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Response of Adrenal Tumor Cells to Adrenocorticotropin: Site of Inhibition by Cytochalasin B†

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ABSTRACT: The ability of cytochalasin B to inhibit the steroidogenic response of mouse adrenal tumor cells (Y-1) to adrenocorticotropin (ACTH) was examined with two aims: to consider the specificity of the inhibitor and to determine at what point(s) in the steroidogenic pathway it acts. Cytochalasin B did not inhibit protein synthesis or transport of [^{3}H]-cholesterol into the cells nor did it alter total cell concentration of ATP. Together with previous evidence, this suggests that the effects of cytochalasin observed are relatively specific in these cells. Cytochalasin inhibits the increase in conversion of [^{3}H]cholesterol to 20α-[^{3}H]dihydroprogesterone (20α-hydroxypregn-4-en-3-one: a major product of the steroid pathway in Y-1 cells) produced by ACTH but does not inhibit conversion of cholesterol to pregnenolone by mitochondrial and purified enzyme preparations from Y-1 cells and bovine adrenal, respectively. Cytochalasin does not inhibit the conversion of pregnenolone to 20α-dihydroprogesterone but was shown to inhibit increased transport of [^{3}H]cholesterol to mitochondria resulting from the action of ACTH. These findings indicate that cytochalasin acts after cholesterol has entered the cells and before it is subjected to side-chain cleavage in mitochondria. In view of the known action of cytochalasin on microfilaments, it is proposed that these organelles are necessary for the transport of cholesterol to the mitochondrial cleavage enzyme and that at least one effect of ACTH (and cyclic AMP) is exerted upon this transport process. The specificity of the effects of cytochalasin is considered in relation to this conclusion.

A previous report from this laboratory demonstrated that cytochalasin B inhibits the steroidogenic response of mouse adrenal (Y-1) tumor cells to ACTH[1] (Mrotek and Hall, 1975); cytochalasin does not inhibit the basal or unstimulated production of steroids. Because cytochalasin is known to inhibit the function of microfilaments, it was proposed that these organelles may play a part in the response of Y-1 cells to ACTH. Moreover, it appeared that the inhibitor acts before the conversion of cholesterol to pregnenolone which is the step of steroid biosynthesis stimulated by trophic hormones (Hall and Young, 1968; Hall and Koritz, 1965; Karaboyas and Koritz, 1965). It was decided to study the effects of cytochalasin B on Y-1 cells from two points of view, namely, to investigate the specificity of action of cytochalasin and to determine the site(s) on the steroidogenic pathway at which inhibition of the action of ACTH is exerted by this inhibitor.

Experimental Section

Culture of Cells. Mouse adrenal tumor cells (Y-1) (American Type Culture) were grown as monolayers in plastic tissue culture dishes (60 × 15 mm) as described previously (Mrotek and Hall, 1975). Cells were allowed to reach confluence before use in these studies. The method of incubating cells with various agents such as ACTH and cytochalasin has also been reported (Mrotek and Hall, 1975). [7α-^{3}H]Cholesterol (0.5 μCi; 0.05 pmol/flask) was added to the incubation medium in 50 μL of dimethyl sulfoxide–ethanol (1:1, v/v).

Assay of Medium and Cells. Following incubation, the steroid content of the medium was measured either as total steroids using a fluorimetric assay (Callard et al., 1965) or as 20α-dihydroprogesterone using a radioimmunoassay (Maroulis and Abraham, 1975). When mitochondria and mitochondrial enzymes were examined, pregnenolone was determined by a radioimmunoassay which has been described previously (Hall et al., 1975).

Levels of ATP in cells plus medium were determined by a fluorimetric method (Means and Hall, 1968). In some studies, cells were incubated with L-[U-^{14}C]phenylalanine (0.5 μCi/10^7 cells) and incorporation of ^{14}C into protein was measured (Means and Hall, 1967).

