EFFECTS OF ESTROGEN ON THE MORPHOLOGY OF HUMAN CHROMOSOMES AND ON THE MITOTIC RATE OF CHROMOSOMES IN VITRO

by

Elizabeth Ann Barrow

Submitted as an Honors Paper
in the
Department of Biology

The University of North Carolina
at Greensboro
1967

Approved by

Laura G. Anderton
Director

Examining Committee

Bruce M Eberhart

Hilde T. Harpster

Paul E. Lutz

My sincere thanks to Dr. Laura Anderton and to Mrs. Joan Hall for their help and encouragement throughout this study.

TABLE OF CONTENTS

	Page
Abstract	
Introduction	1
Materials and Methods	7
Results	12
Discussion	19
Illustrations	24
Literature Cited	38

ABSTRACT

An investigation was made to determine the effect of the natural estrogen estrone, in varying concentrations and over varying periods of time, on the chromosomes of human female peripheral blood lymphocytes *in vitro*. All experiments were carried out according to a modification of Tips' Micro Procedure for Peripheral Leucocyte Culture. One set of leucocyte cultures was exposed to 6.0 μg of estrone for 24, 48 and 72 hours, and another set of cultures was exposed to 0.6 μg of the hormone for the same respective time periods. Chromosome spreads obtained from each culture were analyzed in respect to control groups for changes in morphology and mitotic rate. 100 control and 50 estrogen-treated spreads were analyzed for each experimental group in consideration of morphology, and 500 control and 500 estrogen-treated spreads were analyzed for each group in consideration of mitotic rate. The net effect of estrone at 0.6 μg is an increase in mitotic rate in the 48 to 72 hour range and an increase in polyploidy in the 24 to 48 hour range, with the peak of activity at 48 hours. At 6.0 μg the net effect is a decrease in mitotic rate and an increase in polyploidy in the 24 to 48 hour range, the peak of activity coming, again, at 48 hours. Marked endoreduplication occurs at

48 hours with 6.0 μg of estrone.

Numerous implications may be drawn from such effects as estrogen has been shown to elicit in this investigation. From the current literature three areas suggest possible immediate applications for the conclusions drawn here:

1) "Target"-"non-target" tissues: A need to re-examine these concepts is indicated by the marked response to estrogen exhibitted by lymphocytes.
2) Embryonic differentiation: The varying effect of estrogen over varying periods of time suggests a direct relation to embryonic differentiation, where time is a key factor in the control of tissue response and developmental direction.
3) Cancer research and diagnosis: The link which estrogen has been shown to have with cancerous changes in the human and in animals suggests a possible application of cytogenetic evidence of estrogen action to diagnostic and research techniques in the area of malignant transformation.

INTRODUCTION

The natural estrogens are hormones secreted in the human female by the ovaries, the Graafian follicles, the corpus luteum and, during pregnancy, the placenta, under the influence of FSH from the pituitary. In the normal female the estrogens bring about changes in the vaginal epithelium and prepare the uterus for reception of the fertilized ovum. Estrogens stimulate growth of the mammary glands and affect development of secondary sex characteristics. The natural interaction of all the gonadal and gonadotropic hormones in the human (male or female) is, however, an extremely complex process, and it is difficult to define these various physiological events as specific effects of the hormone estrogen alone. They involve, rather, the intricate interaction of estrogens, progestin and the hormones of the pituitary. It has been, therefore, the effort of research to attempt to determine the effects of estrogen on a more specific level. Investigations have been made to discover not only its general influence in normal development, but its effect on individual tissues, cells and their components as well, and to determine the exact mechanism by which such effects are accomplished.

Certain "target tissues," tissues which respond dramatically and characteristically to the presence of

estrogen, have been the subject of many investigations. Recent studies on the cornification of vaginal epithelium and additional responses of the vagina to estrogen stimulation have been conducted by Kusuda (1963) and Hermreck (1966). Uterine response to estrogen (stromal edema, vasodilation, gland proliferation) has been well documented. Current investigation (Nillson, 1966) concerns additional factors such as the increased adhesiveness of uterine epithelium as a result of estrogen treatment. Other target tissues, including the oviduct, mammary gland and pituitary, have also been studied in detail (Kim, 1963; Cutts, 1964; Schitkova, 1964; Harris, 1965).

Many tissues designated "non-target," however, have been shown to demonstrate pronounced responses to estrogen. Desoille (1961) reported significant leukopenia and a decrease in red blood cells in both male and female rabbits subjected to estrogen treatment. Estrogen has been found to raise the level of certain serum proteins in sheep (Slebodzinski, 1962) and some plasma proteins in man (Musa, 1965). Jarzyna (1966) found that estrogen administration impaired blood clotting in rabbits, and Bennett (1966) reported a reduction in antiplasmin and fibrinogen levels as a result of estrogen treatment in the human. Injection of estrogen has been found to produce an increase in thyroid weight and thyroidal radioiodine uptake in the rat (Boccabella, 1964; Yamada, 1966). Fisher (1963)

reported evidence of direct stimulation of pituitary TSH release by estradiol benzoate. Estrogen has been found to affect mast cells of the rat uterus (Levier, 1965) and L strain fibroblasts in tissue culture (Kuchler). In "The Action of Hormones on Cell and Organ Cultures" Ilse Lasnitzki gives a review of varied and conflicting reports of estrogen-responsive tissue from previous years: dental pulp, bone marrow and spleen from rabbits (Yagi, 1940); chick iris epithelium (Kubo, 1938, 1939); mouse heart fibroblasts (Von Haam, 1940) and chicken heart fibroblasts (Tageguti, 1937); mouse ear epidermis (Bullough, 1954a).

Estrogen thus displays a pronounced and unmistakable influence on a wide range of both "target" and "non-target" tissues. Many attempts have been made to explain the varying results of estrogen activity in the elucidation of such widely differing responses as have been outlined here. A review of the literature will show that considerations of possible mechanisms of estrogen action have followed three major lines of thought. These areas of thought place the effect of estrogen at three different levels of activity:

1) Effect on the cell membrane level: effect on metabolism by altering membrane permeability, thus making substrate and cofactors more available.
2) Effect on the intracellular, cytoplasmic level:
   a) altering of the rate of enzyme production

b) Regulating the rate at which useful energy is made available to the cell

c) altering activity of a preformed protein molecule (converting an inactive protein to one with enzymatic activity)

d) competing with one of the cofactors for a site on an enzyme molecule, thus depressing the over-all activity of the system

e) being involved itself as a coenzyme or cosubstrate in the enzyme system

3) Effect on the nuclear level: involvement in the biosynthetic process of nucleic acid synthesis.

