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THE EFFECT OF DESOXYCORTICOSTERONE ACETATE ON EPIDERMAL MITOSIS OF THE MOUSE IN VITRO

by

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EFFECT OF DOCA ON MITOSIS

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ABSTRACT

In view of the hormonal relations to mitotic activity, a study was done on the effects of desoxycorticosterone acetate (DOCA) on epidermal mitotic activity in vitro.

Mouse ear fragments were incubated at 38° C. in a phosphate-buffered saline medium, to which isotonic glucose, oxygen, and colchicine were added. The various concentrations of the hormone were added to this basic medium in the form of an ether solution then submitted to a gentle stream of air until a fine suspension of the hormone was obtained. After five hours of incubation, the ear fragments were fixed, to be subsequently sectioned and stained for counting mitoses. The number of mitoses in both control and experimental groups was counted per unit length (1 cm) of ear epidermis. The average was then found and the standard deviation determined.

The results obtained showed that the effect of DOCA varies with its concentration. With low concentrations, less than 1 mg. of DOCA per 100 cc. of solution, there is an increase in the average number of mitoses present per unit length of tissue. On the other hand with concentrations exceeding 1.5 mg. per 100 cc. of solutions there is a sharp decrease in the mitotic activity of the mouse ear epidermis. Experiments with 1 mg. of DOCA failed to give

any significant increase or decrease.

It was found by various investigators that the rate of entry of glucose and its subsequent oxidation are the primary energy factors in mitotic activity. On the other hand, it was shown that DOC inhibits the glucose uptake of isolated muscles. As DOC was also found to inhibit oxidation in tissues of a few substrates, it was concluded that the mitotic inhibitory action of this hormone was due to both its interference with glucose entry and its block to oxidative processes.

Less support was found in the literature for the mitogenic action of DOCA. DOCA in small doses was found by some investigators to completely inhibit glycogenesis due to enhanced glycogen breakdown. So it was suggested that the mitosis stimulating action of DOCA at low concentrations was the result of freedom of glucose entry and increased anaerobic glycolysis.

From the results of these experiments the conclusion was reached that actually DOCA is a mitotic inhibitor as well as a mitosis activator depending upon its concentration.

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INTRODUCTION

Cell division in the mammalian epidermis has been studied by many investigators. Thuringer (1928) found that cell division was more frequent in the spinous than in the basal layer. However, in the ear epidermis of mice, the greatest mitotic activity occurs in the basal cell layer, the stratum germinativum (Bullough 1952, Carter 1953). Bullough (1942-44, 1948) observed cyclic and diurnal variations in epidermal mitotic rates of female and male mice respectively. Similar and related rhythmic mitotic changes were reported by other investigators too (Cooper 1939; Storey and Leblond 1951; Carter 1953). These observations and others led Bullough to believe that mitotic activity is related to some of the physiological activities of the body such as rest and sleep (1948, 1954), hormonal function (1942-44, 1946) and carbohydrate metabolism (1949, 1952).

Before reviewing the study of hormonal relations to mitotic activity, it is necessary to discuss the factors involved in normal mitosis.

FACTORS IN NORMAL EPIDERMAL MITOSIS

In vivo and in vitro studies.

Most of the studies on factors involved in normal epidermal mitosis were based on observations in vivo.

Cooper and Franklin (1940) found maximum activity in the ear epidermis of mice at 10:00 a.m. and the least activity at 10:00 p.m. Bullough and Eisa (1950) observed that this diurnal variation in epidermal mitotic rate was directly related to a similar cycle in the levels of liver and epidermal glycogen. That is, during rest the glycogen content of epidermal tissue and the number of mitoses was higher than in periods of activity.

A direct cyclic variation of mitotic activity during oestrus cycle was also demonstrated by Bullough (1942-44, 1949-50). To ascertain the mechanism of this relationship in vivo and in vitro experiments were found of primary importance. Injection of substances such as hormones or starch solutions proved successful in promoting mitosis but often they had unknown side-effects which complicated the interpretation of final results.

In vitro techniques showed that: (1) the influence of any substance on epidermal mitosis could be observed in a much shorter period of time and (2) there were less side-effects. Moreover, maintaining mammalian epidermal mitosis in vitro was no more a problem.

The first studies on epidermal mitosis in vitro are those of Medawar (1947, 1948). Following Medawar's method, Bullough and Johnson (1951) described a simple technique which consisted of incubating ear fragments in a phosphate-buffered saline medium to which isotonic glucose and oxygen

were added. Bullough (1948) suggests also the use of colchicine which is supposed to arrest mitosis at metaphase. Some investigators however, as Carter (1953) do not advise the use of this drug on the basis that its effects vary considerably according to dosage. This will be discussed later.

Conditions for normal mitosis.

Oxygen: Medawar (1947, 1948) and Bullough later (1950) demonstrated that the mitotic rate of epidermis in vitro increases with increasing oxygen tension and that aerobic conditions are essential for epidermal cell division. Medawar also suggested that survival of epidermal cells depends upon the function of glycolytic cycle whereas cell division is a function of respiration.

Carbohydrate: Bullough (1949) observed that the concentration of glycogen within a tissue was a critical factor in controlling the rate of mitosis; sugar deficiency resulting in mitotic depression. Injection of starch solutions resulted in an increased rate of cell division (Bullough 1950). Starch appeared to function as a source of energy since the increase in mitotic activity follows an increase in blood sugar (Bullough 1949). According to Bullough (1952) epidermal mitotic activity is closely dependent on maximum energy production and therefore any factor which interferes, even partially with either glycolysis or the tricarboxylic acid cycle is expected to

interfere with mitosis. Studies on mouse ear epidermis in vitro have shown that active mitosis can develop only when a carbohydrate substrate is efficiently oxidized in the tri-carboxylic acid cycle.

Phosphorus: These assumptions are substantiated by the fact that interference with phosphorylation by the injection of phlorhizin or by the induction of a hypoglycemia with insulin depresses the epidermal mitotic activity (Bullough 1949 a). Bullough (1949 a) observed that by injecting phosphate together with starch an even greater mitosis stimulation was achieved.

Oxygen, sugar and phosphorus therefore seem to be the primary requirements for the initiation of mitosis in vitro.

The normal control of mitosis.

The most interesting studies on the normal control of mitosis are those of Bullough (1942-44, 1948, 1950). He found a direct correlation between estrogens and mitotic activity in that maximum activity occurred on the third day of dioestrus and again early in oestrus (Bullough 1942-44). Injection of small doses of oestrone induced a similar effect on mitotic activity (Bullough 1949-50, Carter 1953). According to Bullough (1952) part of the mitogenic action of oestrone was due to the induction of hyperglycemia following its administration. The rise in blood sugar was

assumed to result in an increase in intracellular glycogen (Bullough 1949).