When cells were incubated with [7α-^{3}H]cholesterol, 20α-dihydro[^{3}H]progesterone was isolated by extracting the medium three times with diethyl ether after addition of carrier 20α-dihydroprogesterone. The combined extracts were taken to dryness and applied to thin-layer chromatograms (Kowal and Fiedler, 1968). 20α-Dihydro[^{3}H]progesterone was measured by liquid scintillation spectrometry and recovery of carrier steroid was determined by A_{240}; values shown in the accompanying data are corrected for recovery which varied within the range 62–83%. The identity of 20α-dihydro[^{3}H]-progesterone was confirmed by recrystallization with authentic 20α-dihydroprogesterone. When cells were incubated with

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[1] Abbreviations used: dibutyryl-cAMP and db-cAMP, N^6,$O^{2'}$-dibutyryladenosine 3',5'-monophosphate; pregnenolone, 3β-hydroxypregn-5-en-20-one; 20α-dihydroprogesterone, 20α-hydroxypregn-4-en-3-one; ACTH, adrenocorticotropin.

TABLE I: Time of Addition of Cytochalasin to Y-1 Cells.[a]

Time of Addition of Cytochalasin (min)	ACTH (units)	Steroid Production (μg/plate h^{-1})
	0	0.233 ± 0.010
	10^{-2}	0.910 ± 0.140
0		0.205 ± 0.005
0	10^{-2}	0.310 ± 0.020
15	10^{-2}	0.555 ± 0.025

[a] Plates of Y-1 cells were incubated with the additions shown as described in the Experimental Section. Following incubation, total steroid production was measured. Incubation was for 30 min and ACTH (10^{-2} U), when present, was always added at zero time. Values are means and ranges for duplicate determinations. The concentration of cytochalasin used was 10^{-5} M.

[7α-^{3}H]pregnenolone (0.5 μCi; 0.025 pmol/flask), the incubation, isolation, identification, and measurement of 20α-dihydro[^{3}H]progesterone were performed in the same way.

Cholesterol Transport. Extracellular [7α-^{3}H]Cholesterol. Cells were incubated in glass bottles with [7α-^{3}H]cholesterol (0.5 μCi; 0.05 pmol/bottle) for various periods of time. Bottles were then placed on ice and the medium was removed. Cells were washed twice in cold buffer (phosphate-buffered saline, pH 7.4) followed by 1% bovine serum albumin to remove extracellular [7α-^{3}H]cholesterol and finally in buffer containing 10 μM cholesterol added in 100 μL of *N,N*-dimethylformamide/L. The cells were then boiled in 10 mL of water which was transferred to extraction tubes and cooled; cell membranes remained on the incubation plates. The water was extracted three times with diethyl ether. The combined extracts were taken to dryness and applied to thin-layer chromatograms to isolate cholesterol (Simpson and Boyd, 1966). The region of the chromatogram containing [^{3}H]cholesterol was cut out and subjected to liquid scintillation spectrometry. Recovery of cholesterol by this procedure was determined by adding [7α-^{3}H]cholesterol at 4 °C to boiled cells, followed by the above procedure of ether extraction and chromatography; values for recovery were 92–96%.

Intracellular Transport. Cells were washed with saline and incubated in Hank's minimal medium containing aminoglutethimide (0.76 mM) for 15 min. [7α-^{3}H]Cholesterol (625 000 cpm; 0.03 pmol) in 20 μL of ethanol was added to each plate and incubation was continued for 30 min. At the end of this time, cells were washed with cold saline and fresh medium containing aminoglutethimide (0.76 mM) with or without ACTH and/or cytochalasin B (10^{-5} M) was added. Incubation was then continued for various times. Following incubation, cells were washed with cold saline and scraped from the plates in 5 mL of fresh saline. Cells were homogenized in glass/glass Dounce homogenizers and mitochondria were prepared as described elsewhere (Penman, 1966). The procedures following incubation were performed at 4 °C. An aliquot of resuspended mitochondria was taken for determination of protein by the method of Lowry et al. (1951) and the remaining suspension was extracted three times with ether. The combined extracts were taken to dryness and the content of tritium was determined by liquid scintillation spectrometry.