Experimental evidence at the present time seems to indicate that the mechanism is actually in agreement with the third line of thought, that of an effect on the molecular level. Gorski (1964) reported that the synthesis of rapidly labeled RNA in the rat uterus, measured by <u>in vivo</u> cytidine--H^3 incorporation into RNA, was increased by 88% within one hour after estrogen administration. He concluded that the synthesis of all RNA types is affected, probably by an increase in the activity of RNA polymerase. Mansour (1965) also proposed that the hormone might act directly on the genome by elucidating DNA-primed RNA biosynthesis, the RNA then pervading tissue cells and producing structural and enzymatic alterations. Bonner (1965) stated that estrogen produces its effects by eliciting the synthesis of RNA,

which then elicits the synthesis of proteins, in the form of key enzymes, and Hechter (1965) proposed the "hormone-gene" thesis, which states that "hormones produce effects by regulating genetic programming expressed in terms of enzyme formation and consequent change in cellular metabolism." Segal (1966) supported the RNA-based concept of estrogen action by a series of experiments in which estrogen action was shown to be inhibited by known inhibitors of RNA synthesis, and estrogen effects were shown to be produced by estrogen-activated biologically active RNA.

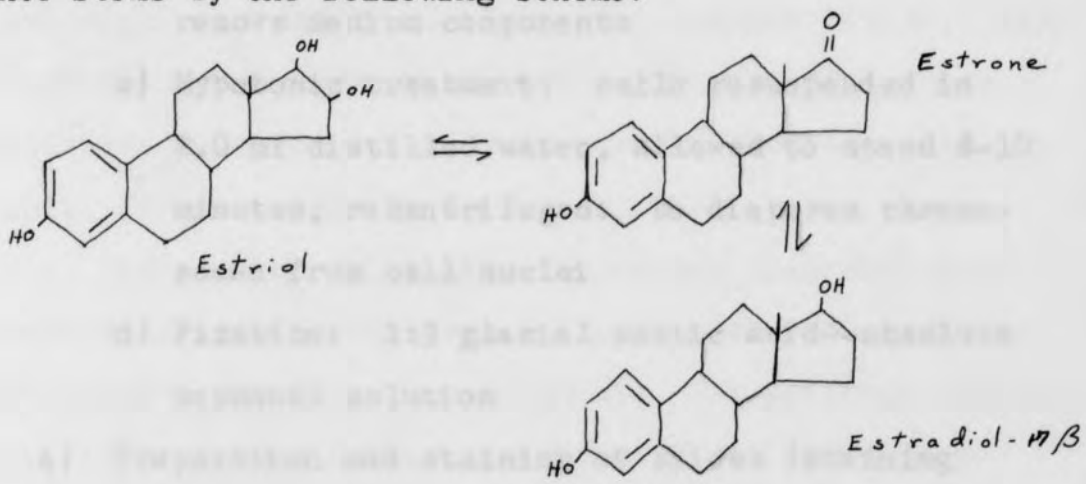
The present state of opinion concerning the mechanism of action of estrogen might, in one sense, make a cytogenetic study in this area unfeasible. RNA synthesis in most cells is at a peak during the interphase of mitosis, while cytogenetic study of the human is generally confined, at this time, to the metaphase chromosomes. In another sense, however, a study of the effect of estrogen on chromosomal morphology and mitotic rate is very applicable to the problem of mechanism. Although present theory ascribes estrogen action to enzyme regulation through RNA-synthesis, the influence of estrogen on different, "non-target" tissues has not been resolved, nor has the problem of differential estrogen effects over varying concentrations and periods of exposure to the hormone. It is therefore important to determine any visible effects which estrogen might have on the actual genetic material of

a non-target tissue, such as the blood lymphocyte. Visible effects, detectable in a cytogenetic study, might include such factors as variations in chromosome morphology (heterochromatin, premature separation of chromatids, coiling, breaks, individual chromosome changes); variations in chromosome number (aneuploidy, polyploidy, endoreduplication); or changes in mitotic rate. Whether such effects could be correlated with the action of estrogen in RNA synthesis, or whether they might simply act as specific and characteristic evidences of estrogen action in the cell, they could be of great value in both theoretical and practical application.

Thus, the present investigation was designed to determine the effects of estrogen, in varying concentrations and over varying lengths of time, on the chromosomes of a "non-target" tissue, human female peripheral blood lymphocytes. An attempt was made to consider all of the cytogenetic factors previously outlined as possible visible effects. It does not appear from the literature that such a study has been conducted before this time, and it would seem that this investigation is not only valuable, but very necessary to the understanding of certain concepts related to estrogen activity and the mechanism by which this activity is governed.

MATERIALS AND METHODS

A study of the effects of estrogen on the chromosomes of human female peripheral blood leucocytes was conducted through analysis of lymphocyte chromosomes after in vitro exposure to varying dilutions of estrogen over varying periods of time. Estrogens are produced in several forms. Of the three principle naturally secreted estrogens the form employed in the present study was estrone. This form is converted, in the body, to the other forms by the following scheme:



Estrone is intermediate between the other two forms in biological strength, its activity being 100 times that of estriol and 1/12 that of estradiol.

All experiments were carried out according to a modification of Tips' Micro Procedure for Peripheral Leucocyte Culture, which may be outlined as follows:

1) Preparation of cultures: 3-4 drops of blood obtained by sterile technique and placed in a

vial containing culture medium (preparation of culture medium is listed on page 11)

2) Incubation: 36.5°-37°C for four days

3) Harvesting of leucocytes:

a) Colchicine treatment: 0.02 cc of colchicine solution (100 μg/ml) added to each culture, cultures incubated 3-5 hours longer: to arrest mitosis at metaphase

b) Treatment with balanced salt solution: 8-10 ml added, followed by centrifugation: to remove medium components

c) Hypotonic treatment: cells resuspended in 2.0 ml distilled water, allowed to stand 8-10 minutes, recentrifuged: to disperse chromosomes from cell nuclei

d) Fixation: 1:3 glacial acetic acid--absolute methanol solution

4) Preparation and staining of slides (staining procedure given on page 11)

Cultures were submitted to experimental treatment during the four-day incubation period. One set of cultures, I, II and III, were exposed to 6.0 μg of estrone for 24, 48 and 72 hours, respectively. Another set, IV, V and VI, were exposed to 0.6 μg of estrone for the same respective time lengths. A control, untreated with estrone, was run with each experimental culture. Such factors as the day

of the subject's menstrual cycle and any occurence of illness, unusual stress or medication, which might affect experimental results, were noted. Normal levels of estrogen in the human female range from 0.1 to 0.3 μg/l, with substantially higher values (up to 1 μg/l) only in the middle third of the cycle (Svendson, 1964). Thus, it was important to note the day of the subject's cycle in order to determine whether high levels of the hormone might be present. It was, of course, necessary to take into account such factors as steroid medication, illness or stress, which might have affected the normal hormone levels. Had any of the subjects recently had X-rays taken this too would have been noted, since chromosome breakage often results from exposure to X-rays.

After harvesting of cultures and preparation of slides a detailed chromosome analysis was conducted for each control and experimental group. The analysis included a count of mitotic index, determination of chromosome number and a study of morphology, considering chromosome breaks, heterochromatic regions, premature separation of chromatids, appearance of rosettes, degree of coiling, and any other morphological variations. Each experimental culture was analyzed in relation to its own control, and care was taken to make uniform studies of all experimental groups. For morphological analysis 100 counts were made for each control group and 50 counts for each of the six

estrone-treated groups. Data on the mitotic index for each group was obtained by taking a count of the number of mitotic figures per 500 cells, for both control and experimental groups.

