THE EFFECTS OF 1,3,5,(10),16-ESTRATETRAEN-3-OL ON MAMMALIAN L-FIBROBLASTS IN TISSUE CULTURE

By

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INTRODUCTION

The observations of Jones et al (unpublished data) that the addition of certain concentrations of 1,3,5,(10),16-estratetraen-3-ol to the water surrounding developing fish embryos resulted in the production of tumors in the caudal region provided the foundation for this project. The purpose of this study was to expand this observation to the effects of this chemical on mammalian L-fibroblasts in tissue culture with respect to the mitotic index, cellular morphology including their nuclei, and general cultural characteristics.

Since tumor formation is typically the result of actively proliferating cells, it was thought that this tendency should be evident from the comparison of the mitotic indices of normal fibroblasts and treated fibroblasts in tissue culture. This expected increase was noted. It was anticipated that further indications of cellular alterations from the standpoint of nuclear changes would be observed in counts made of multinucleate cells. Changes in the basic morphology of the cells were observed in the treated cultures as contrasted with the controls.

Fibroblasts may be obtained from mouse subcutaneous connective tissue and cultured in a variety of media. The L-fibroblast strain was originally isolated by Earle (Paul, 1960) from similar tissue. These cells multiply at a rate such as to require division of stock cultures every

three days, thereby providing an ample number of cells for experimentation. Even though tissue cultures are far removed from the normal environment and in some respects hardly resemble the in vivo counterparts, tissue culture is nevertheless one of the best techniques available for determining the action of foreign substances at the cellular level.

1,3,5,(10),16-estratetraen-3-ol, coded and referred to in the text as chemical # 742, is a synthetic steroid chemically related to the androgens. Treatment of mammalian L-fibroblasts in tissue culture with this chemical was found to produce marked and varied results depending on the concentration: the cellular morphology was radically altered; the mitotic index was increased; and the effects on multinucleation were evident.

LITERATURE REVIEW

Tissue culture as an experimental method has a relatively short history; however the collection of literature over the past thirty years concerned with experimentation involving tissue culture is quite voluminous. The earliest investigator to use a form of tissue culture was an embryologist seeking answers to problems concerning embryonic regulation. Wilhelm Roux in 1885 performed an experiment which laid the foundation on which the technology of tissue culture developed. He found that the medullary plate of a chick embryo could be maintained in warm saline for a few days (Paul, 1960).

Arnold (1887) implanted fragments of alder pith into frogs. He found that the pith would become infiltrated by leukocytes and when removed and placed in warm saline the leukocytes could be maintained for a few days. Ljunggren in 1898 (Paul, 1960) demonstrated that cells from human skin could survive for many days by placing them in ascitic fluid.

The hanging drop method of tissue culture was contributed by Jolly in 1903 who employed this technique in his studies. Later in 1906, Beebe and Ewing attempted to grow an "infective canine lymphosarcoma" in the blood systems of infected and uninfected dogs. (Beebe, 1906). This work marked the beginning of the use of tissue culture as a means of studying

carcinoma.

The early controversy regarding the normal functioning of cultured cells was settled by the investigations of Harrison (1907). Harrison explanted medullary tube tissue from frog embryos onto clots of frog lymph. These tissues survived for some weeks under aseptic conditions and development of axons occurred. This experiment is regarded as the true beginning of tissue culture.

Burrows (1910), Lewis and Lewis (1911) and Carrel (1912) contributed a great deal to tissue culture technology including the use of the plasma clot and the addition of embryo extract to the media.

From the time of Beebe and Ewing, the potentialities of tissue culture as an investigational tool in cancer research were recognized. The National Cancer Institute began to employ these methods early under the direction of Dr. Wilton R. Earle. From his laboratories came such contributions to tissue culture as propagation of cells in suspension, growth of cells directly on glass, and culture growth from a single cell.

1,3,5,(10),16-estratetraen-3-ol, chemical # 742, was prepared by Huffman et al (1955) by the selective benzoylation of estradiol-3,16 B to give the 3-benzoate, which was then esterified with p-toluenesulfonyl chloride to yield C_{16}-tosylate. This compound was refluxed in collidine thereby removing p-toluenesulfonic acid and effecting a double bond at C_{16}-C_{17}.