Mitochondrial Studies. The methods for preparing (Penman, 1966) and incubating mitochondria (Hall, 1972) were those reported in detail previously. Side-chain cleavage was measured by incubating mitochondria with cholesterol (50 nmol/flask) and measuring pregnenolone by radioimmunoassay (Hall et al., 1975). The method of preparing purified side-chain cleavage enzyme system (cytochrome P-450, adrenodoxin, and adrenodoxin reductase) (Shikita and Hall, 1973) and the details of incubation and assay of pregnenolone (Takagi et al., 1975) have been reported. To grow sufficient cells to prepare mitochondria, large plastic bottles were used (House, 1973).

Chemicals. Cytochalasin B was obtained from Aldrich Chemical Co., Inc., Milwaukee, Wisc. [7α-^{3}H]Cholesterol (Lot No. 636-033; 10 Ci/mmol), [7α-^{3}H]pregnenolone (Lot No. 537-168; 20 Ci/mmol), and L-[U-^{14}C]phenylalanine (Lot No. 892-013; 218 mCi/mmol) were obtained from New England Nuclear Corp. The preliminary purification of these radioactive substrates which was performed before use in these studies is given in Hall and Young (1968), Hall and Koritz (1964), and Means and Hall (1967), respectively. Sources of chemicals used in the measurement of ATP have been published (Means and Hall, 1968). In all cases, the best available grade of these chemicals was used. Diethyl ether was purchased from Mallinckrodt Chemical, St. Louis, Mo. ACTH and dibutyryl-cAMP and steroids, other than those given above, were obtained from Sigma Chemical Co. Elipten (aminoglutethimide phosphate; α-(*p*-aminophenyl)-α-ethylglutarimide phosphate) was a gift from Ciba-Geigy Corp., Summit, N.J.

Results

Cells and Steroid Assays. In a large number of preliminary studies with Y-1 cells, the methods used to determine total steroid production, pregnenolone, and 20α-dihydroprogesterone were validated with these cells as expected from results of other workers (Callard et al., 1965; Maroulis and Abraham, 1975) and our own experience with the pregnenolone assay (Hall et al., 1975). For total steroids, the assay showed a sensitivity of 0.1 μg and a standard error of ±0.07 μg within the concentration range used in these studies. The radioimmunoassay for pregnenolone gave a sensitivity of 0.3 pmol with a standard error of ±0.02 pmol, and the assay for 20α-dihydroprogesterone gave values of 0.3 ± 0.1 pmol.

Growth of monolayers to confluence produced a uniform population of cells in each culture dish; neither the determination of the protein content of cells nor cell counts appreciably altered the relative values for total steroids or 20α-dihydroprogesterone. Our results are, therefore, expressed per plate. One plate (60 × 15 mm) contains approximately 2.7 × 10^6 cells at confluence; this corresponds to 1.56 ± 0.06 mg (SE) of protein per plate. One experiment refers to a study using a group of plates containing cells grown at the same time and from the same subculture. Considerable variation in steroid production is seen between experiments so that comparisons are made only within one experiment. The concentration of ACTH used in these studies was 10^{-2} units in a final volume of 2 mL. Neither cytochalasin B nor cholesterol interfered with any of the three steroid assays used and cytochalasin B, which enucleates cells under certain conditions (Prescott et al., 1972), exerted no observable effects on cells or cell nuclei at the concentrations used in these studies. Steroid production by the tumor cells is linear for at least 2 h.

Effect of Cytochalasin B on Adrenal Tumor Cells. Previous studies showed that the increase in steroid production by Y-1 tumor cells, produced by addition of either ACTH or cAMP, is inhibited by concomitant addition of cytochalasin B (Mrotek and Hall, 1975). Table I shows that when cytochalasin is added 15 min after ACTH, the inhibition produced is approximately 60% of that observed when cytochalasin is added at zero time

TABLE II: Addition of Cytochalasin B, Pregnenolone, and [7α-^{3}H]Cholesterol to Y-1 Adrenal Tumor Cells.[a]