Photographs were made and karyograms prepared (according to standards established by the Chicago Conference on Standardization in Human Cytogenetics, December, 1966) for further study of individual chromosome spreads.

Care was taken throughout this investigation to standardize procedure, and methods were particularly designed to eliminate variables as much as possible. Careful sterile technique was, of course, used throughout the investigation. Strict uniformity was maintained in regard to timing. Wherever a range of effective times was suggested in the original procedure, for example, an exact and consistant time was used here. Uniformity of culture medium was preserved. The colchicine and phytohemagglutinin used in this investigation were standardized in relation to each other, in order to eliminate technical error. Slides were made immediately after harvesting of leucocytes in each case, to eliminate error which might have arisen from letting the cultures stand overnight first (a possible variation of this procedure). In the final analysis of the chromosomes care was taken to consider a large number of spreads and to make a large number of counts in order to assure mathematical significance to the data.

Culture Medium

Culture Medium #199 with $NaHCO_3$	4	ml
Fetal Bovine Serum	1	ml
Heparin - 1000U/ml solution	0.1	ml
Penicillin-Streptomycin mixture	0.1	ml
Phytohemagglutinin	0.1	ml

Procedure for Giemsa Stain

Giemsa: 90 ml distilled H_2O
7 ml Giemsa stain
5 ml 0.15 M NH_4OH

1) 1 N HCl (60°C)	:	10 min
2) Distilled H_2O	:	rinse
3) Giemsa	:	8-15 min
4) Acetone I	:	rinse
5) Acetone II	:	rinse
6) Acetone/Xylol (50:50)	:	2 min
7) Xylol I	:	5 min to hrs
8) Xylol II	:	10 min to hrs
9) Mount		

RESULTS

A summary of observations and experimental results for the investigation, as outlined in MATERIALS AND METHODS, is given in tables and graphs in the following pages.

Table 1, page 13, gives an analysis of chromosome number and morphology after 6.0 μg estrone treatment at 24, 48 and 72 hours.

Table 2, page 14, gives an analysis of chromosome number and morphology after 0.6 μg estrone treatment at 24, 48 and 72 hours.

Table 3, page 15, gives a summary of data concerning mitotic index for all experimental groups.

Figure 1, page 16, illustrates percent polyploidy versus length of exposure to estrone at 0.6 μg.

Figure 2, page 17, illustrates percent polyploidy versus length of exposure to estrone at 6.0 μg.

Figure 3, page 18 summarizes mitotic index as a function of time of exposure to estrone for both estrone concentrations.

Photocopies of photographs and karyotypes illustrative of controls and of estrogen effect in the various experimental groups will be found at the end of the text of this paper. The originals for these photocopies are on file in the office of the Biology Department, UNC-G.

Table 1. Chromosome Number and Morphology at 6.0 μg

	Culture Number I		II		III	
	Con[1]	Est[2]	Con	Est	Con	Est
% Modal Number (46)	87	58	87	56	84	82
% Nonmodal Number (other than Polyploid)	12	34	12	32	15	18
% Polyploid	1	8	1	18	1	0
% Partial or Total Endoreduplication	0	0	0	10	0	0
% Rosettes	8	0	8	2	12	6
% Coiling	0	4	0	2	9	20
% Heterochromatin	0	0	0	0	2	0
% Early Separation of Chromatids	1	6	1	2	0	2
% Chromosome #1 Aberrations	1	2	1	0	1	0

[1]Con = Control
[2]Est = Estrone

Table 2. Chromosome Number and Morphology at 0.6 μg

	Culture Number					
	IV		V		VI	
	Con[1]	Est[2]	Con	Est	Con	Est
% Modal Number (46)	90	72	90	80	82	70
% Nonmodal Number (other than Polyploid)	9	18	9	6	18	28
% Polyploid	1	10	1	14	0	2
% Partial or Total Endoreduplication	0	0	0	0	0	0
% Rosettes	11	4	11	8	5	16
% Coiling	0	4	0	18	4	6
% Heterochromatin	0	0	0	0	0	0
% Early Separation of Chromatids	0	2	0	4	4	0
% Chromosome #1 Aberrations	1	2	1	6	2	8

[1]Con = Control

[2]Est = Estrone

Table 3. Summary of Data on Mitotic Index

6.0 μg

	Culture Number I	II	III
Day of Cycle	30	30	22
Length of Exposure	24 hrs	48 hrs	72 hrs
Mitotic Index of Control $(M_C)_1$	0.070	0.070	0.081
Mitotic Index of Experimental (M_E)	0.070	0.080	0.067
Δ Mitotic Index (ΔM) = (M_E-M_C)	0.000	0.010	-0.014

0.6 μg

	Culture Number IV	V	VI
Day of Cycle	26	26	8
Length of Exposure	24 hrs	48 hrs	72 hrs
Mitotic Index of Control (M_C)	0.057	0.057	0.075
Mitotic Index of Experimental (M_E)	0.062	0.093	0.109
Δ Mitotic Index (ΔM) = (M_E-M_C)	0.005	0.036	0.034

---

[1] Mitotic Index (Based on 500 counts per culture) is calculated as $\frac{\text{number mitotic figures}}{\text{number non-mitotic cells}}$