Plate I

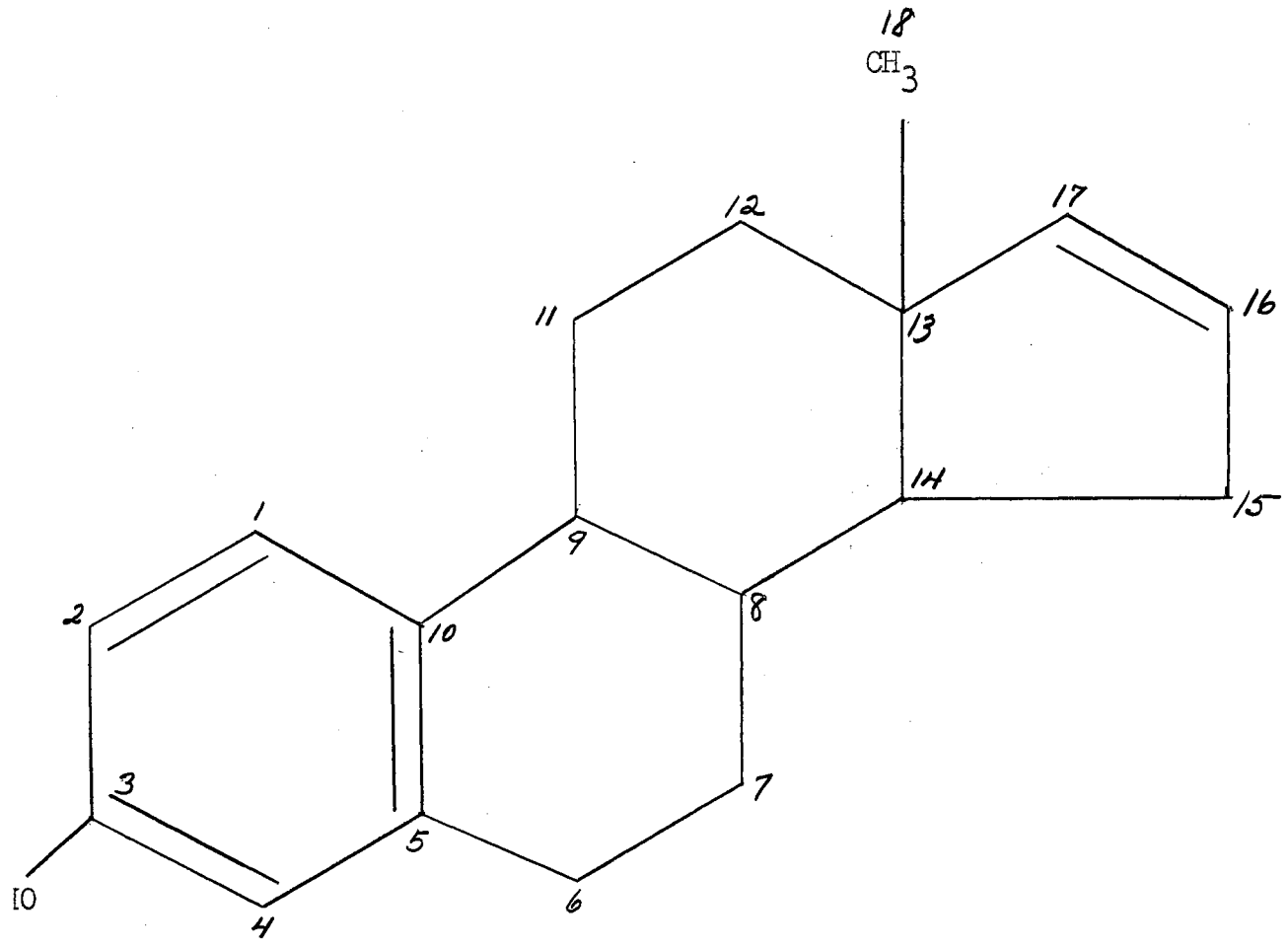

Figure 1. 1,3,5,(10),16-estratetraen-3-ol.

Chemical # 742

The physiological effects of D-substituted Δ 16-steroids are unknown. It was found that chemical #742 did not inhibit pituitary gonadotropin in the parabiotic rat even at dosages of 50 γ (Huffman, 1955). Jones et al (unpublished data) observed that certain concentrations of this steroid when added to the growth media produced tumor formation (apparently of connective tissue origin) in the caudal regions of fish embryos. Preliminary studies of the effects of this chemical on tissue culture of pig kidney done at the Navy Biological Laboratory at the University of California in 1961 indicated that this chemical stimulated cell division (Jones, unpublished data).

The effects of various treatments and compounds on mammalian cells have been reported by many authors. Earle (1943) reported that the addition of methylcholanthrene in concentrations from 0.2 γ to 1,000 γ per cubic centimeter produced injurious effects on cultures of rat and mouse fibroblasts. Later it was determined that upon reinjection these treated cells would produce carcinoma (Earl, 1943).

Creech (1940) found that 1,2,5,6,-dibenzanthrene-choleic acid produced a highly significant increase in cell proliferation as indicated by counts of mitosis and cell outgrowth. The effects of silica on the multiplication of animal cells were studied by Furukana (1960) in which he found silica to be a mitotic stimulator. Other mitotic stimulatory actions were reported by Sacerdote et al (1949) as a result of their experimentation with the addition of cancerous human serum to the growth medium. Nucleoprotein

fractions of adult chicken brain, liver, and heart were all found to stimulate growth of skeletal muscle and fibroblasts in vitro as reported by Harris et al (1958).

Other investigators have found compounds which do not stimulate cell division but inhibit it. Portugal (1951) conducted experiments with testosterone propionate and estradiol benzoate and found that fibroblastic proliferation was inhibited. Growth inhibition was also found by Cagianut (1951) with adrenal corticoid hormone; this effect was not always proportional to the dosage. 2-bromo-1,4-naphtho-hydroquinone-diphosphate gave 50% inhibition of mitosis of chick fibroblasts as reported by Friedmann (1952). Bach (1953) found strong mitotic inhibition with arginase added to the culture medium.

The effect of colchicine as a mitotic poison has been known for some years (Brues, 1936). The nature of its effect has been the subject of numerous investigations. It has been shown that colchicine produces a metaphasic blockage which slows the mitotic rate and brings about a reversion of cell division (Eigsti, 1955). Other compounds which are derivatives of colchicine have been observed to have the same effect; however much higher concentrations may be required. Specific inhibition by mesoinositol of the colchicine effect was found by Murray et al (1951).