Furthermore, Green and Ghadially (1951) demonstrated a possible relation between shock inducing agents, adrenaline and adenosine and mitotic activity. These compounds had a powerful inhibitory action on the pre-prophase stage in the ear epidermis of adult mice. The antimitotic effect of shock producing agents was thought to be due to an indirect interference with carbohydrate utilization through some hormonal mechanism.

It has been reported by Bullough (1952) that insulin, adrenaline, cortisone, ACTH may all depress mitotic activity in normal mice evidently because of their interference in various phases of carbohydrate metabolism. Castration also reduced mitotic activity of the ear epidermis of male mice. Subsequent administration of testosterone propionate however, stimulated the mitotic activity to normal in these mice (Bullough and Van Oordt 1950).

EXPERIMENTAL CONTROL OF EPIDERMAL MITOSIS

Most of the studies on the relationship between hormones and cell division are recent. As already mentioned, injection of either estrogens or androgens resulted in stimulation of mitotic activity in different tissues and organs. However, after a certain number of injections of

oestrone, Bullough (1946) observed that mitosis was inhibited. It was consequently supposed that there were in the body certain substances capable of counteracting the effect of mitogenic hormones (Bullough 1946). Many investigators had already showed a marked interest in the study of mitotic inhibitors and poisons.

Colchicine.

The first well-known mitotic poison is colchicine the effects of which were first discovered by Lits (1934) and Dustin (1934). Colchicine differs from the other mitotic poisons in that it does not inhibit mitosis in the antephase but in the metaphase (Bullough 1952). Brues and Cohen (1936) found that large doses prevent mitosis, whereas Lits (1934) and Paff (1939) working with smaller doses found it to stimulate mitosis. Bullough himself (1949 b) observed that after 5 hours the drug inhibited mitosis in the ear epidermis of the mouse and that during the 5 hours a considerable number of mitoses were not arrested in metaphase but reached telophase.

Hormonal control of mitosis.

Most of the mitotic inhibitors were found effective in the antephase or pre-prophase stage.

Green and Ghadially (1951) found that adrenaline and adenosine inhibited the pre-prophase stage of mitosis in the ear epidermis of adult mice. They also found that cortisone administered topically and parenterally was a

powerful inhibitor of epidermal mitosis (1951).

Most of the adrenal cortical hormones, administered topically to rat skin, induced thinning of the epidermis (Baker 1951). Studies carried on the effects of corticosteroids on growth and differentiation gave further evidence of the antimitogenic action of adrenal cortical hormones. Landauer (1947) first reported that an extract of adrenal cortex would inhibit the growth of chick embryos. Parmer _et al_. (1951) found that cortisone was the most toxic and most powerful inhibitor of growth when injected in new-born rats. The effects of cortisone were so striking that it was possible to detect without any difficulty limited amounts of cortisone-like activity either from small amounts of material or from larger amounts of less active corticosteroids (Stock _et al_. 1951).

It is interesting to note that desoxycorticosterone, one of the adrenal corticosteroids, does not show a marked cortisone-like activity. Since DOC has not been studied as intensively as cortisone, a review of its characteristics is necessary in this paper.

<u>Desoxycorticosterone Acetate (DOCA)</u>.

<u>Chemical and Physiological characteristics</u>: DOC differs from the well-known cortisone in many ways. It is characterized by the lack of an alcoholic or ketonic group at C_{11} position and it is more correctly called 11-desoxycorticosterone. Cortisone however has a ketonic

group at C_{11} and an OH at C_{17}. The structural formula of DOCA is given in Materials and Methods of this present paper. The absence of an oxygen or an OH group on C_{11} is in fact responsible for the effect of DOC on water-electrolyte balance in the body (West and Todd 1951). Cortisone is concerned only with carbohydrate metabolism and has no effect at all on salt metabolism.

It was also found (Verzár and Wenner 1948) that DOC completely inhibits glycogen formation when added to a Ringer's solution containing rat diaphragm whereas cortisone had the opposite effect. Verzár (1952) explains this inhibition as a result of increased glycogen breakdown since DOC was shown to increase glycogen phosphorylation in vitro.

Furthermore the C_{11} oxygenated steroids were more potent growth inhibitors than C_{11} non-oxygenated group (Baker 1951). According to Baker, there might be some parallelism between the capacity of a compound to increase liver glycogen and inhibit growth and that possibly these two actions are dependent on a common process.

Another striking difference between 11-oxy steroids and DOC consists in their respective actions on capillary permeability. Administered alone, DOCA was found (Folley and Greenbaum 1948, Fourman 1952) to enhance capillary permeability in the target organs or elsewhere whereas cortisone had generally been observed to depress permeability (Balourdas and Chambers 1952).

Thus the difference between DOC and cortisone could be tabulated in the following form:

DOC	Cortisone
1. No C_{11} oxygen, no $C_{17}OH$	1. C_{11} oxygen, $C_{17}OH$
2. Effect on water-salt balance.	2. Effect on carbohydrate metabolism.
3. Inhibits glycogen formation in rat diaphragm.	3. Favours glycogen formation in rat diaphragm.
4. Enhances capillary permeability.	4. Depresses capillary permeability.

Effects on growth and development. Taubenhaus and Amromin (1949) reported that DOCA stimulates fibroblasts and encourages the deposition of a homogeneous groundwork of collagen. In a few experiments, DOCA showed cortisone-like activity only when given at higher doses. (Stock et al. 1951). Growth was generally retarded when 2 mg. of DOCA were injected in baby mice. Recently however, Mirand et al. (1953) could develop sarcomas in March albino mice by injection of DOCA in sesame oil. The amount of DOCA used was 0.2 mg. given daily for three months.

These findings seem to contradict each other in that DOCA inhibits tissue growth in some experiments, whereas it favours cell proliferations in other experiments. Whether or not it would inhibit epidermal mitosis in vitro had not been determined and so could not be guessed before-

hand.

These many aspects of the influence of either DOC or DOCA on organs and tissues combined with the observations of Bullough on epidermal mitotic activity and energy relations make it worth studying the effect of this hormone on epidermal mitosis in vitro. The present paper is concerned with the specific effects of DOCA on mitotic activity of the ear epidermis of adult male mice.

Will it show cortisone-like activity or will it act like the sex hormones? Preliminary experiments were performed in order to determine which concentrations of DOCA were most effective and whether or not colchicine should be used.

MATERIALS AND METHODS

The animals.

The animals used in these experiments were albino male mice 3-6 months old, obtained through the courtesy of the Pharmacology Department where they were reared on sterilized meat and barley. They were brought on the day of experimentation and fed once on carrots and barley before the ears were removed. Ten mice were used for every experiment, providing ear tissue for both experimental and control media.

The basic incubation medium.

