379
N81
No. 5659

# CIGARETTE RESIDUES AFFECT STEROIDOGENESIS IN CULTURED Y-1 MOUSE ADRENAL TUMOR CELLS

THESIS

Presented to the Graduate Council of the North Texas State University in Partial Fulfillment of the Requirements For the Degree of

MASTER OF SCIENCE

By

Paula D. Morris, B.S.
Denton, Texas

December, 1979

Morris, Paula D. Cigarette Residues Affect Steroidogenesis in Cultured Y-1 Mouse Adrenal Tumor Cells. Master of Science (Biology), December, 1979, 32 pp., 7 figures, bibliography, 29 titles.

This study (1) quantitatively compared steroid production in cultured Y-1 mouse adrenal tumor cells exposed to Camel and Carlton-smoke derived residues, and (2) localized the effects in the cell.

Basal steroid production was increased by Camel residues but not by Carlton, while ACTH stimulation was interfered with by both residues. Camel basal stimulation was comparable to that of cAMP, and was abolished by Cytochalasin D. The stimulation was also comparable to that of cholera toxin, which activates adenyl cyclase.

Results indicate that residue components dissolve in the membrane stimulating adenyl cyclase at a point similar to or before that utilized by cholera toxin for its stimulating effect.

# TABLE OF CONTENTS

| | Page |
|---|---|
| LIST OF ILLUSTRATIONS . . . . . . . . . . . . . . . . . . . . | iv |
| Chapter | |
| I. SUMMARY. . . . . . . . . . . . . . . . . . . . . . | 1 |
| II. INTRODUCTION . . . . . . . . . . . . . . . . . . . | 3 |
| III. MATERIALS AND METHODS . . . . . . . . . . . . . | 6 |
| Cell Culture<br>Cigarette Residues<br>Experimental Procedure<br>Chemicals | |
| IV. RESULTS. . . . . . . . . . . . . . . . . . . . . . | 9 |
| V. DISCUSSION . . . . . . . . . . . . . . . . . . . . | 11 |
| ACKNOWLEDGEMENTS. . . . . . . . . . . . . . . . . . . . . . | 20 |
| APPENDIX. . . . . . . . . . . . . . . . . . . . . . . . | 28 |
| BIBLIOGRAPHY . . . . . . . . . . . . . . . . . . . . . | 30 |

## LIST OF ILLUSTRATIONS

Figure Page

1. Experimental Procedure. . . . . . . . . . . . . . 21
2. The Effect of Log Doses of Camel and Carlton Residues on Basal Levels of Steroid Production. . . . . . . . . . . . . . . . . . . 22
3. The Effect of Residues on Basal and ACTH Stimulated Steroid Production. . . . . . . . . . . . . . . . . . . 23
4. Residue Effect on Basal and cAMP Stimulated Steroid Production. . . . . . . . . . . . . . . . . . . 24
5. Cytochalasin D Inhibition of Camel Residue Stimulation . . . . . . . . . . . 25
6. The Effect of Cholera Toxin in Conjunction with Camel Residues. . . . . . . . . . . . . . . . . . . . 26
7. The Effect of Pregnenolone Addition on Residue and Control Steroid Production. . . . . . . . . . . . . . . . . . . 27

# CHAPTER I

## SUMMARY

The effect of cigarette residues obtained from non-filtered Camel and heavily filtered Carlton cigarettes on adrenal steroidogenesis was examined in cultured mouse adrenal tumor cells (Y-1). Stimulation of basal levels of steroid production was noted with Camel residues, but not with Carlton. ACTH stimulation was interfered with in both cases. The first possible explanation for this is the action of two separate agents of the many tar components, one or several causing basal stimulation and another causing inhibition of ACTH stimulation. In this instance, the filter might remove the stimulating agent(s), but not the inhibiting. The other possibility is that there may be only one acting agent or set of agents which is removed by the filter sufficiently to prevent basal stimulation, but not ACTH inhibition. The basal stimulation by Camel is similar to stimulation elicited by cAMP, the second messenger in the adrenal system. This stimulation is abolished by Cytochalasin D, which inhibits the function of microfilaments, known to be involved in the rate-limiting step in the steroid pathway. Changes in plasma membrane lipid structure appear to result from the lipid-soluble smoke constituents. When these changes occur, the interaction of the various components of

the ACTH-receptor complex may be prevented. On the other hand, the structural rearrangement may cause the activation of adenylate cyclase in the absence of ACTH, since control steroid production is increased in the presence of Camel residues. This idea is supported, since the level of stimulation is comparable to that of cholera toxin, which is known to activate steroid production by activating adenylate cyclase. The stimulation apparently does not involve microsomal cytochrome P-450, since added pregnenolone, bypassing the rate limiting step, causes no added stimulation.

# CHAPTER II

# INTRODUCTION

The formation of the second messenger, cyclic 3', 5' adenosine monophosphate (cAMP), is generally believed to be catalyzed by the plasma membrane enzyme, adenyl cyclase (12). Cigarette smoking significantly increases urinary and plasma levels of cAMP (25). This increase may be due either to blood-borne cigarette-derived compounds which cause increased epinephrine and vasopressin (ADH) stimulation of target cell adenyl cyclase, or, possibly, to a direct activation of adenyl cyclase in the renal tubules by these compounds (4, 25). These alternatives have yet to be explored.

Cyclic AMP is an important second messenger in many tissues, including adrenal tissue (12). Since smoking increases the circulating levels of cAMP, it could have effects on the cAMP responsive systems of the adrenal. _In vitro_, Mrotek and Hall observed a slow increase in steroid production in response to added cAMP in the media (unpublished results). If this _in vitro_ situation may be extrapolated to the _in vivo_ high cAMP concentration caused by smoking, increased blood levels could directly increase steroid production. In addition, the adenyl cyclase system in the cell might be affected by the smoke constituents. In the adrenal, adrenocorticotropic hormone (ACTH) stimulation

of steroidogenesis is preceded by an increase in cAMP. This increase is mediated by the membrane-bound adenyl cyclase enzyme which is associated with the ACTH receptors (27).

The polycyclic aromatic hydrocarbons (PAH) found in cigarette smoke (29) might interfere with steroid synthesis. In liver microsomes, metabolism of PAH occurs via cytochrome P-450 (5). This enzyme complex has been identified as a component in the steroidogenic process, and is located in adrenal mitochondria and microsomes (7). Both hepatic and adrenal P-450 appear to be similar in function, form, and kinetic properties. For example, Kuntzman and coworkers found that hepatic microsomal P-450 metabolizes both drugs and steroids (16). Ultrastructural studies also reveal a morphological resemblance between adrenal cells and PAH-exposed hepatocytes in which the smooth endoplasmic reticulum (SER) induced by PAH is similar to the SER of the adrenal (8).

Since circulating cigarette residues may affect many organs, endocrine organs, whose synthetic and secretory processes depend on adenyl cyclase and P-450 oxidations, offer an advantage in the identification of the effects of these residues. Previous studies examining cigarette residue effects on cultured cells compared the toxicity of these residues (10). The advantage of the use of cultured secretory cells lies in the ability to examine cigarette effects on a specific physiological function.