Propylene glycol was found to have a slightly toxic effect on tissue cultures as reported by Neukomm (1957) who found methylcholanthrene to have the same effects whether

it be dissolved in acetone or in propylene glycol.

Transformation of cultured cells into malignant cells has been described by Earle et al (1943) who found that cells treated with methylcholanthrene would on reinjection produce sarcomas. Goldblatt (1953) reported that normal cells grown under anaerobic conditions (nitrogen) would become malignant. When exposed to oxygen, these cells would continue to metabolize glucose via anaerobic glycolysis, whereas nonmalignant fibroblasts would revert to aerobic metabolism.

MATERIALS AND METHODS

Standardized stock cultures of L-fibroblasts originally isolated by Earle from the subcutaneous connective tissue of the C3H mouse, were obtained from the Biochemistry Department of Oklahoma State University. These cultures were grown in one ounce glass bottles with three milliliters of medium, and in 150 milliliter dilution bottles with fifteen milliliters of medium. Eagle's medium supplemented with calf serum was used throughout the duration of the study. The culture bottles were placed in an incubator at 37.5°C flat side down. Cell growth was at such a rate as to require division approximately every three days in the stock cultures. These cultures were observed for some weeks before any experimentation was carried out.

Rigorous aseptic technique was used to avoid bacterial contamination. No cultures were lost due to contamination; however some were lost as a result of some suspected defective calf serum.

Glassware was handled in the prescribed manner, with particular attention given to the care of pipettes. All pipettes were soaked in an "alconox" solution; then placed in concentrated nitric acid, usually overnight; and thoroughly rinsed for several hours in an automatic pipette washer with distilled water.

After a thorough rinsing, the pipettes were dried in an

oven and cotton filters put into place. The autoclaving period was fifteen minutes at 250°F. Rubber tubing with a cotton filter was used for suctioning pipettes. At no time was direct mouth contact used to suction material into a pipette.

Rubber stoppers were soaked in "alconox" solution, rinsed with distilled water and autoclaved in large cotton plugged test tubes. All stoppers used on chemically treated cultures were disposed of and not reused. Stoppers on control culture were washed, sterilized and used again.

All one ounce cultures bottles were disposed of after usage; however dilution bottles which did not come into contact with any experimental chemical were always washed and reused. Petri dishes were cleaned and sterilized with the usual technique.

Size 22mm. (7/8") number one coverglasses were washed in sodium bicarbonate solution and allowed to stand overnight in the solution before autoclaving. It was thought some penetration of the bicarbonate might be achieved by allowing them to stand. Steam sterilization was found to be superior to dry heat for the coverglasses to avoid their becoming too brittle.

All work done with open cultures was carried out in a pressurized sterile room equipped with ultraviolet lights, suctioning apparatus, 5% carbon dioxide tank, hood, and storage for sterile equipment. The ultraviolet lights were left on whenever the room was not in use. The working

surfaces were washed with ethyl alcohol before each working period.

In order to avoid the effects of additional chemicals on the test cultures, the "policeman" method of culture division was used instead of the more widely used trypsin method. This operation was found to be simple and effective. It involved the use of a sterile rubbertipped glass rod which was inserted into an open culture bottle under the hood, and rubbed back and forth across the cells on the surface of the glass. One could observe with the unaided eye when the operation was complete by the absence of the film of cells.

When the cells were removed from the surface, three milliliters of medium were added to the one ounce bottles containing the suspension of medium and cells; or fifteen milliliters were added to the dilution bottles, and the contents pipetted up and down at least twenty times with a one milliliter pipette. This process rendered the cell suspension free of clumps and was not found to be overly abusive to the cells.

The regulation of pH was found to be critical in the maintenance of both stock and experimental cultures. The medium was added in a basic condition and then adjusted to approximately pH 7.2 by bubbling 5% gaseous carbon dioxide through the medium. Usually within three days after incubation, the cultures became acid which indicated the need for a change of medium. An effort was made to keep all of the

stock cultures at the same optimum pH.

Since petri dishes allow air to flow in and out rather freely, it was essential to use a carbon dioxide incubator to keep the pH at the desired uniform point. All experimental cultures in petri dishes were placed in this incubator which minimized the possibility of pH change. Evaporation was not a factor since the incubator was at 100% humidity.

1,3,5,(10),16-estratetraen-3-ol was obtained from Dr. Max N. Huffman, Lasdon Foundation, at concentration of one milligram per milliliter of propylene glycol. Dilutions were made by measuring quantities of the stock solution in a tuberculin hypodermic syringe and adding it to a known quantity of triple glass-distilled water.

Aliquots of the diluted # 742 were then measured and added to the test cultures. The dilutions were made in such a way that quantities of 0.5 milliliter or 1.0 milliliter could be added to make the desired concentrations as these larger quantities could be more accurately measured.

The control cultures were treated with an equal amount of triple glass distilled water corresponding to the amount of chemical added to the test cultures. Other experimental preparations were made using propylene glycol added in approximately equivalent amounts to that contained in the test chemical. (2% and 4% propylene glycol cultures were used to test the effects of this substance contained in 20 ppm and 40 ppm # 742 respectively.)