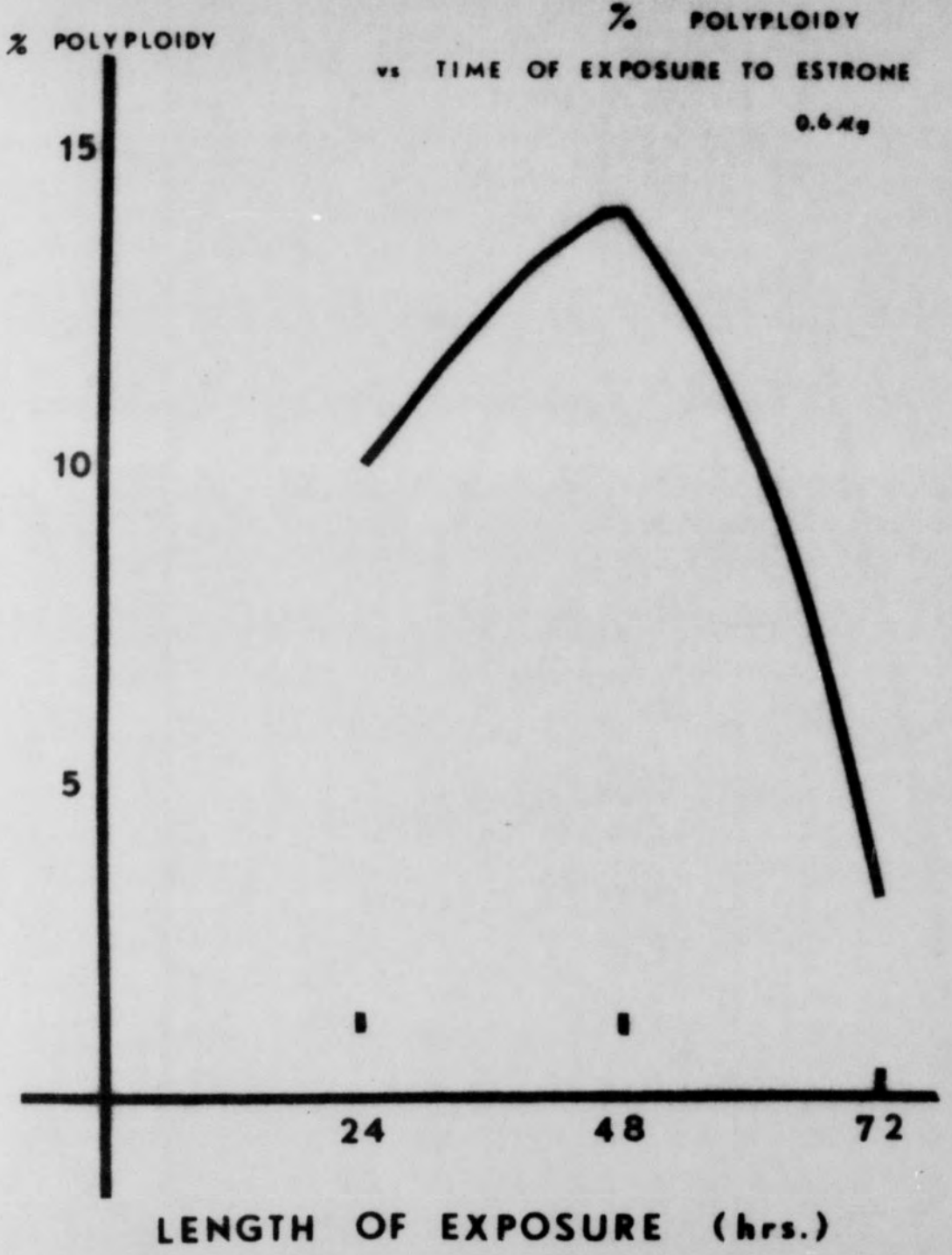

% POLYPLOIDY
% POLYPLOIDY
vs TIME OF EXPOSURE TO ESTRONE
0.6 µg
15
10
5
24
48
72
LENGTH OF EXPOSURE (hrs.)
CONTROL
ESTRONE

Figure 1.