Current cigarette advertising emphasizes the ability of modern filters to reduce tars. Tars are the source of PAH (29). Ghanayem *et al*. demonstrated a reduced biologic response when filtered cigarette smoke condensate or extract was administered to cells in culture (10). In this study, differences between the effects on adrenal cells of a cigarette with no filter (Camel) and one with a very effective filter (Carlton) were compared to determine the effect of non-filterable cigarette residues on the adrenal.

# CHAPTER III

# MATERIALS AND METHODS

## Cell Culture

Y-1 adrenal tumor cells (American Type Culture, Rockville, Md.) were prepared and maintained in plastic flasks, as prepared by Mrotek and Hall (11).

## Cigarette Residues

Cigarette smoke residues were obtained by suction through an aquafilter and tubing into a gas dispersion tube immersed in twenty ml of acetone. A disposable syringe attached to a tube connected to a light opaque smoke chamber provided the suction. Thirty cubic centimeters of suction were administered per puff, at the rate of one puff every thirty seconds. Cigarettes were smoked to within five mm of either the filter (Carlton) or the end of the cigarette (Camel). After ten cigarettes were "smoked" into the acetone, the aquafilter and gas dispersion tube were rinsed with twenty additional ml of acetone which were added to the original extract. The acetone was evaporated and residues were redissolved in seven ml of ethanol. In preliminary experiments, ethanol exerted the least interference with control production levels.

Experimental Procedure

The incubation procedure shown in Figure 1 consisted of a preincubation of cells with extracted residues dissolved in ten microliters (μl) of ethanol per ml of serum-containing media. Treatments and controls were dissolved in non-serum-containing media. Non-serum-containing media were used in the last three incubations to remove serum proteins containing adsorbed steroids. Mrotek, Carraway and Carraway found that proteins adhere to the culture plate and contribute to variability in the assay procedure (unpublished results). In the case of cholera toxin, five one-half-hour incubations were conducted, with the toxin being included in the final three incubations to accomodate the one-hour lag period in the action of the toxin, as determined by Wishnow, et al. (28). The concentration of cigarette residues was maintained throughout all incubation periods for a given treatment. The three ml of media from the last one-half hour incubation, as well as the saline wash of the flask after media removal, were collected in ether. The cells were then trypsinized and mixed with EGTA to disperse them. Trypsin inhibitor was added prior to cell counts, using a Coulter Counter (Model No. ZBI, Coulter Electronics, Inc., Haleah, Fla). The trypan blue exclusion method of Phillips was used to measure cell viability (23). Media were assayed for steroids by extraction with ether and measurement by radioimmunoassay as 20a-hydroxypregn-4-en-3-one

(20a-dihydroprogesterone) by the method of Abraham and Maroulis (1), as modified by Mrotek and Hall (20).

### Chemicals

Cytochalasin D, ACTH and dibutyryl cAMP were obtained from Sigma Chemical Co., St. Louis, Mo. Pregnenolone (3$\beta$-hydroxy-pregn-5-en-20-one) was obtained from Nutritional Biochemicals Corp., Cleveland, Ohio. Cholera toxin was donated by M. Openshaw and Y. Chen of North Texas State University.

# CHAPTER IV

## RESULTS

Solutions of residues prepared from Camel and Carlton dissolved in ethanol (EtOH) contained 6.2 mg/ml, and 0.9 mg/ml, respectively. Since there were approximately 1.43 cigarette equivalents/ml EtOH for both Camel and Carlton, then one Camel cigarette contains 4.34 mg/residue, and there is 0.62 mg residue/cigarette for Carlton. In these experiments, treatments were based on cigarette equivalents rather than milligram equivalents, to obtain comparative results. The doses used caused 50 per cent or less cell death in every case, and results are expressed as picograms (pg)/viable cell. The concentration of EtOH used was 1 per cent, one-half of the 2 per cent maximum concentration generally accepted for use as a solvent. Standard residues prepared by the Council for Tobacco Research, New York, New York [a gift from Ghanayem, _et_ _al_. (10)] were used to establish the twenty-four-hour preincubation time (data not shown).

The response of basal steroidogenesis to log doses of Camel and Carlton given in ten ul EtOH/ml media is shown in Figure 2. There was a discernible stimulation of steroid production by Camel. Although Carlton showed progressive increases in steroid production with increasing concentrations of residue, none of these values appeared to be

different from those for the solvent control. Subsequent studies utilized 0.062 mg/ten μl EtOH/ml media as the preferable cigarette equivalent value.

The ACTH-stimulated increase of 20a-dihydroprogesterone in the media was greatly inhibited in the presence of the Camel residue and moderately inhibited by Carlton residue (Figure 3).

The steroidogenic stimulation exerted by dibutyryl cAMP (dbcAMP) (Figure 4) was unaffected by Camel or Carlton.

Figure 5 illustrates that Cytochalasin D dramatically inhibited the stimulation of Camel residues without affecting baseline production of steroids.

Steroids present in the media were no greater after cholera toxin or residue alone than for the two in combination (Figure 6).

Addition of pregnenolone to bypass the rate-limiting step in steroidogenesis (13, 22) led to increased media 20a-dihydroprogesterone (Figure 7). This production was not enhanced or inhibited by residues.

# CHAPTER V

## DISCUSSION

The adrenal steroidogenic cycle begins with ACTH receptor attachment and subsequent activation of adenyl cyclase (12). Simultaneously, or because of this, microfilaments are thought to increase the supply of cholesterol to the mitochondria, thereby accelerating the conversion of cholesterol to pregnenolone (19). When exogenous cAMP is added to these cells, the effect of ACTH is bypassed (12). Inhibitors of microfilaments interfere with the stimulation of steroid production by ACTH at the rate-limiting step (19, 20), while the addition of exogenous pregnenolone to the cells bypasses the mitochondrial conversion of cholesterol to pregnenolone by entering the cell and serving as substrate in the later steps of the steroidogenic pathway (13, 22). These observations were utilized in this study to indicate the site of action of ethanol-soluble cigarette smoke residues obtained from Carlton and Camel cigarettes.

In Figures 2, 3 and 7, some stimulation of steroid production was elicited by EtOH alone. Although this effect was not investigated further in this study, I feel that it may be due to 1) an effect on receptor conformation, 2) the metabolism of EtOH, resulting in the production of NADPH by the mitochondria, 3) a change in the transport of

cytoplasmic cholesterol to the mitochondria, or, 4) EtOH may solubilize the product steroids, thus increasing transport into and out of the cell or within the medium. An increase in the solubility of steroids within the medium might decrease feedback inhibition, resulting in increased steroid release, as suggested by Peron and Moncloa (21). The last two possibilities seem likely in view of results in greater stimulation than non-ethanol-treated cells. Whatever the cause of stimulation, normal ratios of control and stimulated steroid production are consistently maintained, and the variation caused by EtOH should not affect interpretation of these results.

Some variability in the absolute level of response was seen between experiments. This may have been due to aging of the cultured cell population with increasing numbers of passages. Y-1 cells maintained through thirty passages exhibit less steroid production than cells freshly received from American Type Culture (unpublished results). This observation is consistent with those reported by Sato and Bounassi (24).