In order to observe the cells in a stained preparation, it was found necessary to grow the cells on the coverglasses

so they would adhere during the staining process. This was accomplished by placing a 10 milliliter suspension of cells and medium into a sterile petri dish which contained at least five coverglasses. These were then incubated for 72 hours in the carbon dioxide incubator. The coverglasses were then removed and stained according to the following procedure.

Aceto-orcein stain was prepared by warming 45 milliliters glacial acetic acid in a flask to which was then added one gram of orcein. The flask was stoppered and agitated for a few minutes in cool water until cool. After cooling, 55 milliliters distilled water were slowly added and the solution allowed to stand overnight. The stain was filtered before use.

The coverglasses with adherent cells were removed and placed in the stain for exactly thirty minutes. Fixation and staining were accomplished in the same step. After thirty minutes the coverglasses were removed and placed in two changes of 95% ethyl alcohol for two minutes each; then into two changes of xylol for two minutes each, followed by mounting on a slide with picolyte.

Other techniques were attempted; such as Azure A and Eosin B; Feulgen method; and hematoxylin and eosin with various fixation processes. Hypotonic salt solutions were also tried. Aceto-orcein was found to be a rapid and effective method for the purposes of this study. The nuclei stained a bright red and the cytoplasm a pale pink;

cells in mitosis stained a dark red.

The phase contrast microscope was used for observation of living material. This was found to be ineffective without a perfusion chamber since the cells and medium were exposed to atmospheric oxygen and a pH change was immediately effected. Without a perfusion chamber, the cells began to disintegrate after a short time and mitotic divisions could not be observed. The morphological characteristics observed under phase were found to be much better seen using a stained preparation with a light microscope.

The mitotic index and the percentage of multinucleate cells were determined by counting a total of at least 1,000 cells (Paul, 1960). The percentage was found by dividing the total number of cells in division by the total number of cells observed. By the same method, the percentage of multinucleate cells present was determined.

Randomization was used throughout the counting procedure. This was achieved by dividing the coverglass into four general areas, and the selection of random fields for counting. Many slides from different experiments were used in this overall determination.

At least three general morphological shapes were noted in surveying the slides. In order to typify the morphology, it was decided to employ these three generalized forms as criteria for characterizing each experimental concentration of the chemical and the controls on a percentage basis. Random counts and morphological classification were carried out

by a similar procedure as described above.

Since the purpose of the mitotic index was not to determine the actual mitotic index as such, but rather as a comparative aid in evaluating the effect of the chemical on the number of mitoses present, it was possible to eliminate the ambiguous prophase stage completely and begin with the more definitive and evident metaphase stage. Naturally occurring clumps of chromatin present in normal fibroblasts made it exceedingly difficult, even with the elimination of the prophase stage, to ascertain with any great degree of certainty whether or not a cell was truly in mitosis. An endeavor was made to be consistent in counting only those cells obviously in mitosis.

Multinucleate cells were clear and evident. It was felt these counts were highly accurate and representative.

To substantiate the mitotic index findings, a hemocytometer was used to make comparative counts of cells in the experimental cultures, using the white cell count method to determine the number of cells per cubic millimeter. This was done in triplicate for 20 parts per million (ppm) chemical # 742 and propylene glycol.

As the coverglasses were not in any fixed position in the petri dishes, it was possible for them to float and adhere to one another. Thus, some were completely or partially covered and had few or no cells on them. Estimates of the total number of cells could not be determined from the number of cells on the coverglass for this reason;

instead, the above hemocytometer method was used to estimate the total number of cells.

Cultures to be counted with a hemocytometer were prepared as follows: a culture bottle containing fifteen milliliters medium and a substantial amount of growth was diluted to thirty milliliters by adding additional fresh medium. The cells were "policed off" and pipetted up and down, until it was reasonable to assume that the cells were evenly distributed in the medium. Three ten milliliter aliquots were then placed in three separate bottles. Propylene glycol, chemical #742 and sterile distilled water were added in the desired concentrations to these preparations. Three samples of three milliliters, of each of the above ten milliliter preparations were transferred to separate one ounce bottles. The one remaining milliliter was discarded, and the cultures were incubated for 72 hours before counting. All cells were then "policed off" and suspended in the medium as before. Hemocytometer counts were made of each homogenized culture.

EXPERIMENTAL DATA AND OBSERVATIONS

Three topics will be discussed in the following sections: the mitotic index, multinucleation and morphological changes. These are results which were obtained from 152 preparations all incubated for a period of 72 hours.

All values given in table I are expressed in percent.

TABLE I

MITOTIC INDEX

Culture type	% in mitosis
control	3.64
0.5 ppm # 742	4.50
1.0 ppm # 742	6.60
5.0 ppm # 742	6.06
10.0 ppm # 742	11.14
20.0 ppm # 742	13.64
40.0 ppm # 742	lethal
2% propylene glycol	7.2
4% propylene glycol	lethal

It should be re-emphasized that these determined indices are not intended to indicate the actual mitotic index, but rather are for comparative purposes.

There did not appear to be any stoppage of mitosis or a greater number of cells in any one stage of mitosis in the treated slides as compared with the controls. In the higher percentages of mitosis, the cells were much smaller and presented a more definite cellular membrane.

TABLE II

HEMOCYTOMETER CELL COUNTS

Culture	Average No. of Cells
Control	83.5 per mm^3
20 ppm # 742	380.0 per mm^3
2% propylene glycol	215.0 per mm^3

Each count represents an average of three counts made of identically treated triplicate cultures.

