increased or highly potent inducer or extraordinarily high affinity receptor in epithelioid cells in sarcoidosis may be part of the mechanism for the very high ACE level in sarcoidosis lymph nodes (*5*).

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