Preliminary experiments with standard residues (data not shown) established that twenty-four hours of preincubation with residues was necessary to obtain increased steroidogenesis by the cells. The log doses in Figure 2 show that Camel is a much more effective stimulant of steroidogenesis per live cell than Carlton. In this experiment, 0.062 mg/ml medium (0.0143 cigarette equivalents/ml medium) of

ethanol-soluble smoke residue from Camel gave a noticeably greater stimulation than the next lower dose. Although this was by no means the maximum stimulation concentration, this dose was chosen for further study because of its effect on steroidogenesis and the lack of effect on cell viability.

Studying the effect of the residues on cell response to ACTH represented in Figure 3, Camel dramatically inhibited ACTH-stimulated 20a-dihydroprogesterone production, yet stimulated basal steroidogenesis at the same concentration of residue. Carlton did not stimulate baseline steroid production but inhibited ACTH stimulation, although not as much as Camel. Two explanations are offered for these observations. First, of the many components in the extracts, Camel residues might contain two components, one stimulatory under the conditions of basal steroidogenesis and the other inhibitory to ACTH stimulation. In this case, the Carlton filter might remove the former and a small amount of the latter. Alternatively, perhaps Camel residues contain enough of the stimulating component both to increase basal, unstimulated production and to interfere with ACTH. On the other hand, there did not appear to be enough of this component in Carlton to cause stimulation, yet there was enough to interfere with ACTH interactions with the cell. These two possibilities cannot be distinguished at present.

The stimulation by dbcAMP seen in Figure 4 was not as great as others have reported (19, 20). I have no

explanation for this. Since there is a twofold increase in steroid production following administration of dibutyryl cAMP, this minor depression of response should not affect my interpretation of the results. Carlton again gave no basal stimulation, while Camel stimulated basal production to a level comparable to that produced by dbcAMP and that for dbcAMP plus residue. As for ACTH, no additive effects were seen to result from an interaction of residue with cAMP. Such an observation is consistent with the view that the cell is operating maximally in the presence of dbcAMP or Camel residue.

Cytochalasin B and D have been implicated in the disruption of microfilaments; Cytochalasin D seems to exhibit fewer side effects and is effective at lower concentrations than Cytochalasin B (17, 26). The concentration of Cytochalasin D (0.01 mM) comparable in effectiveness to that used most by Mrotek and Hall (19, 20) in Cytochalasin B studies on my cells was examined in an attempt to determine whether the effects of cigarette residues were mediated by microfilaments. In addition, we examined the effects of 0.1 mM Cytochalasin D and results comparable to those for 0.01 mM were obtained. Since the experiments with the lower concentration were performed on an older cell population, the level of steroid production for all treatments was proportionately reduced. Therefore, I elected to report the effects of the larger concentration. The degree of effect is not

changed. The results obtained using 0.1 mM Cytochalasin D, seen in Figure 5, confirm those of Mrotek and Hall. I find, in addition, that the alteration of microfilaments reduced the stimulation obtained in the presence of Camel residues. No effect of Cytochalasin D on control levels was seen, indicating that Cytochalasin D only affected steps subsequent to those controlled by ACTH or Camel residues. The facts that the residues affect ACTH but not cAMP, and that these effects of the residues were inhibited by Cytochalasin D, suggest that the residue was acting somewhere in the steps leading to activation of adenyl cyclase.

Mrotek and Hall have postulated that microfilaments control the transport of cholesterol to the mitochondria (20). Also, Belloni _et al_. observed that large mitochondrial inclusions believed to be cholesterol are found in adrenal cells from rats treated with 7, 12-dimethylbenzanthracene (DMBA), which is one PAH found in cigarette smoke (3). Belloni suggested that the accumulation of cholesterol resulted from a competitive inhibition for P-450 between cholesterol and DMBA. While the possibility of inhibition of mitochondrial P-450 has not been investigated in this work, it seems likely that if Camel stimulation is mediated by microfilaments, as implied by my results, these cholesterol inclusions may be a byproduct of increased transport of cholesterol to the mitochondria as well as a product of competitive inhibition of P-450.

A more specific delineation of the site of action of cigarette residues on adenyl cyclase became desirable, to confirm my results. The steroidogenic pathway in the Leydig cells of the testis is stimulated by luteinizing hormone and human chorionic gonadotrophin, resulting in increased cAMP production (12, 6). Cholera toxin is known to activate adenyl cyclase in this tissue (2, 9, 11, 18) as well as in adrenal tumor cells (28). For the testis, the activation is presumed to occur through 1) binding of the B protomer of choleragen to cell surface receptors, and 2) penetration of the A protomer through the lipid membrane to activate the cyclase (22). Because of the lipid solubility of the residues, I suspected that the residues were activating steroid production by dissolving in the lipids of the membrane and increasing cAMP production by causing adenyl cyclase conformational changes. In Figure 6, it is seen that Camel stimulates basal steroid production to a level approximately equal to that caused by cholera toxin. No additive effect of the residues upon baseline stimulation was observed in the presence of cholera toxin. Carlton had no appreciable effect on basal or cholera-toxin-stimulated production. These observations are consistent with the idea that the components of Camel residues may be stimulating either in the same manner as cholera toxin or at some point previous to this site, i.e., an indirect conformational effect on the adenyl cyclase located in the lipid portion of

the membrane. If this view is correct, then the observation that cigarette residues inhibit the activity of ACTH is explained by the fact that each interferes with the other. If ACTH acts to stimulate in a two-step fashion by first binding to its receptors and then activating adenyl cyclase (14), one can visualize ACTH as binding but not activating in the presence of Camel residue. On the other hand, it would appear that the cigarette residue might activate by way of a two-step sequence, as does cholera toxin. In the presence of the residue, the events occurring within the lipid portion of the membrane occur; however, the interaction of ACTH with the receptor portion of the membrane may be conformationally interfered with; thus reduced steroid production results.

In view of the presence of an abundance of cytochrome P-450 in adrenal cells, it was necessary to investigate whether the residues had any additional effects on steroidogenesis other than those on the membrane. The possibility of an effect on microsomal P-450 was examined by the addition of pregnenolone, which is able to bypass the rate-limiting activities associated with the relocation of cholesterol to mitochondrial P-450 (13, 22). Cells exposed to Camel residues to which pregnenolone was added exhibited conversion of pregnenolone to 20a-dihydroprogesterone in amounts comparable to the ranges obtained for control. I conclude that no effect of the residues on the microsomal enzymes was measurable within the sensitivity of my methods of assay. Therefore,

I suggest that the residue stimulation does not involve microsomal enzymes, nor does there appear to be an effect on steroid release since the concentration of 20a-dihydroprogesterone in cells with added pregnenolone remained the same both in the presence and absence of Camel residues. The possibility that mitochondrial P-450 is inhibited remains to be investigated, although it seems unlikely in view of the stimulation I obtained using Camel residues.

My results are consistent with the hypothesis that ethanol-soluble components of cigarette residues obtained from a non-filtered cigarette dissolved in the membrane of cultured adrenal tumor cells. Here they affected the conformation of the intermembranous molecules associated with the ACTH receptors, inhibiting the ability of ACTH to stimulate cAMP production. The residues alone may have activated adenyl cyclase independently of the receptors, by the same conformational changes. In this event, microfilaments were activated to increase cholesterol transport to the mitochondria, resulting in activation of the rate-limiting step of steroidogenesis. Residues obtained from filtered cigarettes may differ either in absolute content or in bulk of constituents. There appeared to be enough of the membrane-soluble component present in Carlton residues to inhibit ACTH stimulation, but not enough of the stimulating component to activate adenyl cyclase. The stimulation does not appear to

be occurring due to an effect of the residues on microsomal P-450.