The basic medium was phosphate-buffered saline devised by Bullough and Johnson (1951). It had the following composition:

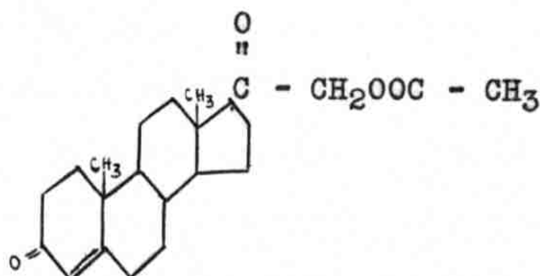
0.90%	NaCl	100 cc.
0.15%	KCl	4 cc.
1.22%	$CaCl_2$	3 cc.
2.11%	KH_2PO_4	3 cc.
3.82%	$MgSO_4.7H_2O$	1 cc.
1.30%	$NaHCO_3$	3 cc.

To nine parts of this mixture was added one part of 0.1 Molar phosphate buffer, pH 7.4, made by dissolving 17.8 gm. of $Na_2HPO_4.2H_2O$ in 20 cc. of 1 N - HCl. diluted to 1 liter with distilled water.

As the concentration of sugar within a tissue was found by Bullough (1949) to be a critical factor in controlling the rate of mitosis, 8 cc. of isotonic glucose - 5.22 gm/100 cc. - were added to 100 cc. of this phosphate-buffered saline to give an optimum concentration of 0.02 M.

The Hormone.

The hormone tested was the acetate derivative of 11-desoxycorticosterone (DOC) known as 11-desoxycorticosterone acetate (DOCA), M.W. = 372, kindly provided by the Biochemistry Department of the American University of Beirut. DOCA was found by Hechter et al. (1951) to be qualitatively equivalent to the free compound. Its structural formula is the following:



The optimum activity of this hormone in vitro was found to be at pH = 7.4 (Hayano and Dorfman 1953). As DOCA belongs to an essentially insoluble group of steroids (Hayano and Dorfman 1951-52), the various amounts were added to the basic incubation medium, described above, in the form of an ether solution which was then submitted to a gentle stream of air until a very fine suspension of DOCA of the required concentration was obtained. The

different concentrations of hormone used in both preliminary and final experiments are found on Tables I, III, V, VII, IX, X and XII at the end of the present paper.

Tissue incubation method.

The tissue incubation method used was the one described recently by Bullough (1952, 1954). In preliminary experiments this method proved to be most satisfactory and runs as follows. Ear fragments were put in Warburg flasks having a capacity of 19 cc. The first contained 5 cc. of basic incubation medium and the second, 5 cc. of DOCA suspension in the basic medium.

To get an accurate estimate of the mitotic activity developing during the course of each experiment, 0.016 mg. of colchicine dissolved in 0.04 cc. of saline was used. This amount of colchicine solution was filled in the side arm of each flask. The advantages and disadvantages of using this drug were already discussed in the introduction. Its activity was tested in preliminary experiments. The flasks were then attached to manometers and maintained in a water-bath at a constant temperature of 38°C. The flasks were filled with oxygen gas from a tank by replacement of air. The manometers were then gently rocked during the 5 hours of the experiment. No aseptic conditions were necessary since Bullough did not observe any bacterial growth during the short period of incubation. Nevertheless, all instruments and glassware were thoroughly cleaned.

Histological technique.

At the end of the incubation, the ear fragments were fixed in Bouin's alcoholic fluid for 48 hours. They were then washed with 50% alcohol and dehydrated by consecutive changes in 75%, 85%, 95% and absolute alcohol. The tissues were then cleared in xylol, infiltrated for 2 days and embedded in paraffin wax. They were cut into serial sections of 7 micra thickness. Every 4th or 5th section out of 40 - 50 was affixed to a slide. They were then stained with Delafield's hoematoxylin and eosin.

Counting technique.

The number of cells undergoing division was counted in 10 sections from each ear fragment. As the sections were perpendicular to the surface of the ears, both upper and lower epidermises were seen under the microscope at the same time and therefore mitosis was counted in all cells observed. A nucleus was considered to be in mitosis when the nuclear membrane had disappeared and the chromatin material was aggregated and deeply stained (Carter 1953).

The frequency of mitosis is expressed per 1 cm. length of tissue and a minimum total length of 20 cm. in each of the control and experimental group of ear fragments was reached. The average number of mitoses per unit length (1 cm) for each group was then calculated and the standard deviation estimated according to the formula:

$$\delta = \sqrt{\frac{fd'^2}{n} - \left(\frac{\varepsilon fd'}{n}\right)^2}$$

where, δ is the standard deviation

f is the frequency of mitoses

d' is the deviation from the mean

n is the total number of mitoses in the group.

The interpretation of the results and their significance was based on the calculation of "t" values expressed as follows:

$$\text{"t"} = \frac{\text{diff. between the means}}{\delta \text{ diff.}}$$

where $\delta \text{ diff.} = \sqrt{\delta^2_{\bar{m}_{control}} + \delta^2_{\bar{m}_{exp.}}}$

$\bar{m}$ being the mean calculated from:

$$\bar{m} = a + i\left(\frac{\varepsilon fd'}{n}\right)$$

a is the assumed mean

i is the interval between class values.

Experimental procedure.

All the experiments were performed at 2:00 p.m. during the afternoon sleep-time of the animals, since it was found that mitosis in the epidermis of mice is most active between 10:00 a.m. and 4:00 p.m. (Bullough 1948). The animals were handled gently, care was taken not to frighten them to avoid any mitotic disturbances which might result from fear or shock (Bullough 1949 c, Green and

Bullough 1950).

For the experiment each mouse was submitted to a slight ether anesthesia and the ears were detached with fine scissors. The left ears were kept separately from the right ones for control and experimental groups respectively.

Next, their thin peripheral region was cut into pieces each about 2 x 4 mm., 10 such pieces from the left ears were put in the basic medium. From the right ears 20 pieces were obtained, 10 for each concentration of DOCA. The different media were kept ready in Warburg flasks at the beginning of the experiment. The solutions were prepared fresh for each experiment.

Colchicine, as already described, was placed in the side arm of the flasks which were then attached to the manometers and placed in the water-bath for incubation. Oxygen gas was filled into the flasks at that time and then the manometers were gently rocked.

After 1 hour, when all the mitoses originally present in the epidermis were judged to have passed through the metaphase, the colchicine was washed into the main vessel. The incubation was continued then for a further 4 hours during which time the colchicine was supposed to arrest all newly developing mitoses in the metaphase (Bullough 1948).

At the end of 5 hours of incubation, the tissues were removed from the solutions, dried on a filter paper,

and fixed in Bouin's alcoholic fluid to be sectioned and stained for mitotic counts as already described.

RESULTS

Preliminary experiments.