Substrate	Additions	20α-Dihydro-[^{3}H]progesterone (dpm/plate)
[7α-^{3}H]Cholesterol	None	64 900 ± 1900
	Cytochalasin B	74 700 ± 7100
	ACTH	140 700 ± 7600
	ACTH + Cytochalasin B	65 100 ± 200
[7α-^{3}H]Pregnenolone	None	215 000 ± 7000
	Cytochalasin B	212 000 ± 4000

[a] Y-1 cells were incubated with and without the additions shown. The concentrations of the two additions were ACTH 10^{-2} units and cytochalasin B 10^{-4} M. Following incubation, 20α-dihydro[^{3}H]-progesterone was isolated by thin-layer chromatography and measured by liquid scintillation spectrometery. Values with [7α-^{3}H]pregnenolone as substrate are means and ranges of triplicate determinations. Values with [7α-^{3}H]cholesterol as substrate are means and ranges of duplicate determinations.

TABLE III: Effect of Cytochalasin on Production of Pregnenolone by Mitochondria and by Cytochrome P-450.[a]

Cytochalasin B concn (M)	Source of Enzyme	Pregnenolone (nmol/min)
None	Mitochondria	1.92 ± 0.4
10^{-4}	Mitochondria	1.91 ± 0.4
None	Purified bovine enzyme	3.52 ± 0.08
10^{-7}	Purified bovine enzyme	3.23 ± 0.50
10^{-6}	Purified bovine enzyme	3.23 ± 0.53
10^{-4}	Purified bovine enzyme	3.33 ± 0.66

[a] Mitochondria were prepared from Y-1 adrenal tumor cells and were incubated (1 mg of protein/mL) with cholesterol (50 nmol) in a volume of 2 mL. Details of the method of incubating mitochondria are given elsewhere (Hall, 1972). Cytochrome P-450, adrenodoxin, and adrenodoxin reductase prepared from bovine adrenocortical mitochondria were incubated with cholesterol, and pregnenolone was measured on aliquots of the incubation medium following incubation by means of a radioimmunoassay. Details of the submitochondrial enzyme system have been published (Shikita and Hall, 1973).

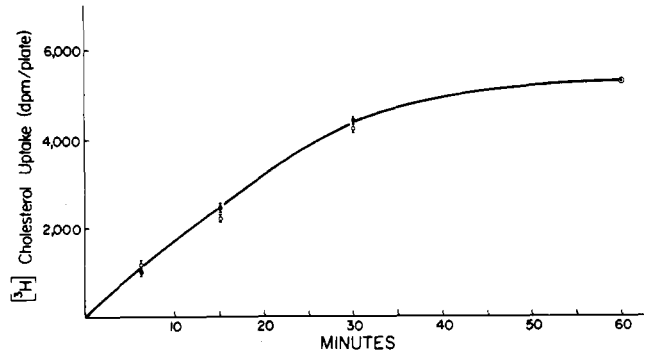


FIGURE 1: Y-1 cells were incubated with [7α-^{3}H]cholesterol (0.5 μCi; 0.05 pmol/plate) with and without cytochalasin B (10^{-4} M) for the times shown. Cellular [7α-^{3}H]cholesterol was determined as described in the Experimental Section. The values overlap to such an extent that only one line is drawn and symbols for ACTH and ACTH plus cytochalasin have been omitted (see Results). Values are means and ranges for duplicate determinations. (●) Control; (○) cytochalasin B.