Since most people smoke cigarettes in times of stress or to help them cope with general stress, and since cortisol is important in aiding the body in stressful situations, then, if my conclusions are correct, the smoking of a cigarette may actually give a small burst of cortisol during smoking, which would help aid in a stressful situation. However, if a truly stressful situation arises and ACTH is released by the pituitary to cause increased cortisol production, this natural stimulation is interfered with, resulting in an inability to cope well with such situations.

## ACKNOWLEDGEMENTS

I am grateful for the excellent technical assistance of Bill Rainey and Kathy Ebenhack. I am also indebted to Dr. D. Busbee, Dr. E. Cantrell, and Burhan Ghanayem, who provided advice and cigarette residues to initiate these experiments. Dr. Busbee also generously donated equipment essential to my research. Mike Openshaw and Dr. Y. Chen provided cholera toxin.

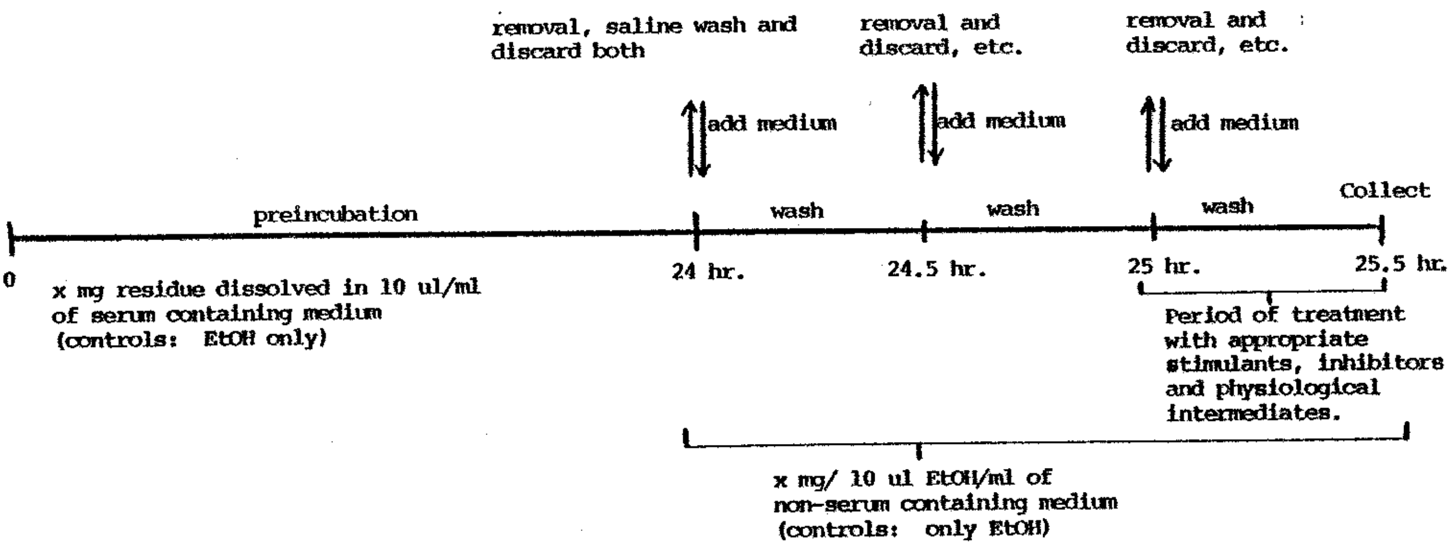
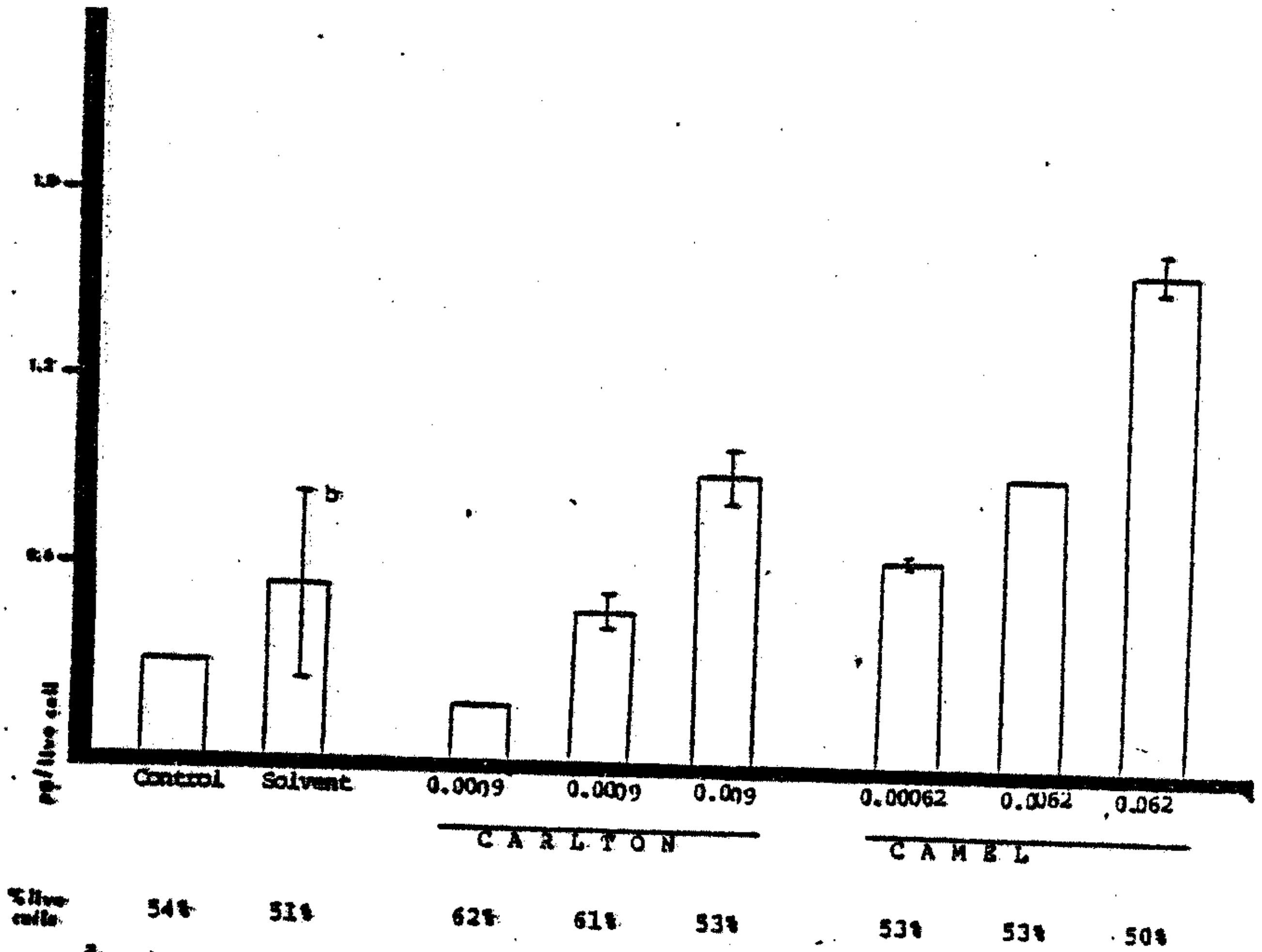
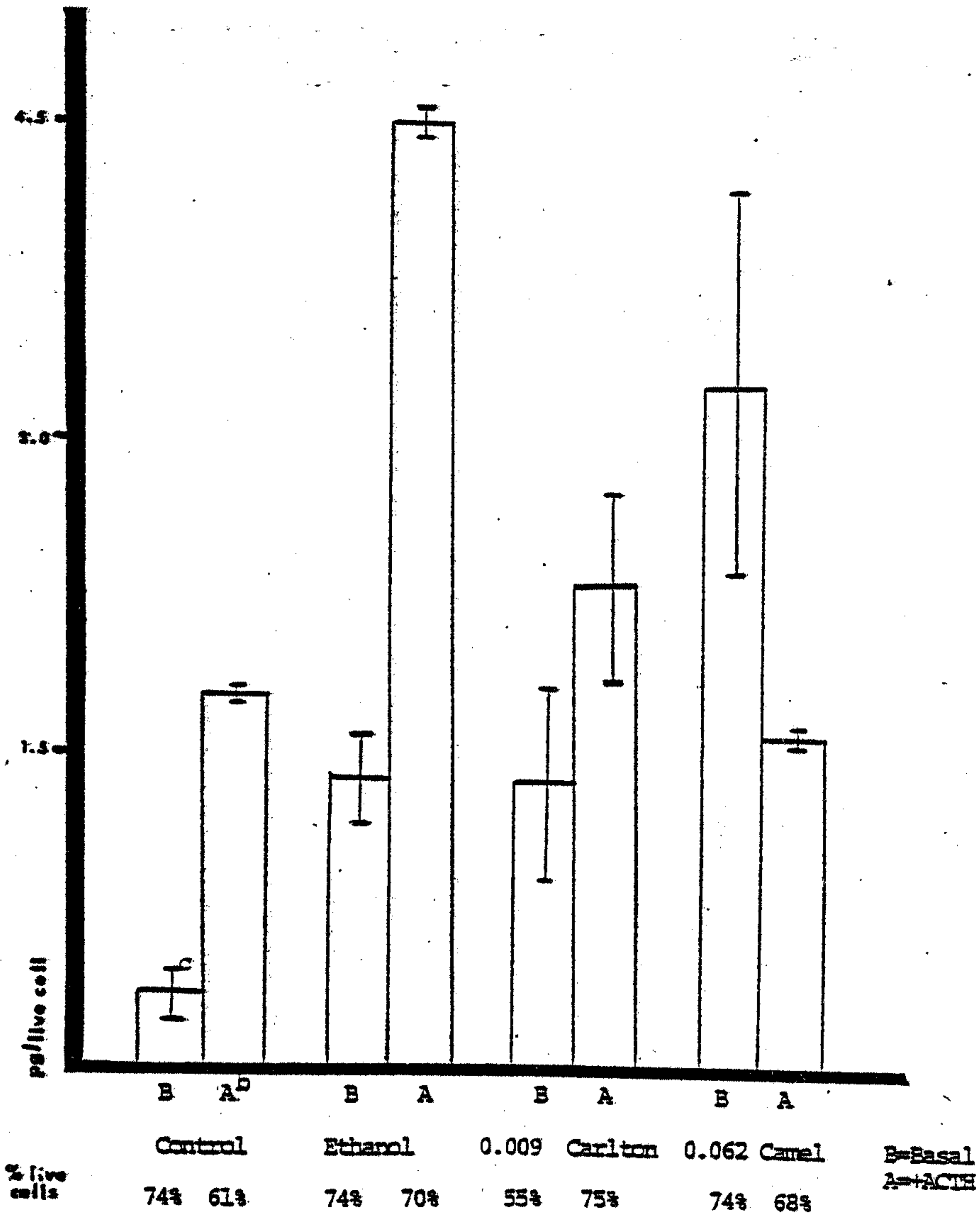