Without colchicine: Tables I, III and V give the results of preliminary experiments in which the effect of different concentrations of DOCA was tested in the absence of colchicine. Graphs 1, 2 and 3 correspond to the results of Tables I, III and V respectively. It can be seen on these graphs that there is a marked increase in the number of mitoses when concentrations less than 1 mg. of DOCA per 100 cc. of solution are used, and that concentrations higher than 1.5 mg. of DOCA result in mitotic depression. Whether DOCA is mitogenic, without a significant effect or inhibitory was judged from the "t" values summarized in Tables II, IV and VI and corresponding to the results of Tables I, III and V respectively, despite the fact that only two mice were used for each experiment.

With colchicine: In Table VII are summarized the results of experiments in which colchicine was used for comparative purposes to see whether or not colchicine should be used in the final experiments. The average number of mitoses resulting from both low and high concentrations of DOCA was calculated and a graph drawn (Graph 4). Two conclusions were reached: (1) the action of DOCA is the same whether or not colchicine is used, as it is seen from Table VIII containing the "t" values for the results of Tabel VII;

(2) when colchicine is used more mitoses were found probably because, in the absence of colchicine, some of the dividing cells went to completion during the incubation period. The comparison between experiments with and without colchicine is made in a separate table (Table IX) and better seen on Graph 5.

Decisive Experiments.

All the final experiments were performed in the presence of colchicine.

A few effective concentrations of DOCA were used for these experiments the results of which are summarized in Tables X and XII. These figures can be considered reliable since ten mice were used for each experiment, repeated three times. It is to be noted that the use of a big number of animals resulted in larger standard deviations but despite this fact the "t" values for all except for 1 mg. exceeded 2, that is they were significant. Table XI contains the "t" values for the results of Table X, and Table XIII gives the "t" values for the results of Table XII. Therefore the increase or decrease observed in the number of dividing cells was not the consequence of chance deviation in counting but due to the specific effect of DOCA present. Experiments with 1 mg. of DOCA failed to give a significant increase in mitotic activity. The slight increase which can be seen on Graph 6 is merely the result of chance deviation. From the results of Tables X and XI, it can be concluded

therefore that DOCA acts as a mitogenic hormone at concentrations below 1 mg.

On the other hand, Table XII and "t" values in Table XIII give evidence of the inhibitory action of this hormone. All the "t" values exceed 2 and therefore the decrease in the number of mitoses with these high concentrations of DOCA is definitely due to the influence of this hormone.

Graph 7 gives a better idea of the sharp decrease in mitotic activity. It is interesting to note however from the results of Tables V, VII and XII that both in preliminary and final experiments no complete inhibition of mitosis was observed even with 20 mg. which is known to produce narcosis when injected in the body.

DISCUSSION AND CONCLUSIONS

The results summarized in Tables I - XIII inclusive give some evidence of the effects of DOCA on factors involved in normal mitotic activity. It is interesting to note that different concentrations of DOCA, provided all the other conditions are kept constant, produce different effects on cell division. High concentrations inhibit mitotic activity in the mouse epidermis, low concentrations rather stimulate it.

The results therefore have to be considered from two angles: (1) how does DOCA stimulate mitosis? (2) how does it inhibit it? To the second, a further angle may be added, namely, how does a change in concentration produce a reversal of action on the part of this hormone?

To answer these questions fully is beyond the scope of this paper since the very mechanism which stimulates a cell to enter prophase is still unknown. Nevertheless, an attempt will be made to interpret the results of the present experiments on the basis of what has been already observed and proved by physiologists concerned with these and related problems.

<u>DOCA as a mitotic inhibitor.</u>

It was mentioned in the Introduction that epidermal mitotic activity is closely related to maximum energy production. As stated by Bullough (1954) both the rate of

entry of glucose into the cell and its subsequent conversion into energy are responsible for the development of active cell division. The mitosis depressing action of DOCA in vitro might be therefore explained on the basis of its interference with either glucose penetration or glucose oxidation. In fact, it was found recently that both are affected by the presence of DOCA. Leupin and Verzár (1949, 1950) observed that the normal glucose uptake of isolated muscle was inhibited by DOC in vitro. Decrease in permeability to glucose was suggested to account for the decrease of glucose uptake.

Furthermore, Hayano and Dorfman (1951-52) found that at concentrations as low as 1.5 mg. DOC inhibited the oxidation, in various brain tissue preparations, of a number of substrates, including hexoses, Krebs cycle components and amino acids. The results showed an inhibition of 30 - 90% in each instance which is somewhat similar to the percentage of inhibition in epidermal mitosis at concentrations of DOC exceeding 1.5 mg. The mitosis depressing action of DOCA may be purely chemical in nature, that is DOCA unites with the available oxygen of the culture medium and does not give it up easily. There is no strong evidence for such a hypothesis since incubation of DOC with various surviving rat tissues as observed by Schneider and Hortsmann (1951) resulted in alteration of the side chain of DOC and the change was reductive.

The inhibitory action of DOC is more logically explained on a physiological basis, that is DOC interferes with the respiratory mechanism of the cells. Since cell division is greatly supported by energy coming from respiration (Medawar 1947, 1948) interference of DOCA with some of the respiratory enzymes may account for the inhibitory action of this hormone in vitro. In fact DOC is known to inhibit a few enzymes as d-amino acid oxidase and also the oxygen consumption of different tissues as liver, kidney and brain at concentrations of 5 mg. or less (Hayano and Dorfman 1951-52).

These considerations and others give some evidence for the actual interference of DOCA with both glucose utilization and oxidative processes of the cells. Observations done by various investigators, some of whom were mentioned in the Introduction, on the effect of DOC on growth and development favour the hypothesis that DOC is actually a mitotic inhibitor.

The mitogenic action of DOCA.

It is hard to explain how low concentrations of DOCA resulted in accelerated cell division as there is much evidence to the contrary. Nevertheless an attempt will be made to analyze some peculiarities of DOCA action in order to find out a possible hypothesis for its mitosis stimulating effects.

Since mitotic activity depends upon energy production,

and DOC was found to inhibit aerobic oxidation of glucose, there must be some source providing the energy supply for active cell division. A review of the relations existing between DOC and glycolysis might help in finding out this source of energy supply.

Verzár and Montigel (1941) showed that glycogen phosphorylation in the muscle was enhanced by DOC in vitro. Later on, Verzár and Wenner (1948) found that glycogenesis was completely inhibited in the isolated rat diaphragm in vitro. In a subsequent study, Verzár (1951-52) suggested that the inhibition of glycogen production in vitro was due to an increased breakdown of this substance through the mechanism of enhanced phosphorylation.

Furthermore, Bullough (1954), in his studies of hormonal relations of mitotic activity, considered that the one factor normally limiting the epidermal mitotic rate is the slowness of the glucokinase reaction. This reaction is poised mid-way and is capable of being either stimulated or depressed.