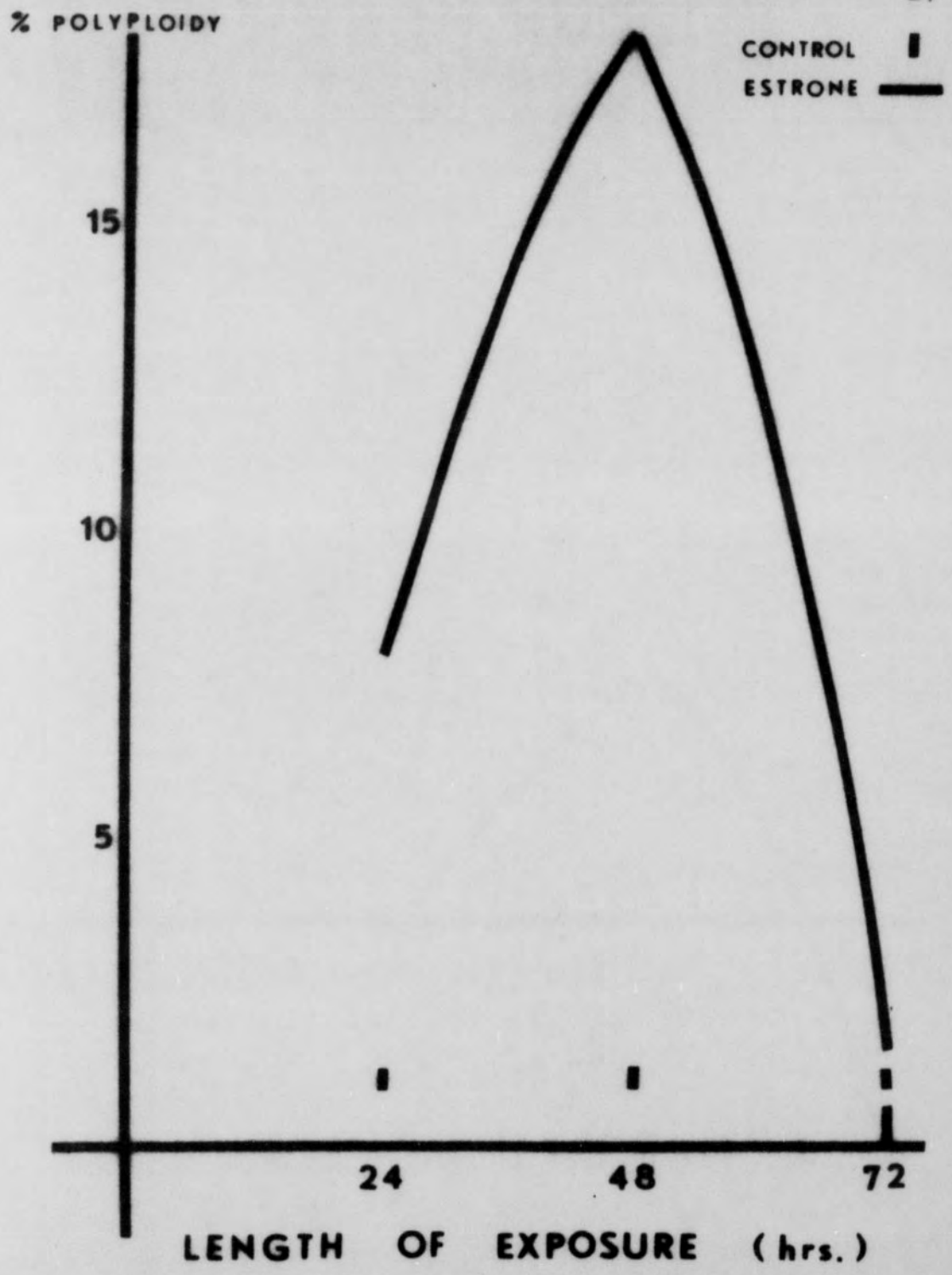


Figure 2.
% POLYPLOIDY vs TIME OF EXPOSURE TO ESTRONE 6 µg

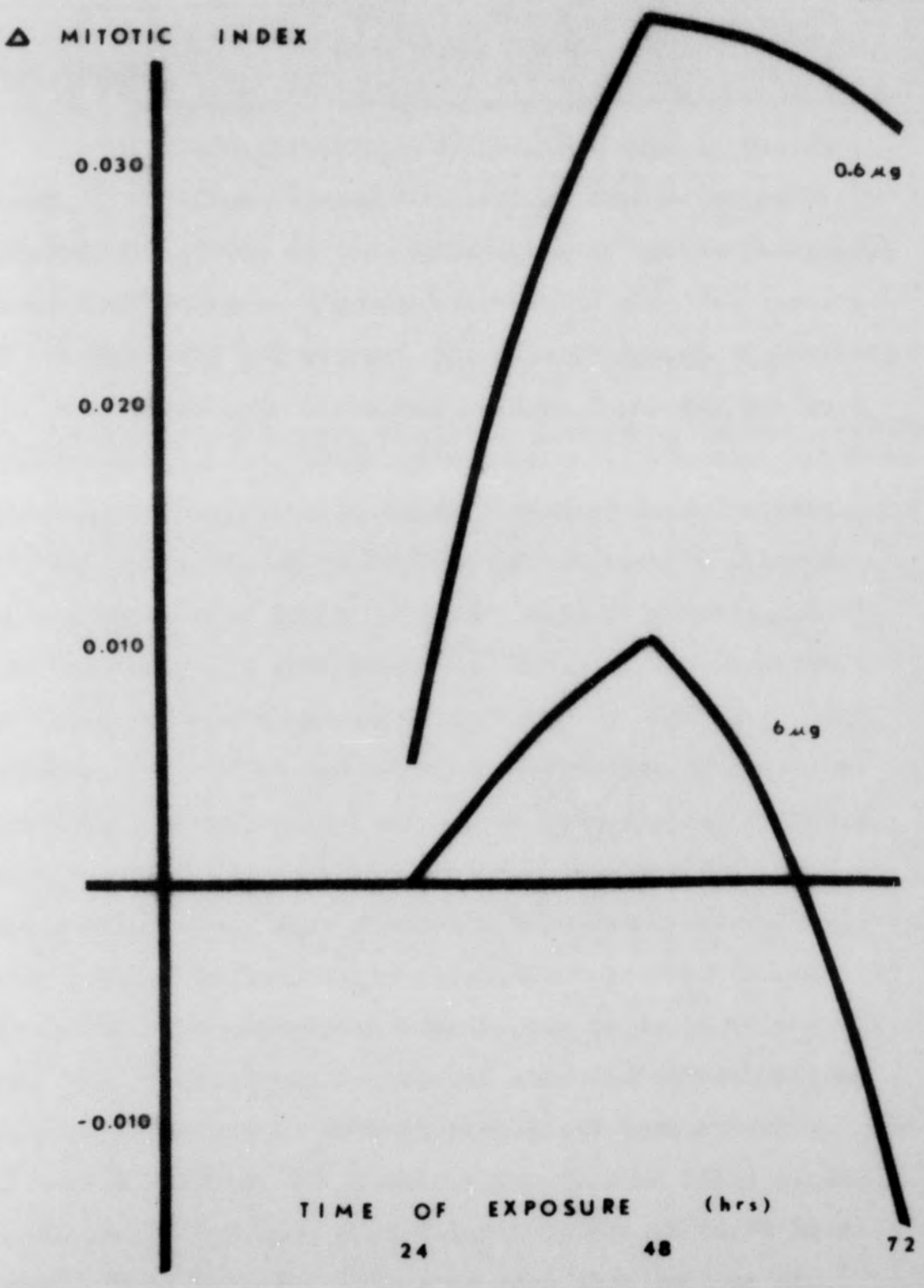


Figure 3.
Δ MITOTIC INDEX vs TIME OF ESTRONE EXPOSURE

DISCUSSION

It can be clearly seen from the data in Tables 1, 2 and 3, and from Figures 1, 2 and 3, that estrogen has a significant effect on the chromosomes of female peripheral blood lymphocytes. A great increase in nonmodal numbers of chromosomes, and a great increase in amount of coiling, can be seen in some instances (Tables 1 and 2), but such effects are, on the whole, inconsistent. The most striking effects, rather, seem to be the increase in polyploidy (Tables 1 and 2), which follows an interesting pattern with a sharp peak in the 48 hour range (Figures 1 and 2), and the change in mitotic index (Table 3), which gives a striking pattern as graphed in Figure 3. Sandberg (1966) reports that the normal level of polyploidy in a lymphocyte culture may range from 0.7 to 2.0 polyploids (based on a count of 250 cells). The level of polyploidy seen in the present study thus reaches a significant percentage when related to Sandberg's results and to the control groups of this experiment itself, and it is important to note that this effect is seen at estrogen concentrations only slightly higher than physiological concentration. The effect of estrogen on mitotic index is also dramatic at these concentrations, particularly in the 48 to 72 hour range. From Figure 3 it may be seen that estrone at 0.6 μg gives a small increase in mitotic index at 24 hours and greater increases at 48 and 72 hours. The effect at

6.0μg is no change at 24 hours, a small increase at 48 hours and a large decrease at 72 hours. It would seem likely, then, that the net effect of estrone at 0.6μg is an increase in mitotic rate in the 48 to 72 hour range and an increase in polyploidy in the 24 to 48 hour range, with the peak of activity at 48 hours. At 6.0μg the net effect is a decrease in mitotic rate (with a small increase at 48 hours) and an increase in polyploidy in the 24 to 48 hour range, the peak of activity coming, again at 48 hours. The occurence of marked endoreduplication at 48 hours with 6.0μg of estrone suggests a particularly strong, perhaps toxic, effect at this level.

Numerous implications may be drawn from such effects as estrogen has been shown to elicit here. The involvement of hormones in embryonic differentiation, for example, makes an understanding of hormone action over varying periods of time necessary. Watterson (1959), at the Symposium on Endocrines in Development, emphasized the importance of repeated hormone action over a period of time, due to the changing capacity of embryonic material to respond: "the agent [hormone] involved more likely operates at different times, repeatedly, producing different results, depending on the time at which it works. It is absurd, therefore, to inquire whether a particular response is determined hormonally or genetically. Even if it appears superficially to be determined hormonally, the

hormone source is determined by the genome plus the embryonic history that is behind it when the product is first encountered." The problem of hormone action in embryonic development is thus a complex relation between embryonic history and the nature of a hormone action over given periods of time.

The effects which estrogen has been shown to elicit in cultured lymphocytes must also reflect on the concept of the "target tissue." The marked response shown by chromosomes of a "non-target tissue" in this study suggests that further cytogenetic studies should be conducted on other non-target areas. A determination of the chromosomal response pattern in these tissues would be invaluable both for the elucidation of the general mechanism of action of the hormone and for medical applications. Steroid treatment is a common medical technique at the present time. The synthetic progesterone-estrogen contraceptive is being used secondarily for many problems ranging from stimulation of conception to regulation of the menstrual cycle. (Maisel, 1965), In such clinical use of the steroids, effects on the target organs are considered carefully. What, however, could be the long-range effects on non-target tissues, which are not considered? What effect might estrogen treatment have, for example, on the blood protein level or the lymphocytes? What effect on the cells of the heart? A study of specific, visible chromosomal