Fig. 1--Experimental procedure.



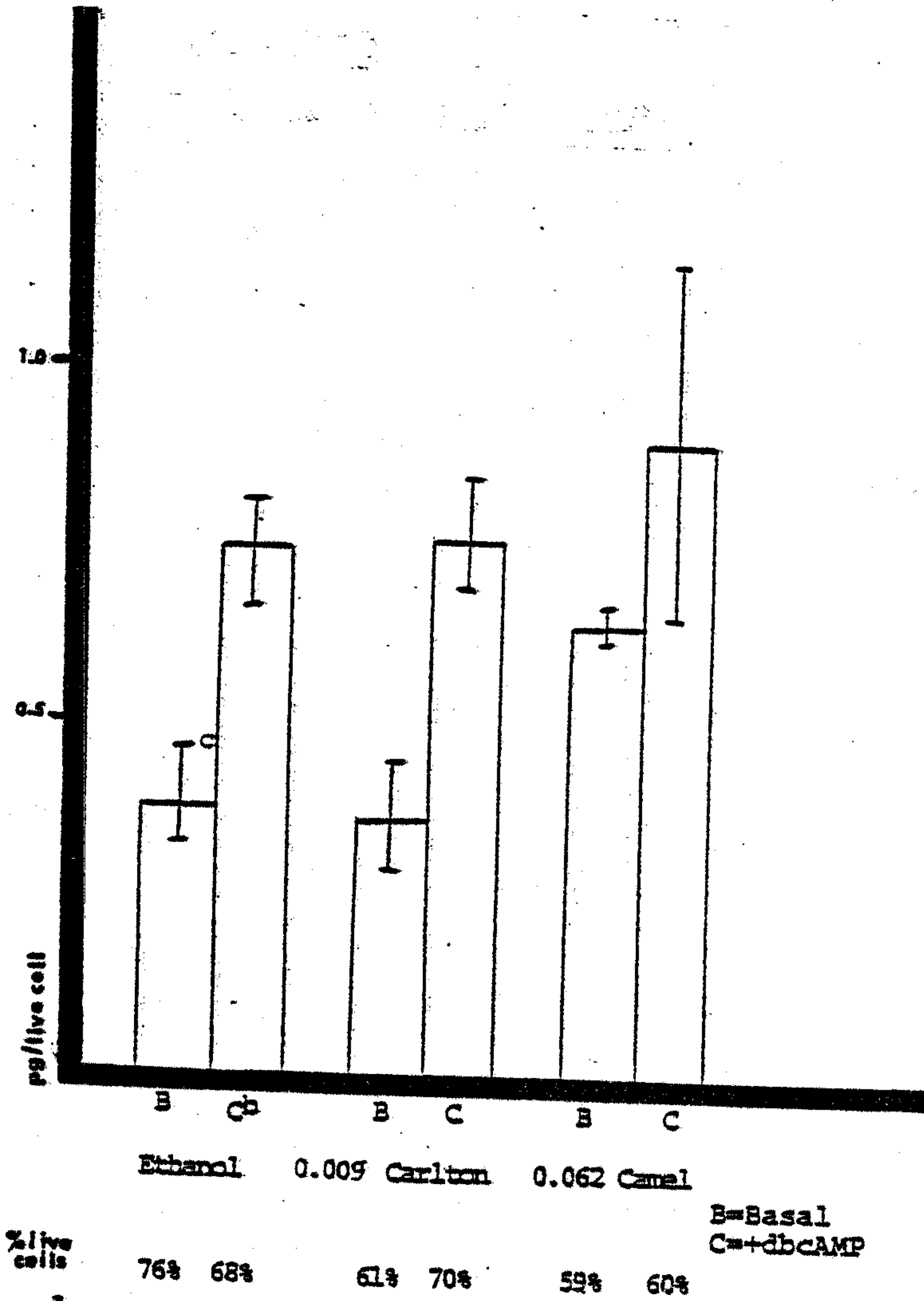

[a]Residue doses are expressed in mg/10ul EtOH/ml medium.
[b]Error bars represent Standard Deviation in two determinations.