On the other hand, it has to be noted that in all experiments so far mentioned in connection with the inhibitory action of DOC, concentrations not lower than 1.5 mg. were used. It is possible that, at such low concentrations as 0.5 mg., DOCA does not interfere with the penetration of glucose into the cells. Therefore, after considering these facts, it might be suggested that the mitogenic action of

DOCA is due to increased glycogen breakdown followed by stimulated anaerobic glucolysis.

This explanation is tentative however. It is possible that DOCA exerts its action through some other unknown mechanism. The problem has not been elucidated yet and needs to be further studied.

Reversal of action of DOCA.

Such a reversal has been already observed by a few investigators. Verzár and Wang (1950) found a reversal of glycogenesis to glycogenolysis on the part of DOC depending upon the dosage given to the animal.

It might be that changes in permeability are the primary factors responsible for both aspects of DOCA behaviour. Minute amounts of this hormone do not probably interfere with the permeability of the cell to glucose, whereas high concentrations depress it as already mentioned in connection with the glucose uptake of muscles in vitro.

Conclusions.

Although the specific action of DOCA on energy yielding processes is not quite clear, it can be concluded from all the considerations done in this paper that DOCA depresses epidermal mitotic activity because of its interference with both permeability of the cells to glucose and subsequent aerobic oxidations.

On the other hand, the mitogenic action of DOCA is

probably due to the following phenomena: (1) increased glycogen breakdown and (2) stimulated glucolysis. It has to be noted that this latter activity is possible only, if, as suggested already, DOCA does not interfere with the permeability of the cells to glucose.

The inhibitory aspect of DOCA has got strong evidence on its side but the mitogenic aspect of this hormone needs to be further studied and experimented on other tissues than the epidermis.

SUMMARY

The effect of desoxycorticosterone acetate (DOCA) on epidermal mitosis was tested in vitro by incubating mouse ear fragments in a phosphate-buffered saline to which isotonic glucose, oxygen and colchicine were added. The different concentrations of DOCA were found to have different effects. Concentrations lower than 1 mg. per 100 cc. of solution stimulated mitosis; higher concentrations (1-20 mg.) rather inhibited the normal mitotic rate of epidermal cells.

It was suggested that increased glycogen breakdown along with stimulated anaerobic glucolysis might be responsible for the mitogenic action of the hormone, provided that in the presence of low concentrations of DOCA glucose entry into the cells is not affected. As to the inhibitory action of this hormone on epidermal mitotic activity there was found evidence for the actual interference of DOCA with glucose penetrations into the cells and its subsequent oxidation.

T A B L E S

TABLE I

THE AVERAGE NUMBER OF MITOSES PRESENT PER UNIT SECTION LENGTH (1 cm) OF EAR EPIDERMIS IN CONTACT WITH DOCA WITHOUT USING COLCHICINE

% Conc. of DOCA	Group I	Group II	Group III
Control	5.9 ± 0.7	6.1 ± 0.3	5.4 ± 0.2
0.25 mg	9.7 ± 0.4	9.1 ± 1.3	8.6 ± 1.2
0.50 "	9.9 ± 1.2	10.4 ± 0.9	9.5 ± 1.7
0.75 "	8.1 ± 0.7	9.3 ± 1.1	7.2 ± 0.8
1.00 "	6.5 ± 0.9	6.7 ± 0.2	7.0 ± 1.5

TABLE II

"t" VALUES FOR THE RESULTS OF TABLE I

% Conc. of DOCA	Group I	Group II	Group III
0.25 mg	4.4	2.2	2.6
0.50 "	2.8	4.5	2.4
0.75 "	2.1	2.1	2.2
1.00 "	0.5	0.9	1.1

TABLE III

THE AVERAGE NUMBER OF MITOSES PRESENT PER UNIT SECTION LENGTH (1 cm) OF EAR EPIDERMIS IN CONTACT WITH LOW CONCENTRATION OF DOCA AND IN THE ABSENCE OF COLCHICINE

% Conc. of DOCA	Group I	Group II	Group III
Control	6.2 ± 0.5	5.7 ± 0.33	5.8 ± 0.4
0.5 mg	10.1 ± 0.7	9.8 ± 1.3	10.3 ± 1.2
1.0 "	7.1 ± 1.3	6.5 ± 0.9	6.2 ± 1.4
1.5 "	4.9 ± 0.8	5.2 ± 0.5	4.4 ± 1.2
2.0 "	3.4 ± 0.65	4.1 ± 0.6	3.7 ± 0.56

TABLE IV

"t" VALUES FOR THE RESULTS OF TABLE III

% Conc. of DOCA	Group I	Group II	Group III
0.5 mg	4.8	3.05	3.57
1.0 "	0.63	0.84	0.27
1.5 "	1.3	0.84	1.11
2.0 "	3.5	2.4	3.08

TABLE V

THE AVERAGE NUMBER OF MITOSES PRESENT PER UNIT SECTION LENGTH (1 cm) OF EAR EPIDERMIS IN CONTACT WITH HIGH CONCENTRATIONS OF DOCA

% Conc. of DOCA	Group I	Group II	Group III
Control	5.3 ± 0.6	6.0 ± 0.7	5.9 ± 1.2
5.0 mg	3.1 ± 0.7	2.9 ± 1.1	2.7 ± 0.5
7.5 "	3.3 ± 0.2	2.4 ± 0.9	2.5 ± 1.3
10.0 "	1.2 ± 0.1	1.7 ± 0.3	1.3 ± 0.4
20.0 "	0.9 ± 0.5	0.5 ± 0.1	1.1 ± 0.2

TABLE VI

"t" VALUES FOR THE RESULTS OF TABLE V

% Conc. of DOCA	Group I	Group II	Group III
5.0 mg	2.3	2.3	2.4
7.5 "	3.1	3.2	2.0
10.0 "	6.8	5.6	2.9
20.0 "	5.6	7.8	4.0

TABLE VII

THE AVERAGE NUMBER OF MITOSES PRESENT PER UNIT SECTION LENGTH (1 cm) OF EAR EPIDERMIS IN CONTACT WITH DOCA AND COLCHICINE

% Conc. of DOCA	Group I	Group II	Group III
Control	7.9 ± 0.5	8.2 ± 1.3	7.4 ± 0.9
0.5 mg	10.5 ± 0.3	11.4 ± 0.8	10.8 ± 0.4
1.0 "	7.5 ± 1.1	7.7 ± 0.9	8.1 ± 1.3
5.0 "	3.9 ± 1.2	4.2 ± 0.8	3.7 ± 1.4
10.0 "	1.1 ± 0.2	1.4 ± 0.1	1.7 ± 0.6

TABLE VIII

"t" VALUES FOR THE RESULTS OF TABLE VII

% Conc. of DOCA	Group I	Group II	Group III
0.5 mg	4.4	2.1	3.4
1.0 "	0.2	0.3	0.4
5.0	3.1	2.6	2.2
10.0	12.8	5.2	5.2