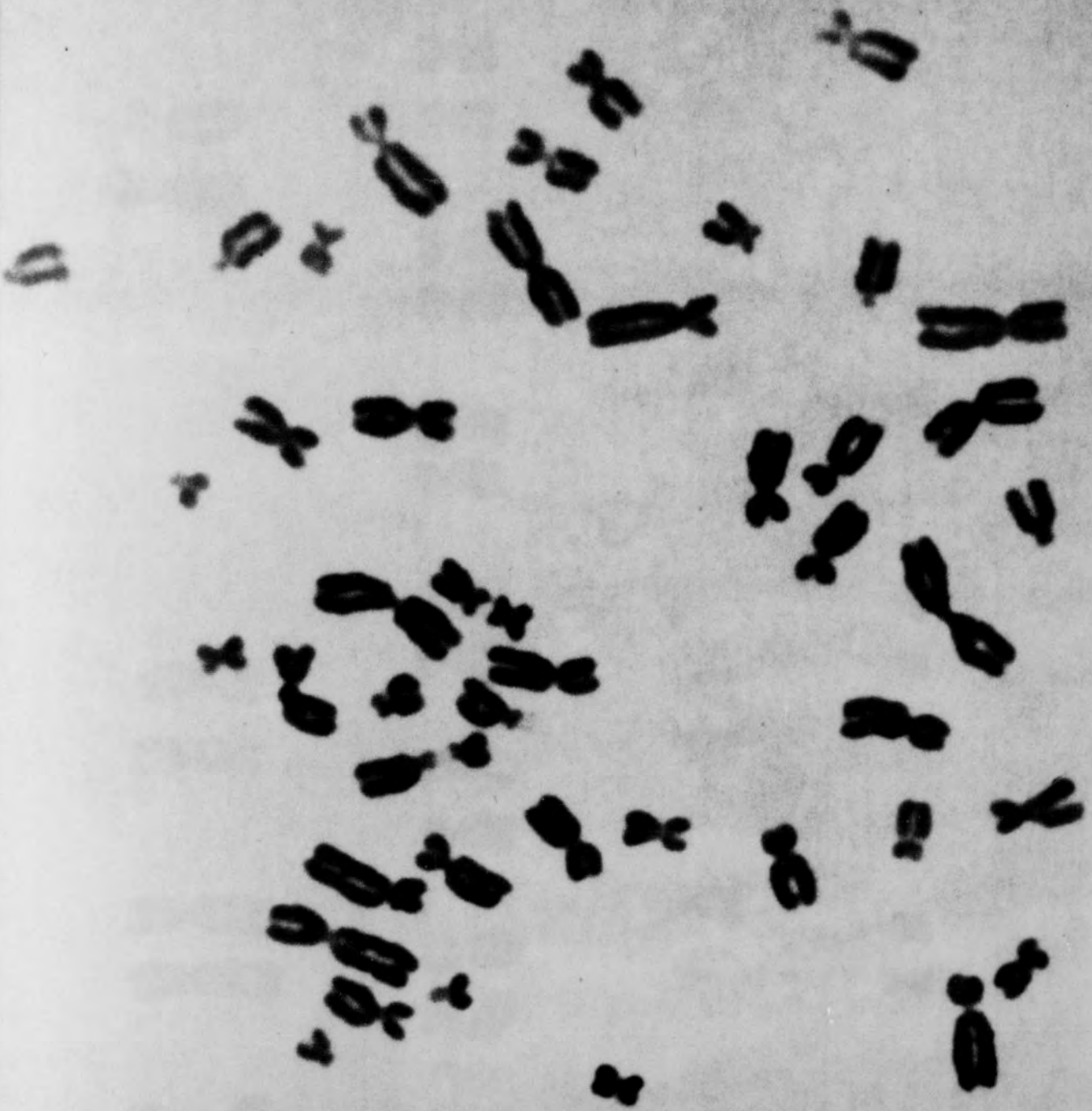
response to the hormone estrogen in various tissues might make it possible to detect tissue response as a result of clinical hormone treatment.

A final example of application of the present study may be seen in the frequent documentation of estrogen effects on cancer cells or in the stimulation of cancerous changes. In 1933 Cook wrote "cell proliferation which characterizes the oestrous state is in some respects reminiscent of the early stages of malignant growth" (as quoted by Sobotka, 1938). Later Sobotka (1938) stated the possibility that biodehydrogenation of sterols or bile acids might be the physiological method for the production of the sex hormones, and that further dehydrogenation by a faulty mechanism might give rise to tumor stimulating substances. Since then many researchers have documented relationships between estrogen and carcinogenic effects. Kim (1966) reported stimulation of mammary tumors as a result of estrogen treatment in the rat. Cutts had reported similar effects in 1964, while Lagova (1964) gave evidence for inhibition of mammary cancer in the rat by thioTEPA and estrogen. Zumoff (1966) reported a relationship between biotransformation of estrogens and breast cancer in the human. Relationships have been shown between estrogen and liver cancer in the rat (Science, 1966), uterine cancer in mice (Min-Hsin, 1964), and some forms of human cancer (Rauramo, 1963; Daly, 1963;

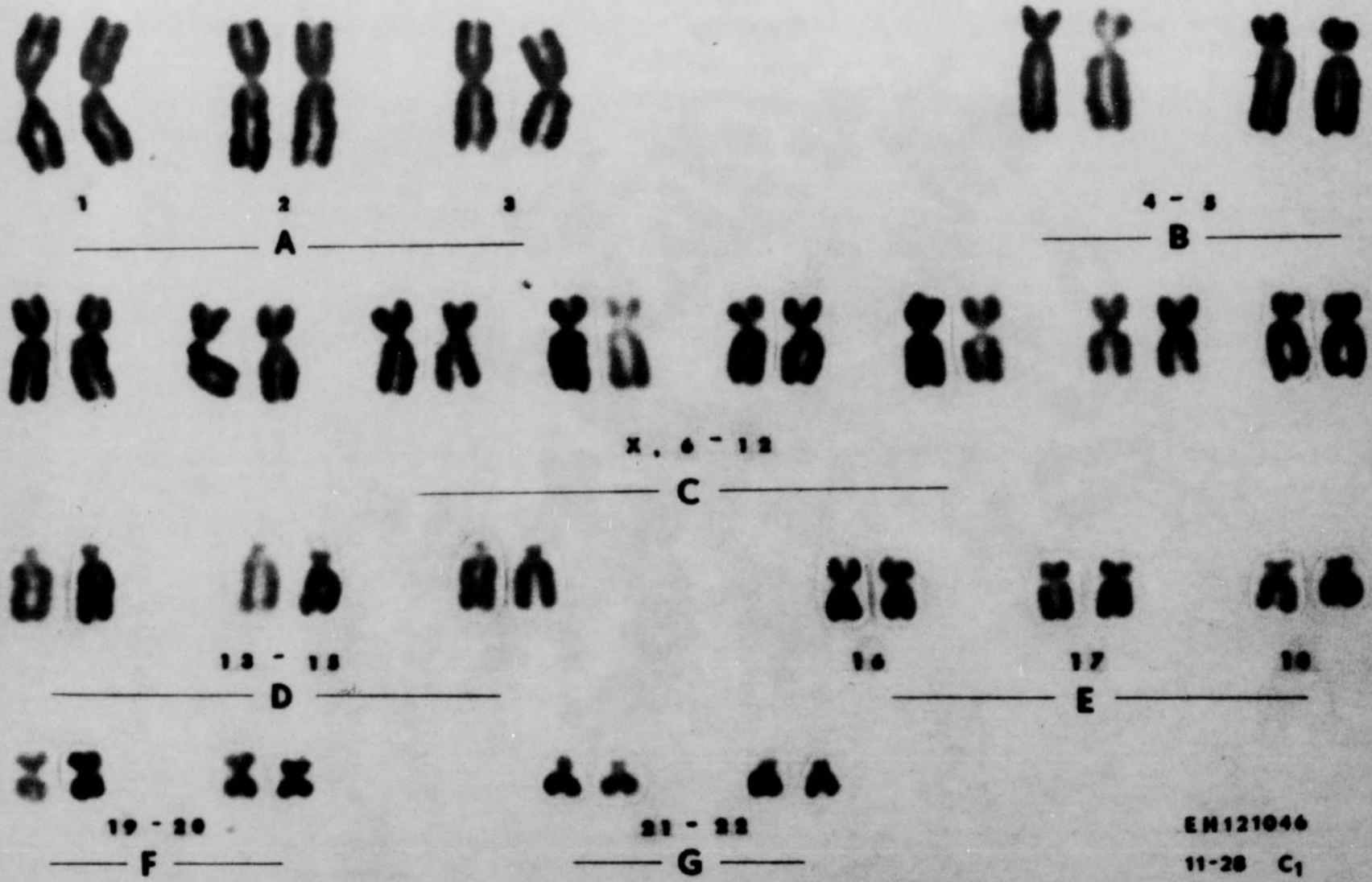
Timonen, 1963). Lancet (1966) reports the study of the relation between the endocrine system and some human cancers as one of the two major areas of cancer research today. Here, then, might be one of the major applications of a relation between estrogen and cytogenetic studies. The establishment of estrogen effects in the present study could be related to cellular changes in cancerous tissue. A record of the effects of estrogen on the chromosomes of non-cancerous or pre-cancerous cells might be developed into a valuable research or diagnostic technique to be used in the detection of early cancers and the study of the actual mechanism of malignant transformation.