Fig. 2—The effect of log doses of Camel and Carlton Residues on basal level steroid production.[a]




[a]Residues expressed in mg/10ul EtOH/ml medium.
[b]ACTH was added 4.9077 mU/ml medium.
[c]Error bars show Standard Deviation in two determinations.

Fig. 3—The effect of residues on basal and ACTH stimulated steroid production.[a]

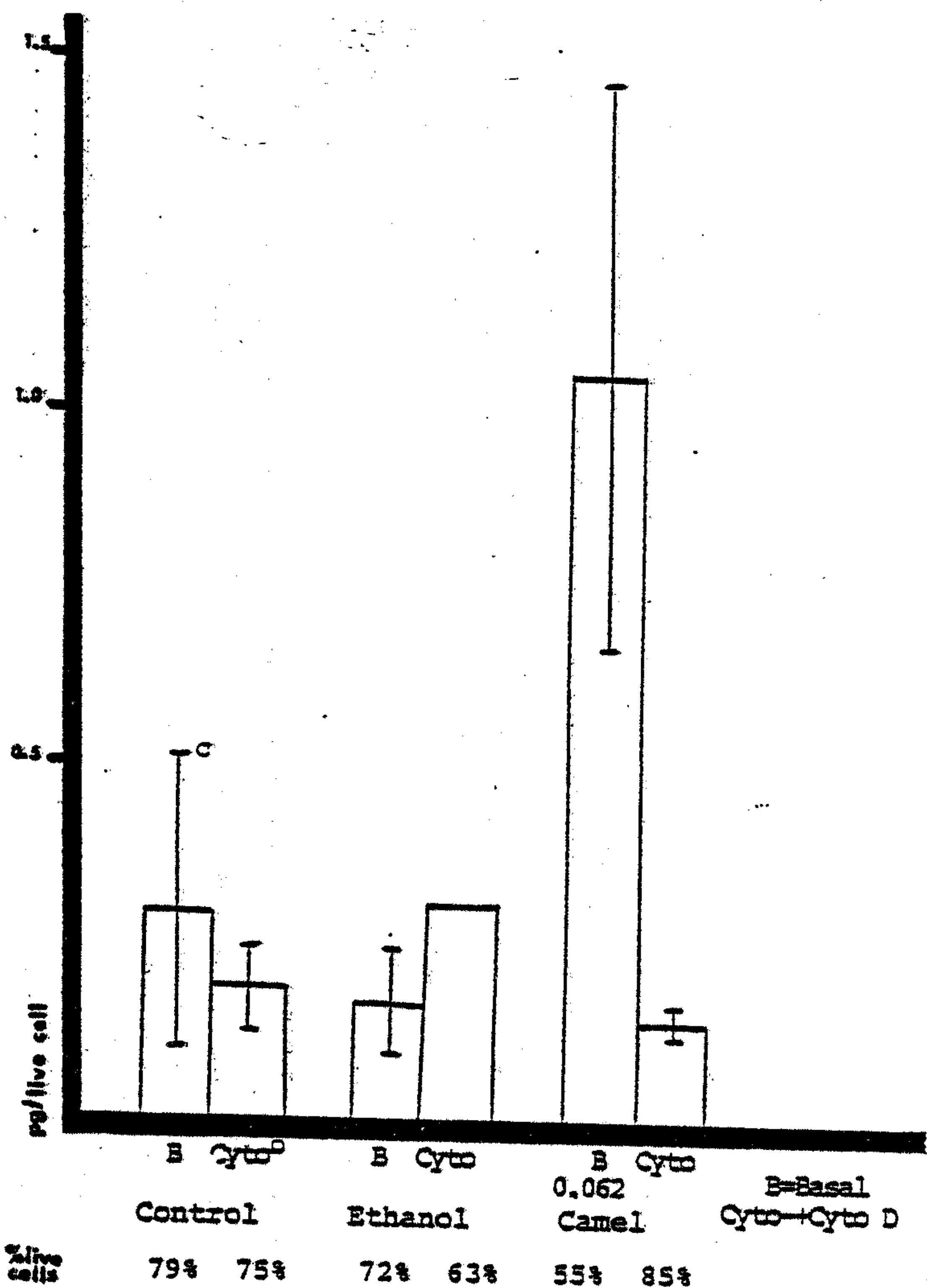



[a]Residues expressed as mg/10ul EtOH/ml medium

[b]dbcAMP was $10^{-3}$M final concentration in the medium

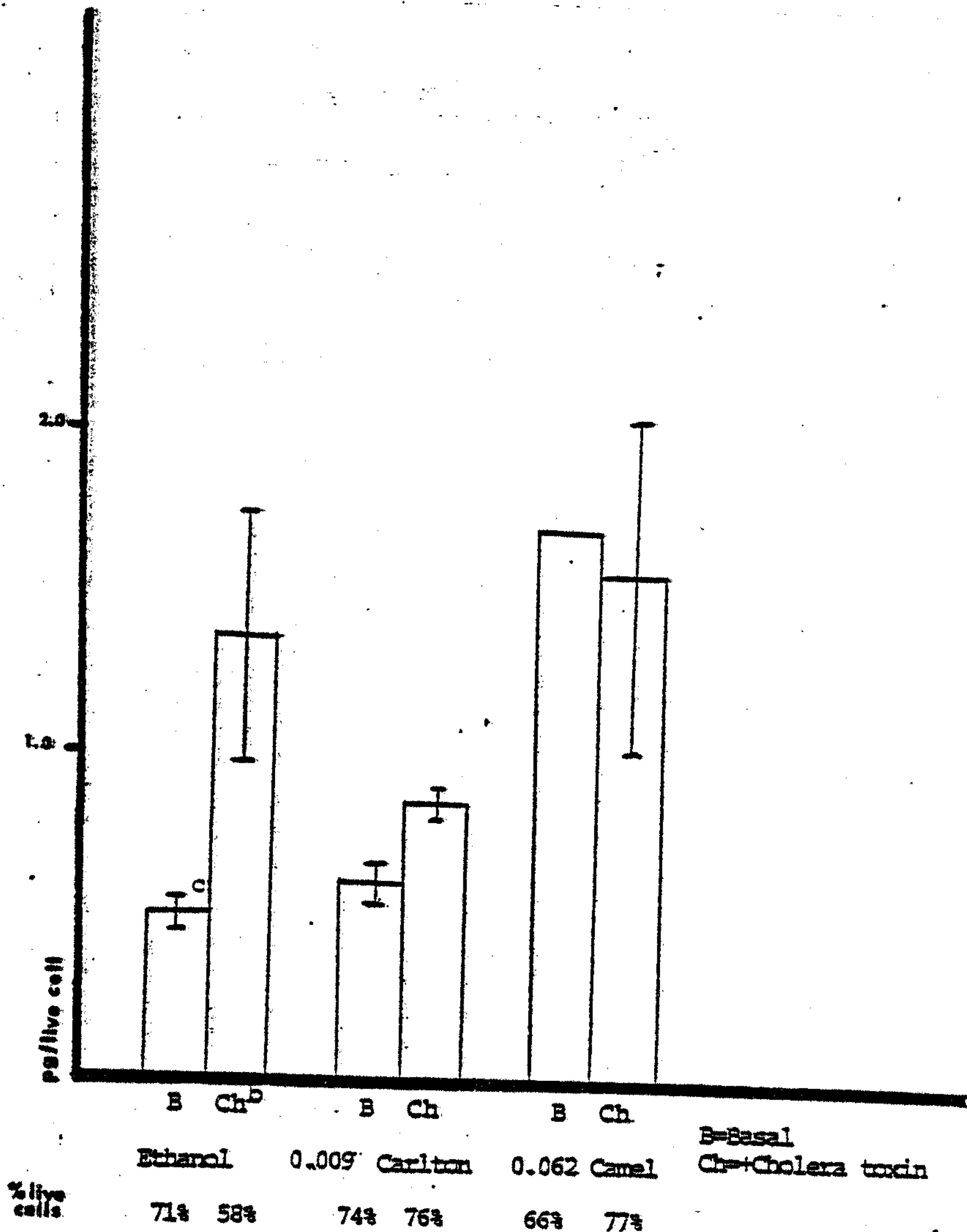
[c]Error bars represent Standard Deviation in two determinations.