TABLE IX

COMPARISON BETWEEN AVERAGE NUMBER OF MITOSES PRESENT PER UNIT LENGTH (1 cm) OF EAR EPIDERMIS WHEN TREATED AND WHEN NOT TREATED WITH COLCHICINE

% Conc. of DOCA	Without Colchicine		With Colchicine	
	Min.	Max.	Min.	Max.
Control	5.3	6.2	7.4	8.2
0.5 mg	9.5	10.4	10.5	11.4
1.0 "	6.2	7.1	7.5	8.1
5.0 "	2.7	3.1	3.7	4.2
10.0 "	1.2	1.7	1.1	1.7

TABLE X

THE AVERAGE NUMBER OF MITOSES PRESENT PER UNIT SECTION LENGTH (1 cm) OF EAR EPIDERMIS IN CONTACT WITH LOW CONCENTRATIONS OF DOCA AND COLCHICINE

% Conc. of DOCA	Group I	Group II	Group III
Control	7.1 ± 0.5	8.0 ± 0.7	7.7 ± 0.1
0.5 mg	10.1 ± 0.8	10.9 ± 1.2	11.1 ± 0.5
1.0 "	7.9 ± 0.3	8.1 ± 0.6	8.2 ± 1.4

TABLE XI

"t" VALUES FOR THE RESULTS OF TABLE X

% Conc. of DOCA	Group I	Group II	Group III
0.5 mg	2.6	2.6	2.3
1.0 "	0.7	0.1	0.2

TABLE XII

THE AVERAGE NUMBER OF MITOSES PRESENT PER UNIT SECTION LENGTH (1 cm) OF EAR EPIDERMIS IN CONTACT WITH HIGH CONCENTRATIONS OF DOCA AND COLCHICINE

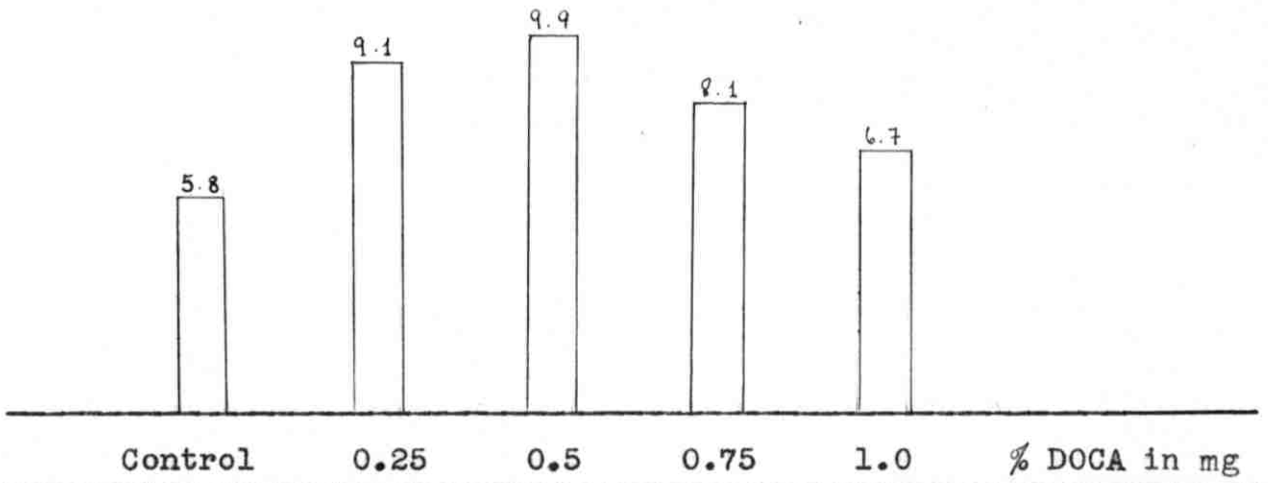
% Conc. of DOCA	Group I	Group II	Group III
Control	7.9 ± 2.8	9.2 ± 3.0	8.1 ± 2.4
5.0 mg	4.1 ± 0.3	3.7 ± 0.9	3.8 ± 0.2
10.0 "	1.3 ± 0.6	1.2 ± 0.7	1.1 ± 0.7