MULTINUCLEATION

The vast majority of cells termed "multinucleate" were binucleate cells; however cells with as many as seven clearly discernable nuclei were found. An effort was made to distinguish between cells in telophase and binucleate cells on the basis of the cytoplasmic constriction and location and appearance of the nuclei.

TABLE III

PERCENTAGE OF MULTINUCLEATE CELLS

Culture	% multinucleate cells
control	3.88
0.5 ppm # 742	5.80
1.0 ppm # 742	6.30
5.0 ppm # 742	4.10
10.0 ppm # 742	2.10
20.0 ppm # 742	2.70
40.0 ppm # 742	lethal
2% propylene glycol	2.57
4% propylene glycol	lethal

Evidence of amitosis in treated and control cultures was seen in several instances during the study.

CELL MORPHOLOGY

Morphological changes were most apparent in the treated cultures. Three morphological forms were generally seen: the large rounded cell with an abundance of cytoplasm and few cytoplasmic processes; the smaller stellate cell with definite cytoplasmic projections usually at opposite poles; and the small fusiform cell. These three forms are repre-

sented in Plate I as types I, II, and III respectively. The percentages of occurrence of the three morphological types are given in table IV.

TABLE IV

PERCENTAGES OF MORPHOLOGICAL TYPES

Culture	Type I	Type II	Type III
Control	52.57	37.62	9.79
0.5 ppm # 742	26.55	62.71	10.73
1.0 ppm # 742	20.37	63.70	15.92
5.00 ppm # 742	13.85	49.86	51.80
10.00 ppm # 742	8.30	16.60	75.09
20.0 ppm # 742	2.97	18.85	78.39
40 ppm # 742		LETHAL	
2% propylene glycol	25.23	62.39	12.39
4% propylene glycol		LETHAL	

It should be pointed out that the cell cultures treated with propylene glycol assumed more of an epithelial appearance and did not fully conform to the criteria of classification as to type.

Plate II

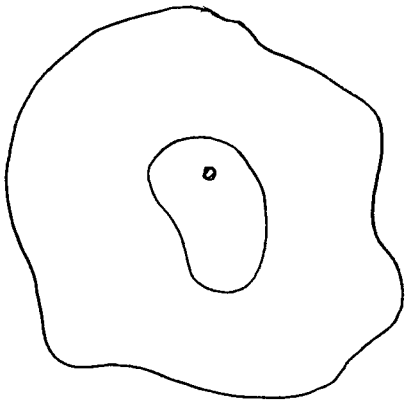

Figure 1. Type I

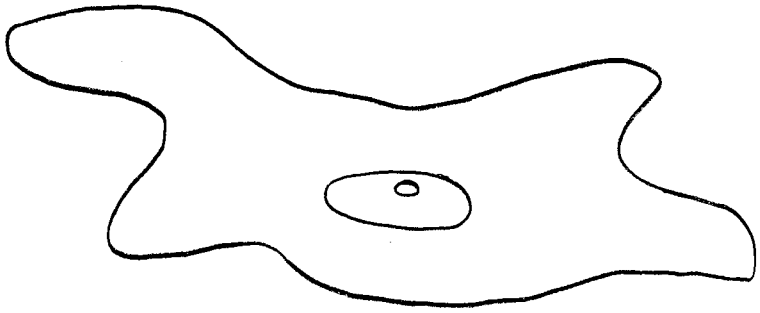

Figure 2. Type II

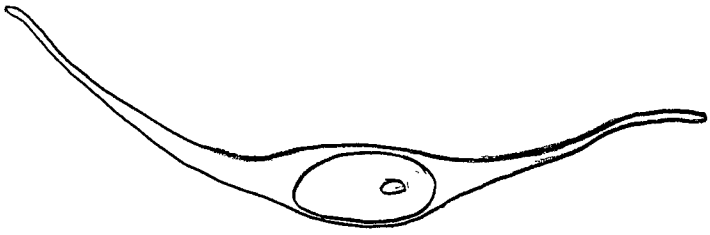

Figure 3. Type III

DISCUSSION

It is not a safe assumption to consider fibroblasts in tissue culture as fully differentiated cells. Cowdry (1960) points out a well known fact that in tissue cultures, cells tend to lose differentiation, not gain it. Plate III of the control cultures readily demonstrates this statement. These cells are most assuredly not the typical fibroplastic elements which would be seen for example in a preparation of loose connective tissue. Many of them resemble macrophages rather than fibroblasts.

Transformations of macrophages into fibroblasts, fibroblasts into macrophages, and lymphocytes and monocytes into macrophages and fibroblasts have been described (Smith, 1953). Even transformation of epithelial tissue into macrophages has been observed, as well as fibroblasts into bone cells (Maximow, 1957). From these supposedly normal phenomena, it is clearly evident that these cells possess the capacity for undergoing change.

The problem of malignancy is also involved with fibroblasts in tissue culture. Earle (1943) found that normal fibroblasts which had been cultured for a long time occasionally underwent a malignant transformation. These cells would on reinjection produce sarcomas (Earle, 1943). The generalized description of malignant cells as seen in pathological preparations usually include such criteria as

hyperchromatic nuclei, reduction of cytoplasm in relation to the nucleus and variation in both size and shape of the nuclei. In tissue culture, these criteria are obscured due to the effects of the removal of the cells from their natural environment to an artificial medium.