Fig. 4--Residue effect on on cAMP stimulated steroid production.[a]



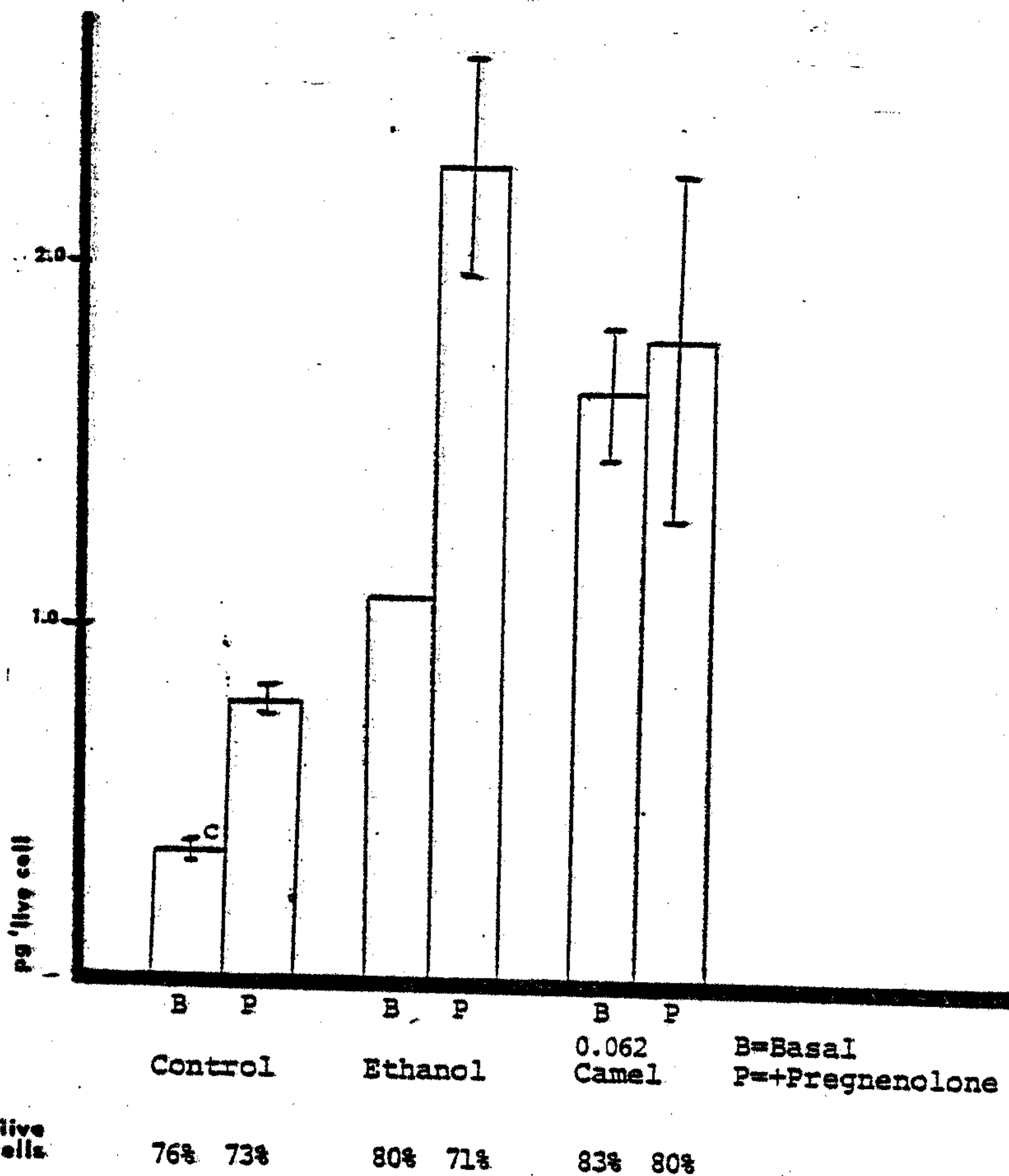

[a]Residue expressed in mg/10ul EtOH/ml medium.
[b]Cytochalasin D was $10^{-4}$M final concentration in the medium.
[c]Error bars represent Standard Deviation in two determinations.

Fig. 5—Cytochalasin D inhibition of Camel residue stimulation.[a]




[a]Residue doses are expressed in mg/10ul EtOH/ml medium.
[b]Cholera toxin was 50 ng/ml medium.
[c]Error bars represent Standard Deviation in duplicate determinations.

Fig. 6—The effect of cholera toxin in conjunction with Camel residues.[a]




[a]Residue doses are expressed in mg/10ul EtOH/ml medium.
[b]Pregnenolone was 50 ng/ml medium.
[c]Error bars represent Standard Deviation in duplicate determinations.

Fig. 7--The effect of pregnenolone addition on residue and control steroid production.[a]

# APPENDIX

I suggest the following for future research to elucidate the effects of unfiltered cigarette residues on steroidogenesis:

1) It is known that ACTH stimulation of Y-1 cells requires a period of recovery before further stimulation may occur. A study to test if residue stimulation is also refractory might give a comparison between ACTH and residue stimulation.

2) My conclusions suggest the need for an intact cell for stimulation of steroid production by the residues. This suggests a study of residue effect on cell homogenate fractions for further proof of this conclusion.

3) Cholera toxin catalyzes the hydrolysis of NAD to nicotinamide and ADP ribose (18). If residues were shown to do the same, this would be further proof of the similarity of cholera toxin and residue action.