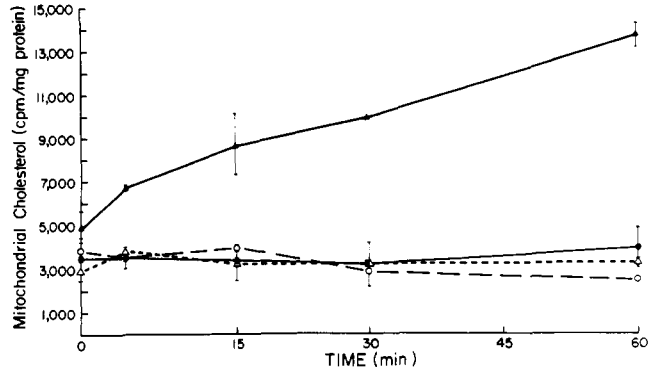


FIGURE 2: Y-1 cells were incubated with aminoglutethimide (0.76 mM) for 15 min. [7α-^{3}H]Cholesterol (625 000 cpm; 0.03 pmol/flask) was added and incubation was continued for 30 min. Cells were washed and fresh medium was added as follows: (△—△) Aminoglutethimide only; (●—●) cytochalasin and aminoglutethimide; (▲—▲) ACTH and aminoglutethimide; (○—○) ACTH, cytochalasin, and aminoglutethimide. Incubation was continued for the times shown. Following incubation, mitochondria were prepared and the content of [^{3}H]cholesterol was determined. Values are means and ranges for duplicate determinations, except where insufficient tissue was available for more than one determination.

(0.910–0.555 and 0.910–0.310, respectively). Since the duration of incubation was 30 min and the production of steroids is linear during this time, cytochalasin must inhibit the action of ACTH rapidly. Cytochalasin does not decrease control levels of steroid production ($P > 0.7$ in 12 determinations). It was also observed that when cytochalasin is added 5 min before ACTH, the inhibition is approximately the same as that seen when the two agents are added simultaneously (data not shown).

It can be seen from Table II that ACTH increases the conversion of [7α-^{3}H]cholesterol to 20α-dihydro[^{3}H]progesterone and that cytochalasin B inhibits this increase. Table II also shows that cytochalasin does not decrease the conversion of [7α-^{3}H]pregnenolone to 20α-dihydro[^{3}H]progesterone.

Protein Synthesis and Concentration of ATP. Cytochalasin does not inhibit incorporation of phenylalanine into protein by Y-1 cells nor does it affect the level of total cell ATP (data not shown).

Cytochalasin and Side-Chain Cleavage. Side-chain cleavage of cholesterol (cholesterol → pregnenolone) was measured in mitochondria from Y-1 cells and with a highly purified reconstituted side-chain cleavage enzyme system prepared from bovine adrenocortical mitochondria. It can be seen from Table III that a concentration of cytochalasin, which inhibits steroidogenesis (10^{-4} M) in the Y-1 cells, does not decrease the conversion of cholesterol to pregnenolone by either mitochondria or purified enzyme.

Cytochalasin and Transport of [^{3}H]Cholesterol into and within Y-1 Cells. Cytochalasin does not inhibit transport of [7α-^{3}H]cholesterol into Y-1 adrenal cells (Figure 1). In these studies, cells were incubated with [7α-^{3}H]cholesterol. Following incubation, [7α-^{3}H]cholesterol was isolated on thin-layer chromatograms and the ^{3}H content was measured. The amount of [^{3}H]cholesterol converted to steroids was less than 2% of the [^{3}H]cholesterol taken into the cell, so that intracellular [^{3}H]cholesterol provides a satisfactory measure of transport. In the experiment shown in Figure 1, values for ACTH and ACTH plus cytochalasin lie on the same line and cannot be presented because of confusion in the overlapping symbols.

Figure 2 shows the results of an experiment in which cells were preincubated with [^{3}H]cholesterol and a concentration of aminoglutethimide which we have found completely inhibits

TABLE IV: Reversal of Inhibition by Cytochalasin B of the Steroidogenic Response to Cyclic AMP.[a]

	Total Steroids (μg/h)	
	1st Incubation	2nd Incubation
Control	0.21 ± 0.03	0.28 ± 0.03
Cytochalasin B	0.19 ± 0.02	0.23 ± 0.04
db-cAMP	4.17 ± 0.06	3.90 ± 0.02
db-cAMP + cytochalasin B	2.12 ± 0.11	2.47 ± 0.13
db-cAMP + cytochalasin B	1.47 ± 0.31	*3.47 ± 0.17