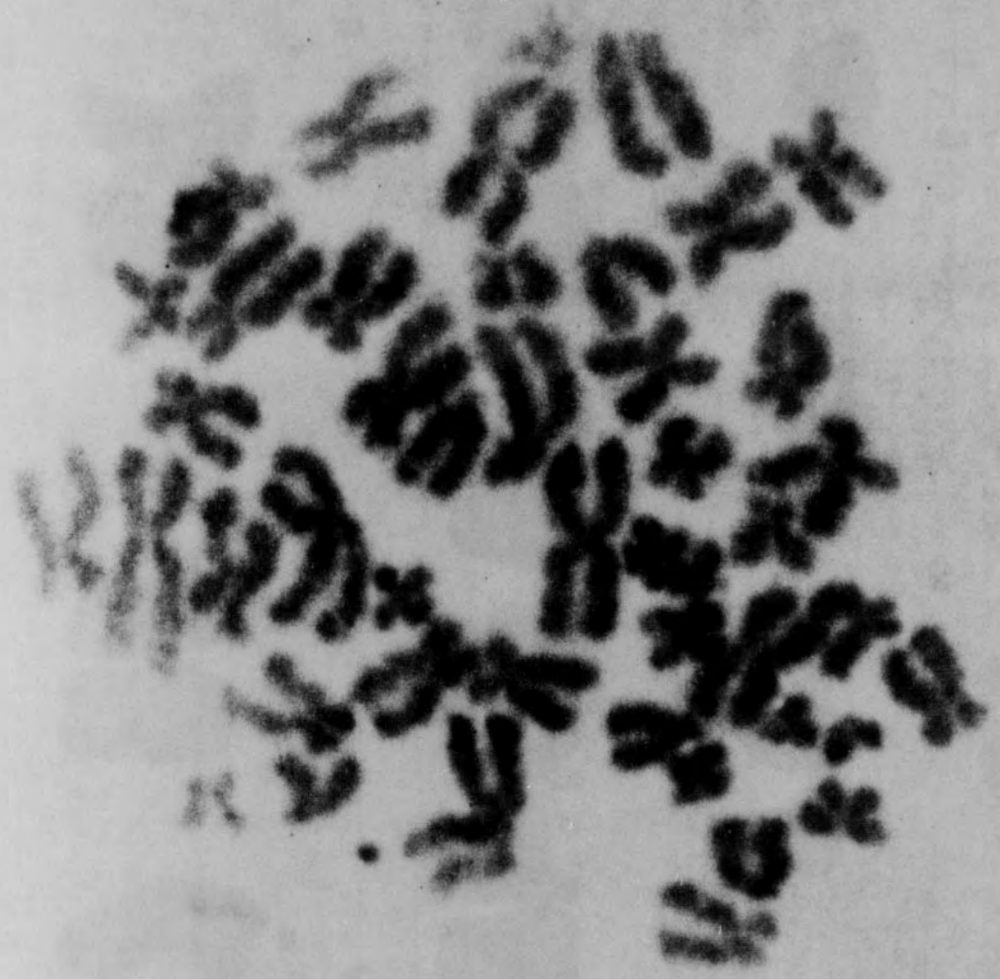
Thus, it can be seen that the practical and theoretical implications of the present study are varied and far-reaching. This is, in a sense, a pilot study, since it suggests many possibilities for future investigation in the area of hormonal effects described by cytogenetic evidence. The range for such future studies is, indeed, unlimited, since application could be made to an infinite range of tissues, using a wide variety of hormones. The scope of such studies could thus be made to encompass many fields of investigation, and the value to scientific knowledge, both theoretical and applied, could be immeasurable.