4) To test Belloni's conclusion, along with mine, that cholesterol is accumulating in the mitochondria upon cigarette constituent addition; loading of cells with radioactive cholesterol and localization during residue stimulation might prove interesting.

5) Further elucidation might be obtained by adding radioactive residue components to the cells and localizing their accumulation, if any, in the cell.

6) Perhaps in a biochemical laboratory, a high-performance liquid chromatographic fractionation of Carlton and Camel constituents to compare content and then to isolate the active component would provide conclusive information on residue activity.

7) Of course, a study of the effects of cigarette components on the adrenal in the *in vivo* situation is highly desirable to support the validity of these results.

BIBLIOGRAPHY

1. Abraham, G. G. Maroulis, and J. Marshall. Evaluation of Ovulation and Corpus Luteum Function Using Measurements of Plasma Progesterone. Anal. Lett. 8 (1975) 377.

2. Azhar, S. P. Fitzpatrick and K. Menon. Role of Gangliosides in Gonadotropin and Cholera Enterotoxin Stimulation in Isolated Rat Ovarian Cells. Biochem. and Biophys. Res. Comm. 83 No. 2 (1978) 493-500.

3. Belloni, A., G. Mazzochi, C. Robba, A. Gambino and G. Nussdorfer. An Ultrastructural, Morphometric and Autoradiographic Study of the Effects of 7, 12-Dimethyl Benzanthracene on the Rat Cortex. Virch. Arch. B. Cell. Path. 26 (1978) 195-214.

4. Broadus, A., N. Kaminsky, J. Hardman, E. Sutherland, G. Liddle. Kinetic Parameters and Renal Clearances of Plasma Adenosine 3', 5'-Monophosphate and Glutamine 3', 5'-Monophosphate in Man. J. Clin. Invest. 49 (1970) 2222-2236.

5. Campbell, P. Liver Enzyme-Membrane Responses in Drug Induction in _The Interaction of Drugs and Subcellular Components in Animal Cells_, London (1968) 97-117.

6. Cooke, B., M. Lindh, and F. Janszen. Correlation of Protein Kinase Activation and Testosterone Production After Stimulation of Leydig Cells with Luteinizing Hormone. Biochem. J. 160 (1976) 439-446.

7. Estabrook, R., D. Cooper and O. Rosenthal. The Light Reversible Carbon Monoxide Inhibition of the Steroid C-21-Hydroxylase System of the Adrenal Cortex. Biochem. Z. (1963) 338-741.

8. Fawcett, D., J. Long and A. Jones. _Recent Progress in Hormone Research._ E. B. Astwood, Ed. N.Y. 25 (1969) 315.

9. Finkelstein, R. Localization of Cholera Toxin _In Vivo_. CRC Crit. Rev. Microbiol. 2 (1973) 533-623.

10. Ghanayem, Burhan. 1979. Effects of Cigarette Smoke Condensates on Cultured Human Lymphocytes and

Separation of Benzo(a)pyrene Metabolites by High Performance Liquid Chromatography. M.S. Thesis. North Texas State University, Basic Health Sciences. 82 pp.

11. Gill, D., and R. Meren. ADP-ribosylation of Membrane Proteins Catalyzed by Cholera Toxin: Basis of the Activation of Adenylate Cyclase. Proc. Nat'l. Acad. Sci. USA. 75 No. 7 (1978) 3050-3054.

12. Grahame-Smith, D., R. Butcher, R. Ney and E. Sutherland. Adenosine 3', 5'-Monophosphate as the Intracellular Mediator of the Action of Adrenocorticotropic Hormone on the Adrenal Cortex. J. Biol. Chem. 242 (1967) 5535.

13. Hall, P., and S. Koritz. Influence of Calcium Ions and Freezing Upon the Conversion of Cholesterol 7a- H to Corticosterone - H by Homogenate of Rat Adrenal. Endocrinology 75 (1964) 135-137.

14. Insel, P. Membrane-Active Hormones: Receptors and Receptor Regulation in: Biochemistry and Mode of Action of Hormones II. H. Rickenberg, Ed. International Review of Biochemistry. 20 (1978) p. 20.

15. Kimura, T. <u>Functions of the Adrenal Cortex.</u> K. McKerns, Ed. N.Y. Vol. II (1968) p. 993.

16. Kuntzman, K., D. Lawrence and A. Conney. Michaelis Constant for the Hydroxylation of Steroid Hormones and Drugs by Rat Liver Microsomes. Mol. Pharm. (1965) 163.

17. Lin, S., D. C. Lin and M. D. Flanagan. Specificity of the effects of cytochalasin B on transport and motile processes. Proc. Nat'l. Acad. Sci. 75 (1978) 329-333.

18. Moss, J., V. Manganiello and Martha Vaughan. Hydrolysis of Nicotinamide Adenine Nucleotide by Choleragen and its A Protomer: Possible Role in the Activation of Adnylate Cyclase. Proc. Nat'l. Acad. Sci. USA. 73 No. 12 (1976) 4424-4427.

19. Mrotek, J., and P. Hall. The Influence of Cytochalasin B on the Response of Adrenal Tumor Cells to ACTH and cyclic AMP. Biochem. Biophys. Res. Comm. (1975) 891.

20. Mrotek, J., and P. Hall. Response of Adrenal Tumor Cells to Adrenocorticotropin: Site of Inhibition of Cytochalasin B. Biochem. 16 (1977) 3177-3181.

21. Peron, F., F. Moncloa and R. Dorfman. A direct inhibitory action of corticosterone on adrenocortical biosynthesis. First International Conference of Endocrinology, Copenhagen (1960a) 707-708.

22. Peron, F. and S. Koritz. On the Localization of the Stimulation In Vitro by Ca++ and Freezing of Corticoid Production by Rat Adrenal Homogenates. J. Biol. Chem. 235 (1960) 1625-1628.

23. Phillips, H. Tissue Culture Methods and Applications. P. Kruse, M. Patterson, Ed. N.Y. (1973), p. 406.

24. Sato, G. and V. Bounassi. In: Symposium on the retention of functional differentiation in cultured cells. Wistar Inst. Symp. Monograph, Wistar Inst. Press, Philadelphia 1 (1964) 27-34.

25. Takahashi, K., H. Takao, T. Takai, K. Gotoh, S. Natsukura and H. Imura. Effects of Cigarette Smoking on cyclic AMP, cyclic GMP, Dopamine -Hydroxylase and Nicotine in Human Plasma and Urine During Water Diuresis. Life Sciences 21 (1977) 803-810.

26. Tannenbaum, J., S. W. Tannenbaum and G. C. Godman. The binding sites of cytochalasin D. II. Their relationship to hexose transport and to cytochalasin B. J. Cell Phys. 91 (1977) 239-248.

27. Taunton, O., J. Roth and I. Pastan. Studies on the Adrenocorticotropic Hormone Activated Adenyl Cyclase of a Functional Adrenal Tumor. J. Biol. Chem. 244 (1969) 247.

28. Wishnow, R., E. Lifrak and C. Chen. Mode of Action of Vibrio Cholerae Enterotoxin in Cultured Adrenal Tumor Cells. J. of Inf. Dis. 133 (1976) S108-S114.

29. Wynder, E., and D. Hoffman. Tobacco and tobacco smoke. N.Y. and London (1967).