In plate III(untreated fibroblasts) some hyperchromatic nuclei with little or no cytoplasm may be seen. Other treated cultures also demonstrate some of these characteristics to a greater degree. However, the scope of this study did not include reinjection tests to determine the percentage of tumor production of the treated cells as compared with the controls. Since this was not done, it can only be said this appearance was present in some of the cultures.

It was thought at the beginning of this study that the cells would not tolerate relatively high concentrations. The reverse was found to be true. The 20 ppm # 742 cultures survived very well, but at 40 ppm # 742 and equivalent propylene glycol cultures there were no cells remaining.

As table I indicates, there was an increase in the number of mitotic figures in the treated cultures. There was a fairly consistent rise in the percentages from 4.5% in the 0.5 ppm # 742 cultures to 6.60% in the 1.0 ppm # 742 cultures. However, at 5.0 ppm # 742 there was no increase. The appearance of these cells at this concentration was more abnormal than at any other non lethal concentration.

At 10.0 ppm # 742, the index was roughly twice that

observed at 5 ppm # 742, but 20 ppm # 742 gave only approximately a 2% increase over the 10.0 ppm # 742. Apparently the cells are more responsive to concentrations between 0.5 ppm to 10.0 ppm than between 10 ppm and 20 ppm # 742.

Neukomm (1957) reported that propylene glycol has a slight effect on growth when used as a solvent for methylcholanthrene. In the present study, propylene glycol was found to increase the number of cells in mitosis, but not to have nearly as marked an increase as with the # 742.

Table IV shows clearly the trend from the low concentration to the high concentration, toward the fusiform or type III cell. The few type I cells remaining at the highest concentration are distinctly smaller. The data indicates the fusiform cells probably have their origin from the large rounded type I cells and the type II stellate cells.

From the observation of many thousands of cells, it is postulated that perhaps the following takes place. Mitosis occurs and the daughter nuclei separate. One nucleus migrates to the periphery of the cell toward a cytoplasmic process, and the other remains in the central portion of the cell. This process with the enclosed nucleus is "pinched off" from the mother cell thereby giving two cells: one rounded and the other fusiform. This is, of course, hypothetical, but many nuclei were seen to be in this condition in what appeared to be a stage of separation.

The multinucleate cells were restricted to type I

morphology for the most part with only a few cases occurring in type II cells, and were very rarely found in type III. The nuclei were not necessarily of the same size or shape. Oftentimes, a large ovoid nucleus was found to be surrounded by several smaller nuclei.

The consensus regarding the origin of multinucleate cells points to amitosis as being the causal factor. (Bucher, 1953). It has also been shown under experimental conditions that an abortive mitotic division without division of the cytoplasm will result in a binucleate cell. Since evidence of amitosis was observed, this perhaps is an explanation. On the other hand, karyokinesis may be complete and the cell exist for some time before cytoplasmic division. Thus, some of the binucleate cells may be in a delayed telophase stage.

If the latter statement is accepted, as the mitotic index increases, it would be expected that the percentage of multinucleate cells would also increase due to a higher number of cells in the delayed telophase stage. This was found only between 0.5 ppm # 742 and 1.0 ppm # 742. (See table III.) Subsequently a marked decrease even below that found in the control groups was found; however it should be emphasized that at concentrations of 10.0 ppm and 20.0 ppm # 742 there was a definite accompanying morphological change to the type III fusiform cell which rarely exhibits multinucleation.

The effects of propylene glycol are also apparent, for

with 2% of this substance the percentage of multinucleate cells did not differ significantly from the 20.0 ppm # 742, but there was not the same morphological change as in the 20.0 ppm # 742.

Studies on preparations of steroid chemicals for intravenous injection reveal the discovery of a hemolysing effect of propylene glycol on erythrocytes (Rothchild, 1952). This would tend to indicate an action on the cell membrane itself, and perhaps would have an influence on the morphology which in turn would reduce the multinucleation at higher concentrations.

Plate III

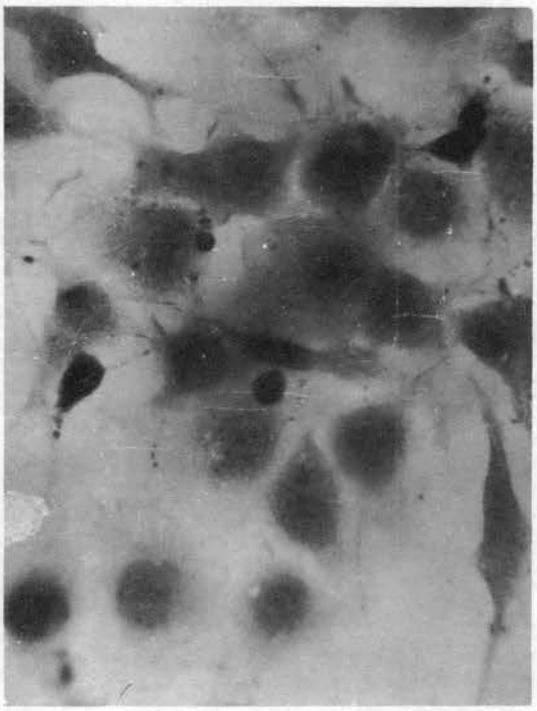

Figure 1. Control.