[a] Incubation of Y-1 cells was performed with the additions shown for 1 h (1st incubation). The serum containing medium was removed for measurement of steroids, cells were washed, and incubation was resumed in fresh medium for 1 h (2nd incubation). The composition of medium and the various additions were the same as in the first incubation except in one case (indicated by an asterisk) in which the fresh medium contained dibutyryl-cAMP but not cytochalasin B. The concentration of cytochalasin used was 10^{-5} M. Values shown are means and ranges of duplicate determinations.

conversion of that substrate to steroids (data not shown). Three of the four conditions studied, namely, no cytochalasin, cytochalasin, and ACTH plus cytochalasin show no evidence of net transport of [^{3}H]cholesterol to mitochondria, since values do not exceed control during a period of 60 min. Either there is little transport of cholesterol to mitochondria under these conditions or cholesterol leaves mitochondria by some mechanism not involving side-chain cleavage at the same rate as it is transported to mitochondria during the 60 min of incubation.

On the other hand, ACTH produced a striking increase in mitochondrial [^{3}H]cholesterol and cytochalasin has prevented this response. The inhibitor did not alter levels of mitochondrial [^{3}H]cholesterol in the absence of ACTH (Figure 2). A second similar experiment gave the same results and, in addition, it was shown that more than 95% of the radioactivity associated with mitochondria was present as [^{3}H]cholesterol.

Reversal of the Inhibitory Effect of Cytochalasin B. Previous studies revealed that removal of cytochalasin by decanting medium, washing the cells once with sodium chloride (0.9% in water, w/v), and continuing to incubate with fresh medium caused the response to ACTH to be restored (Mrotek and Hall, 1975). Table IV shows that the same is true for cAMP (0.05 mM).

Discussion

Cytochalasin B is known to inhibit microfilaments (Wessels et al., 1971), but at high concentrations it also inhibits glucose transport competitively (Lin and Spudich, 1974). It was previously found that in Y-1 cells cytochalasin has the same effect upon the action of ACTH whether glucose is absent from the medium or present in high concentration (0.5 M)—a concentration which would have been expected to overcome the inhibitory effect of cytochalasin on the transport of this hexose (Mrotek and Hall, 1975). Moreover, the effective concentration of cytochalasin on the response of Y-1 cells to ACTH is below that required to inhibit glucose transport in other cells (Mrotek and Hall, 1975; Zigmond and Hersch, 1972; Estensen and Plagemann, 1972; Taylor and Wessels, 1973). The effect of cytochalasin on the response of Y-1 cells to ACTH cannot be ascribed to a nonspecific action on plasma membranes, since this substance also inhibits the effect of the second messenger (cAMP) (Mrotek and Hall, 1975), which is believed to be formed at an accelerated rate as the result of an effect of ACTH on this plasma membrane (Taunton et al., 1969).

Estensen et al. have suggested criteria for determining the specificity of inhibition by cytochalasin B (Estensen et al., 1971). The present studies meet the three criteria proposed, i.e., rapid onset (Figure 1), continuation of cell activities not requiring microfilaments (in the present case protein synthesis, total cell ATP, cholesterol transport into cells, steroid synthesis from pregnenolone, and side-chain cleavage), and rapid recovery after removal of cytochalasin which was reported previously for ACTH (Mrotek and Hall, 1975) and is shown here for cAMP (Table IV). It is, moreover, of interest to note that the unstimulated level of steroid synthesis is not decreased by cytochalasin; this strongly suggests that the inhibitor acts principally or only on the response to ACTH as far as steroid synthesis is concerned. As pointed out elsewhere (Mrotek and Hall, 1975; Hall, 1972), there is reason to believe that mitochondria may contain sufficient cholesterol to support unstimulated synthesis of steroids but not sufficient to permit the expected response to ACTH.

As far as the first objective of these studies is concerned, it appears that there are no grounds for dismissing the action of cytochalasin B on the response of Y-1 cells to ACTH as nonspecific. However, it must be added that it is not possible to exclude an effect of the inhibitor upon some cellular function not examined here.