TABLE XIII

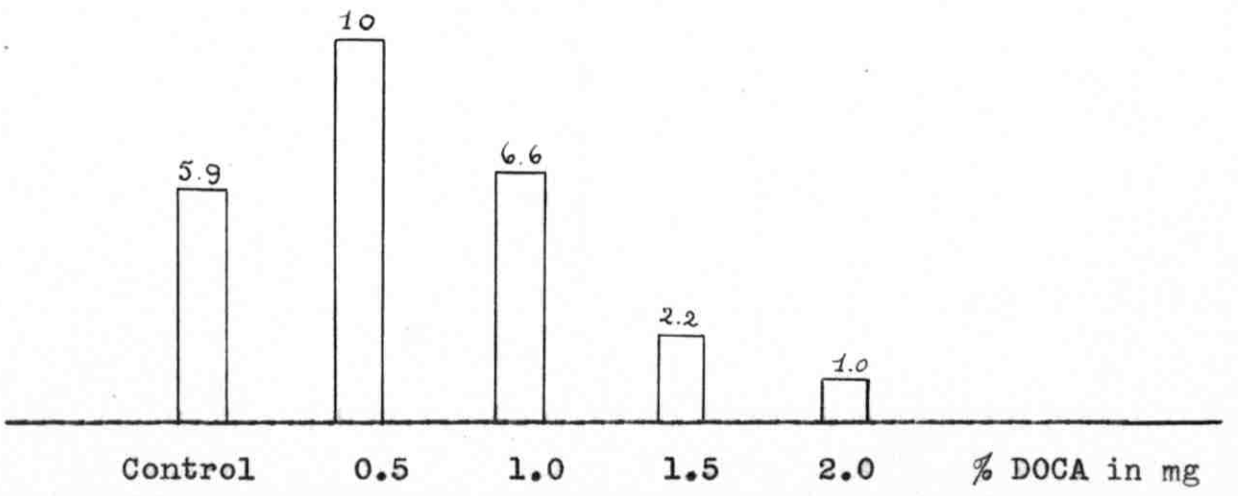
"t" VALUES FOR THE RESULTS OF TABLE XII

% Conc. of DOCA	Group I	Group II	Group III
5.0 mg	2.0	2.3	2.6
10.0 "	2.3	2.6	2.8

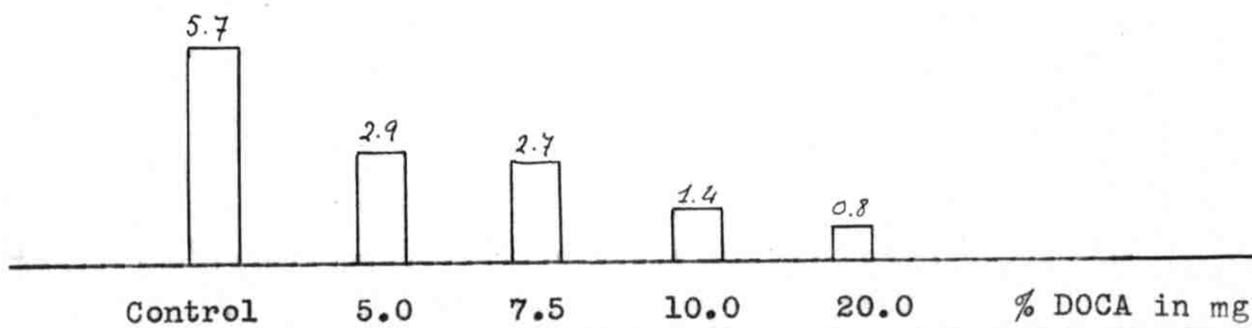
G R A P H S



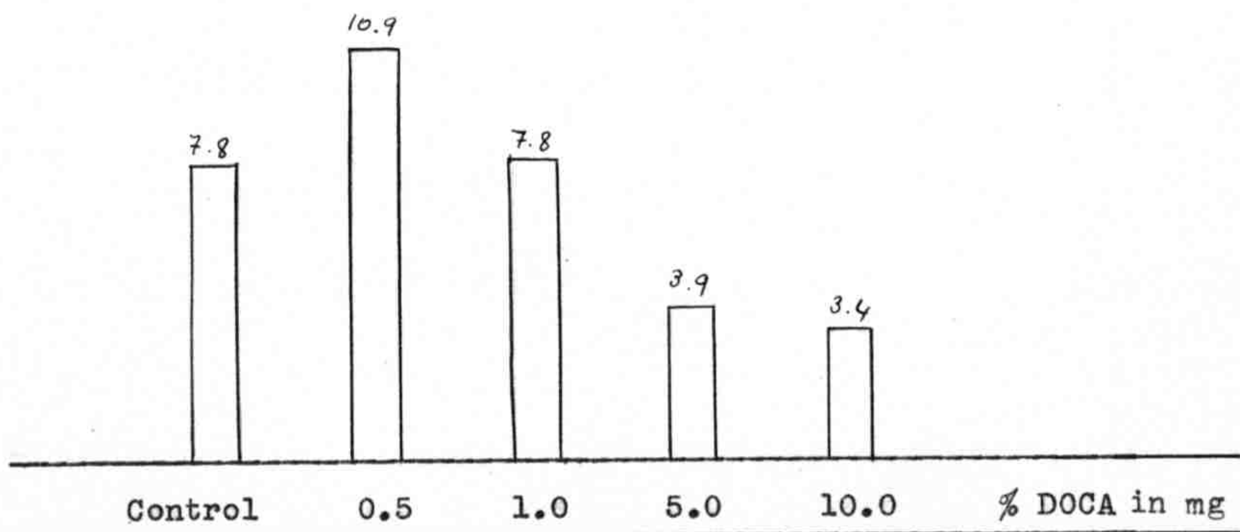
GRAPH 1. THE RELATIVE AVERAGE NUMBER OF MITOSES IN CONTROL AND EXPERIMENTAL GROUPS WHEN COLCHICINE IS NOT USED.



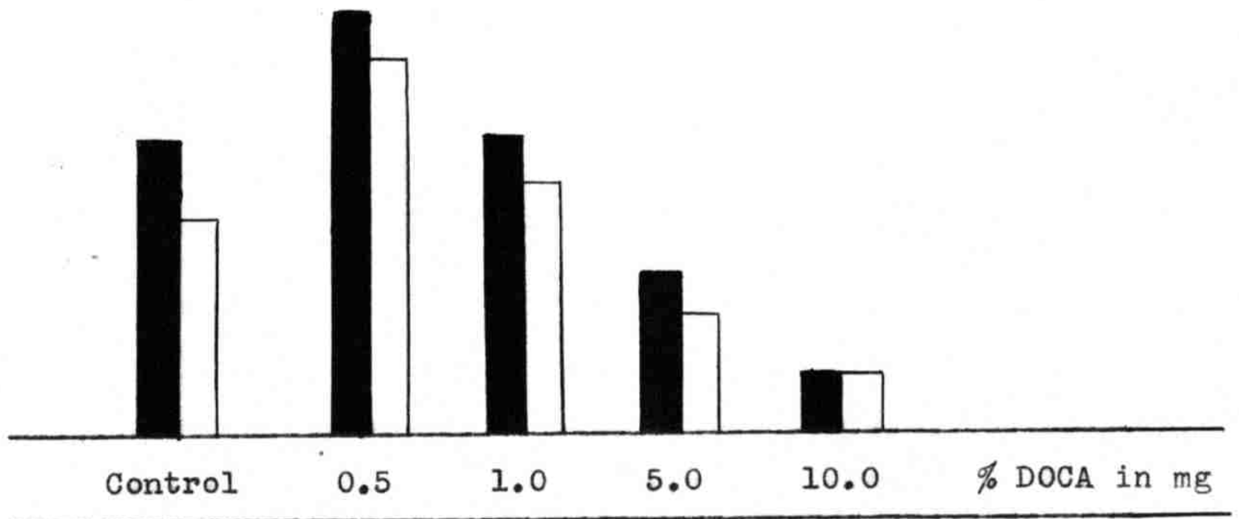
GRAPH 2. THE RELATIVE AVERAGE NUMBER OF MITOSES IN CONTROL AND EXPERIMENTAL GROUPS WHEN COLCHICINE IS NOT USED.



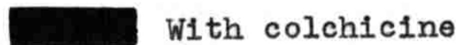
GRAPH 3. THE RELATIVE AVERAGE NUMBER OF MITOSES IN CONTROL AND EXPERIMENTAL GROUPS WHEN COLCHICINE IS NOT USED.