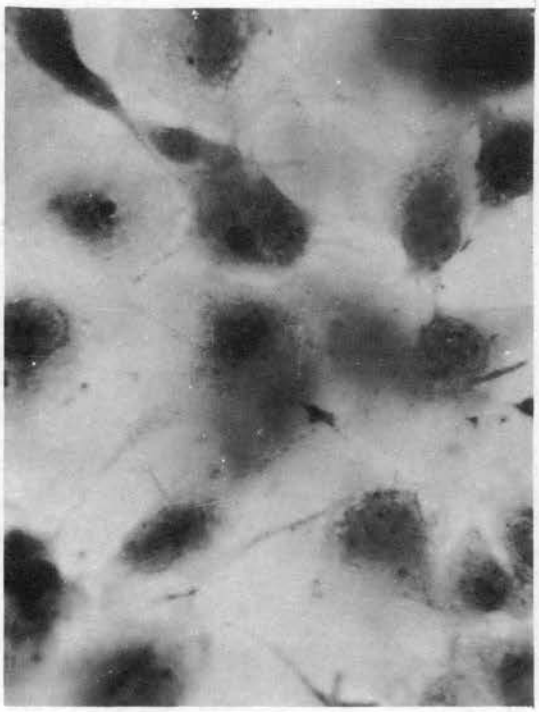

Figure 2. Control.

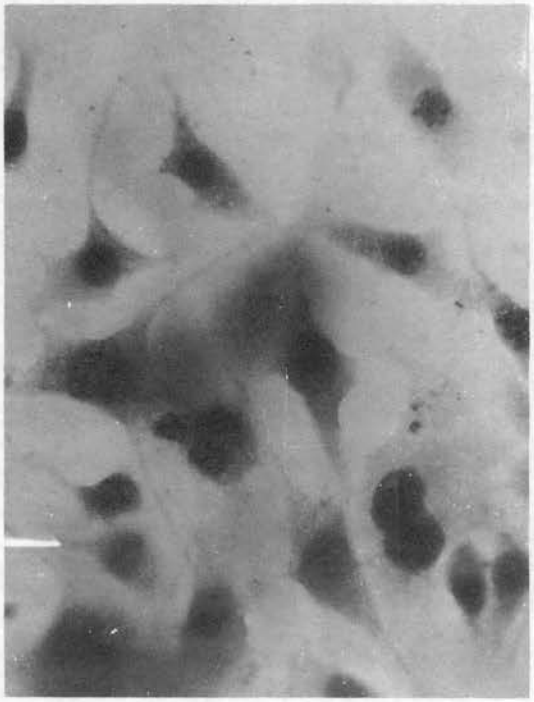

Figure 3. 0.1 ppm # 742.

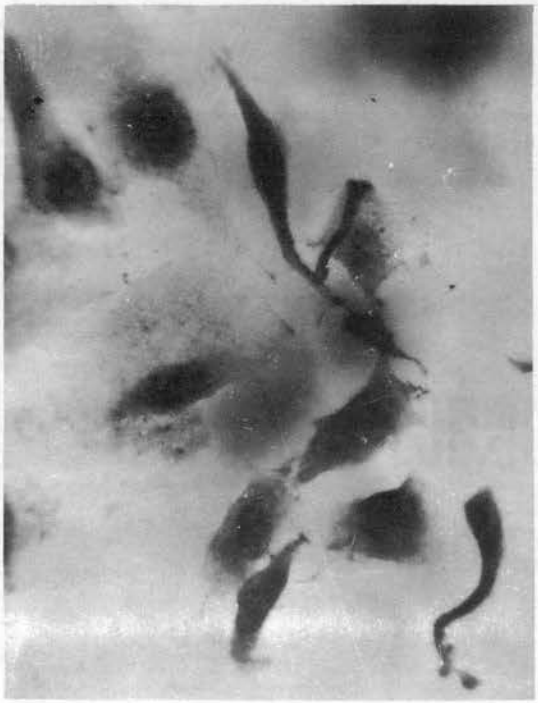

Figure 4. 0.5 ppm # 742.

Plate IV

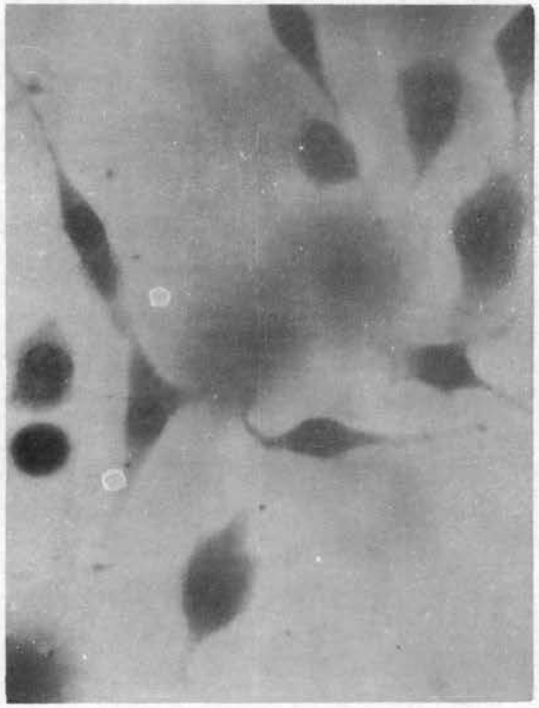

Figure 1. 5ppm #742

Figure 2. 5 ppm # 742

Figure 3. 10 ppm # 742.