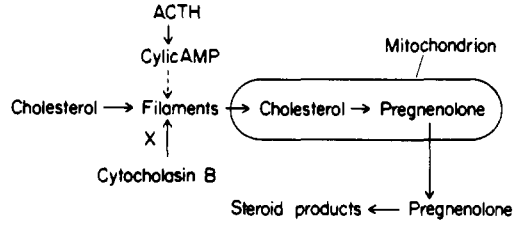
The present experiments enable us to answer the second question approached in these studies; cytochalasin B acts between intracellular cholesterol and the conversion of that steroid to pregnenolone. It is clear that cytochalasin is without effect upon the conversion of pregnenolone to one of the final steroid products of the pathway (Table II). It is, of course, well known that ACTH does not act beyond pregnenolone (e.g., Hall and Young, 1968). Moreover, neither tumor cell mitochondria nor the purified side-chain cleavage enzyme system from bovine adrenocortical mitochondria is inhibited by cytochalasin when conversion of cholesterol to pregnenolone is measured (Table III). Again, transport of [^{3}H]cholesterol into the tumor cells is not inhibited by concentrations of cytochalasin that block the response to ACTH (Figure 1). This is consistent with the fact that cytochalasin inhibits the steroidogenic response to ACTH with endogenous steroid substrates which presumably include cholesterol and do not require transport into the cell.

In addition, inhibition by cytochalasin is also observed when the conversion of exogenous [^{3}H]cholesterol to 20α-[^{3}H]-dihydroprogesterone is measured (Table II). This substrate must enter the cells and then reach the mitochondria; yet, side-chain cleavage in the mitochondria is not inhibited (Table III). Moreover, the movement of [^{3}H]cholesterol to mitochondria in cells in which side-chain cleavage was inhibited by aminoglutethimide was increased by ACTH, and this increase was prevented by cytochalasin. The increase produced by ACTH confirms the findings of Mahaffee et al. (1974) who showed that cholesterol accumulates in mitochondria from adrenal cortex when side-chain cleavage is inhibited by aminoglutethimide. The findings of these authors support the idea that the mitochondrial [^{3}H]cholesterol measured in our studies does, in fact, reflect transport of cholesterol to mitochondria from extramitochondrial sources, at least in the case of the additional level seen with ACTH (Figure 2). The inhibition by cytochalasin of the increased transport of cholesterol produced by ACTH suggests that the effect of the inhibitor is exerted on the transport of cholesterol to mitochondria. Since the present studies show that the action of cytochalasin is specific, it is tempting to conclude that microfilaments are involved in the transport of cholesterol to the mitochondira.

A number of lines of evidence suggest that transport of cholesterol to the mitochondrial enzyme system may be important in the regulation of steroidogenesis. For example, ACTH increases conversion of cholesterol ester to free cholesterol (Davis and Garren, 1966) and decreases the size of lipid droplets in the adrenal cortex (Zoller and Malamed, 1975). In either case, the cholesterol released from storage must reach the mitochondrion. Nothing is known at present of how this is brought about. One possibility, which deserves consideration, is that some contractile element within the cell is involved in the intracellular transport of cholesterol. If cytochalasin turns out to exert some as yet unknown effect on Y-1 cells, this effect must occur at the cholesterol transport step even though something other than or in addition to filaments is involved. Incidentally, inhibition of deesterification cannot explain our results which were obtained with free (unesterified) cholesterol.

These considerations raise questions about the mechanism of action of ACTH. If the hormone cannot stimulate steroidogenesis when microfilaments are inhibited and if this inhibition interferes with the transport of cholesterol to mitochondria, two things follow: (1) stimulation of steroidogenesis by ACTH requires active filaments and (2) the active filaments must be used to accelerate transport of cholesterol to the side-chain cleavage enzyme. We believe that our views can be summarized by Scheme I.

SCHEME I



X indicates inhibition

Since cytochalasin inhibits the responses to both ACTH and cAMP, it seems justifiable to indicate the nucleotide as mediating the effect of ACTH (Scheme I). However, other steps between cAMP and filaments may be involved, as indicated by the interrupted arrow. These findings encourage further studies to determine the mechanism of cholesterol transport within the adrenal cells.

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