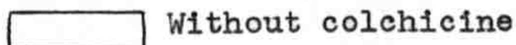


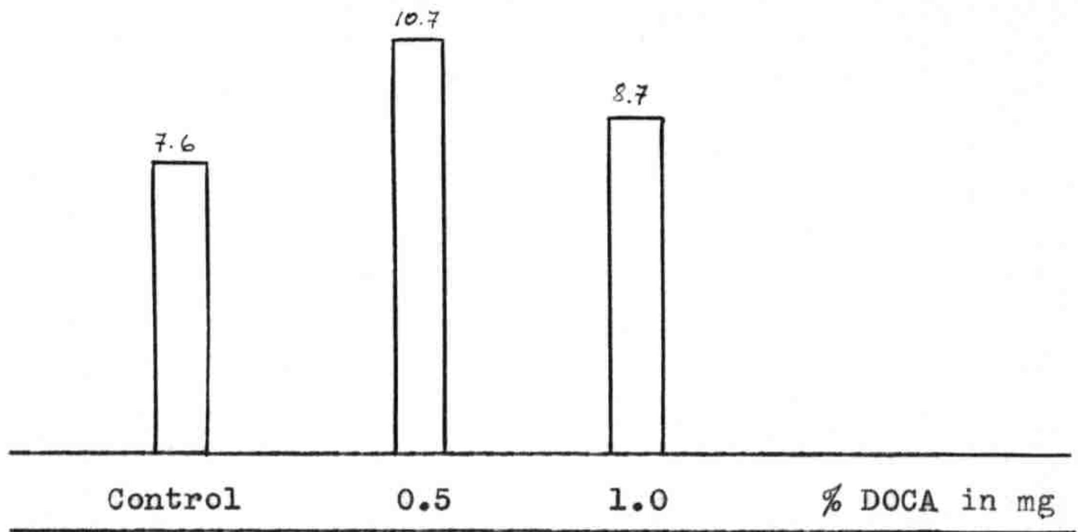
GRAPH 4. THE RELATIVE AVERAGE NUMBER OF MITOSES IN CONTROL AND EXPERIMENTAL GROUPS WHEN COLCHICINE IS USED.



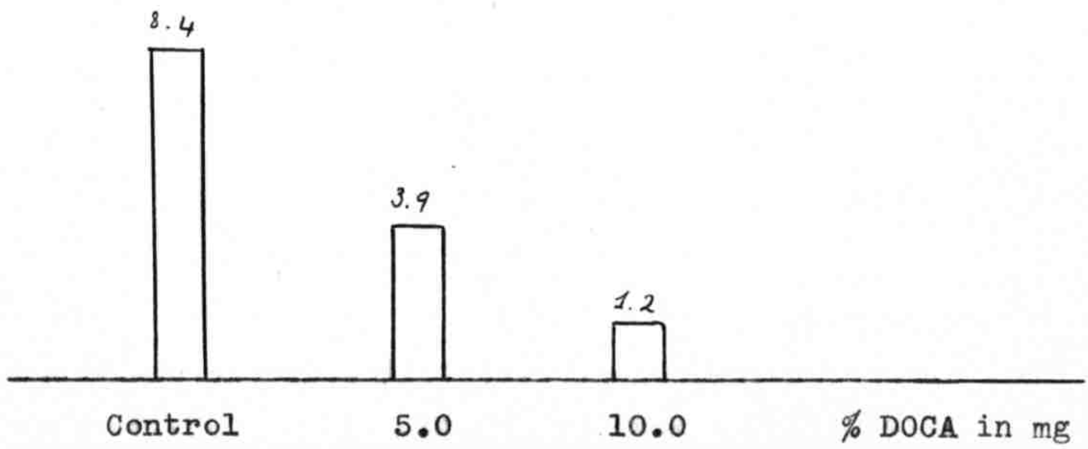
GRAPH 5. AVERAGE NUMBER OF MITOSES WHEN COLCHICINE IS USED COMPARED TO THE AVERAGE NUMBER OF MITOSES WHEN COLCHICINE IS NOT USED.

 With colchicine

 Without colchicine



GRAPH 6. THE RELATIVE AVERAGE NUMBER OF MITOSES IN CONTROL AND 2 EXPERIMENTAL GROUPS.



GRAPH 7. THE RELATIVE AVERAGE NUMBER OF MITOSES IN CONTROL AND 2 EXPERIMENTAL GROUPS.

P L A T E S

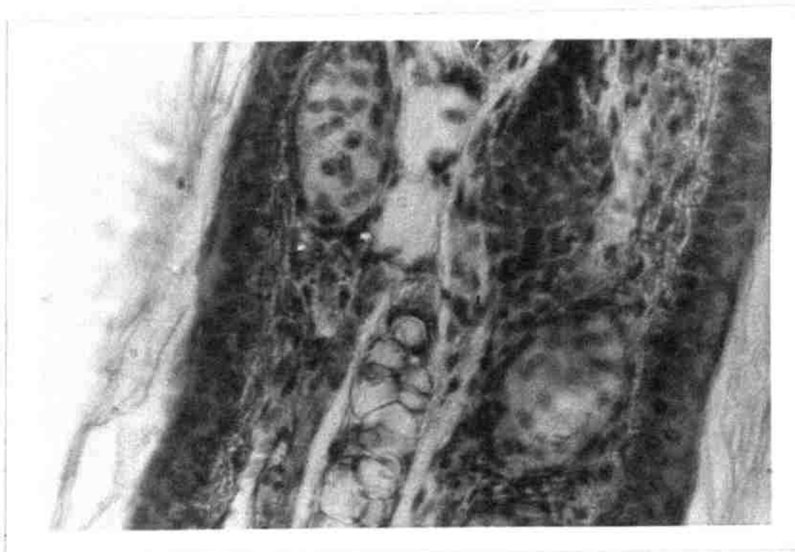


FIG. 1. MICROPHOTOGRAPH OF NORMAL MOUSE EPIDERMIS SHOWING MITOTIC FIGURES.

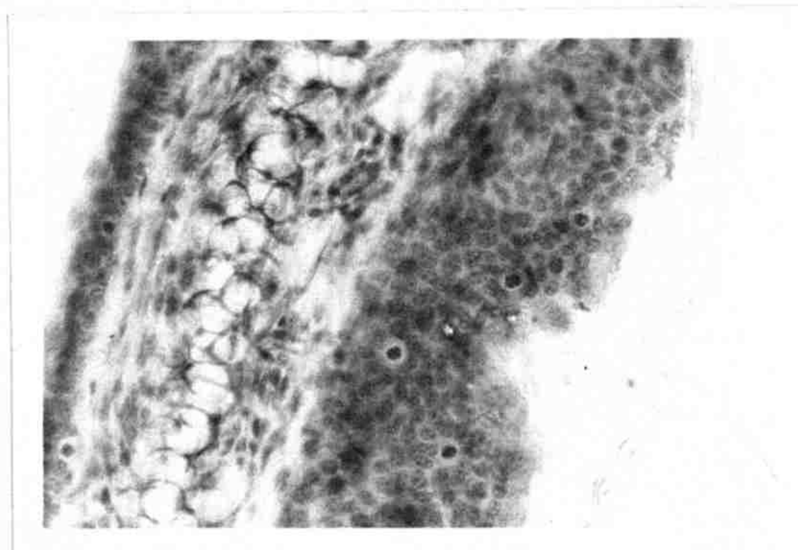


FIG. 2. MICROPHOTOGRAPH OF MOUSE EPIDERMIS IN 0.5 MG/100 CC OF DOCA SHOWING MITOTIC STIMULATION.

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