Figure 4. 10 ppm # 742.

Plate V

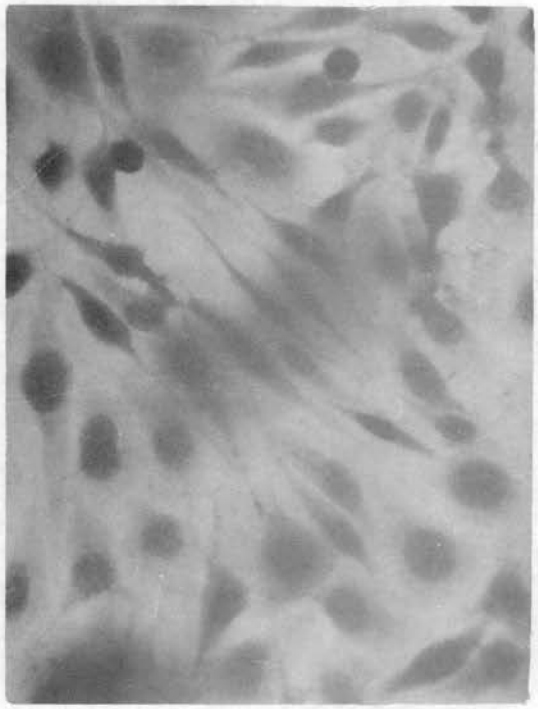

Figure 1. 20 ppm # 742

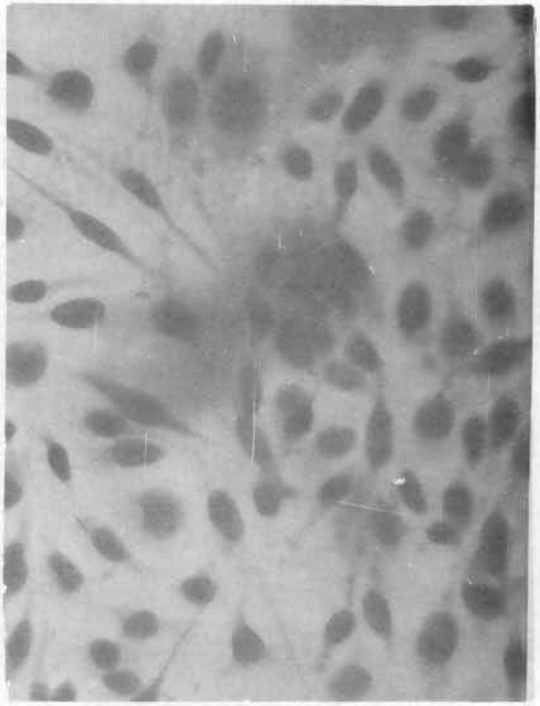

Figure 2. 2% Propylene glycol

Figure 3. 20 ppm # 742

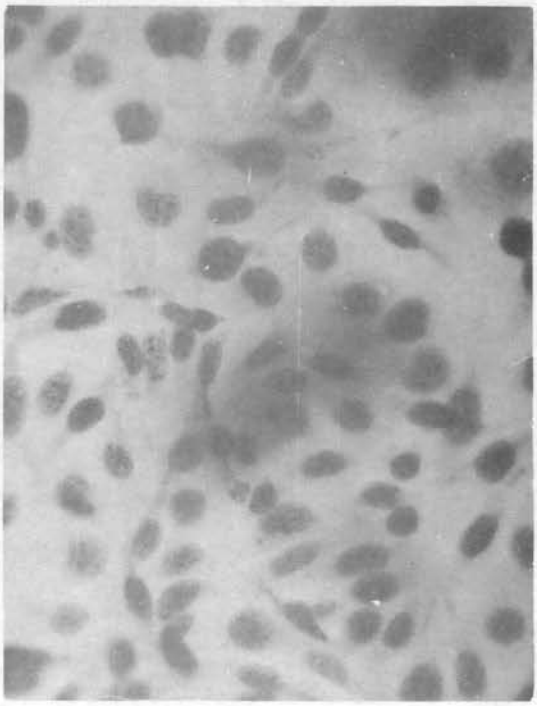

Figure 4. 2% Propylene glycol

SUMMARY

L-fibroblast cells were obtained and cultured in Eagle's medium in one ounce bottles with three milliliters of medium, and in dilution bottles with fifteen milliliters of medium. After a period of observation of some weeks, ten milliliter aliquots were placed in petri dishes containing coverslips and were treated with varying concentrations of 1,3,5,(10),16-estratetraen-3-ol dissolved in propylene glycol. These preparations were placed in a carbon dioxide incubator for a period of 72 hours. Controls were run with each experiment.

The coverglasses with adherent cells were removed and stained with aceto-orcein. The mitotic index, percentage of multinucleation, and classification of morphological forms were determined from these preparations.

An increase in the mitotic index which was not correlated with the concentration was noted. An increase in the percentage of multinucleate cells roughly corresponding to the increase in the mitotic index was observed at the lower concentrations; however at higher concentrations the percentage fell below that found in the controls. Cell forms were grouped into three generalized types: type I, the large rounded cell; type II, the smaller stellate cell; and type III, the fusiform cell. A consistent change in morphology due solely to the test chemical was found to be

effected from the types I and II to the type III with an increase in the concentration of the chemical.

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VITA

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