ILLUSTRATIONS

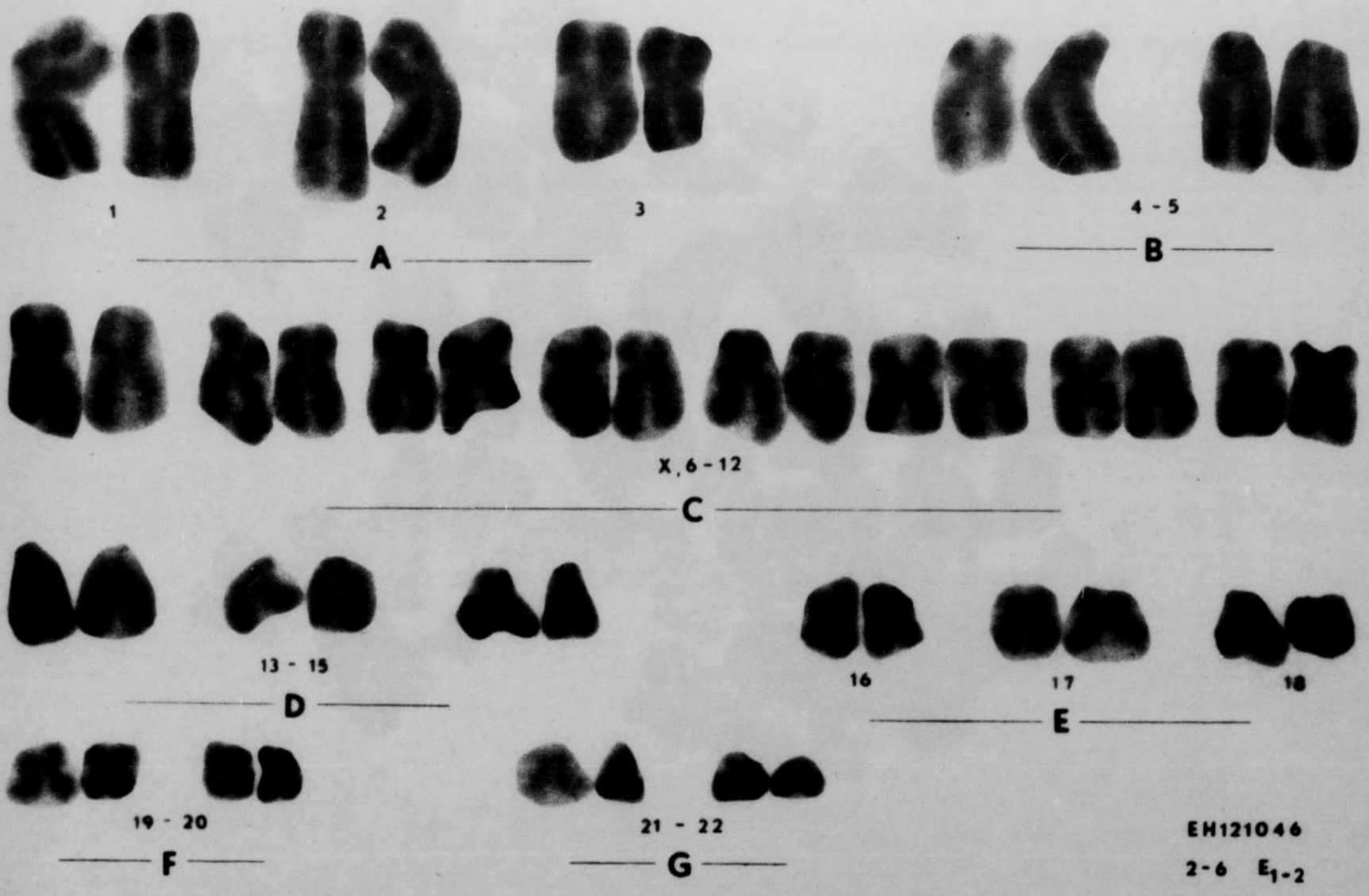


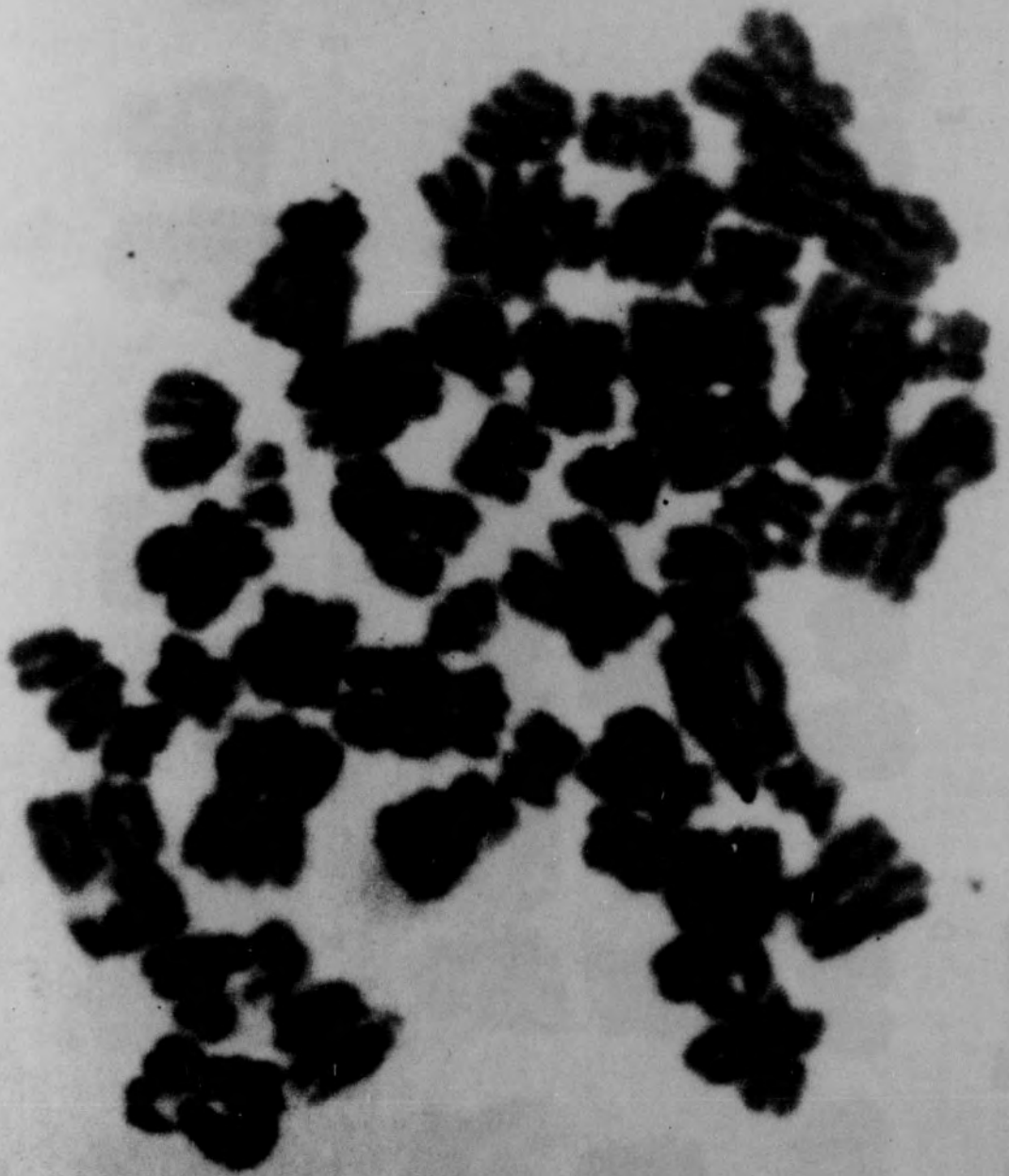
EH121046
11-28 C1
Control


1
2
3
A
4 - 5
B
X , 6 - 12
C
13 - 15
D
16
17
18
E
19 - 20
F
21 - 22
G
EH121046
11-28 C1



EH 12 1046
2-6 E_{1-2}
6.0 ug estrone
24 hours


1
2
3
A
4 - 5
B
X, 6 - 12
C
13 - 15
D
16
17
18
E
19 - 20
F
21 - 22
G
EH121046
2-6 E1-2

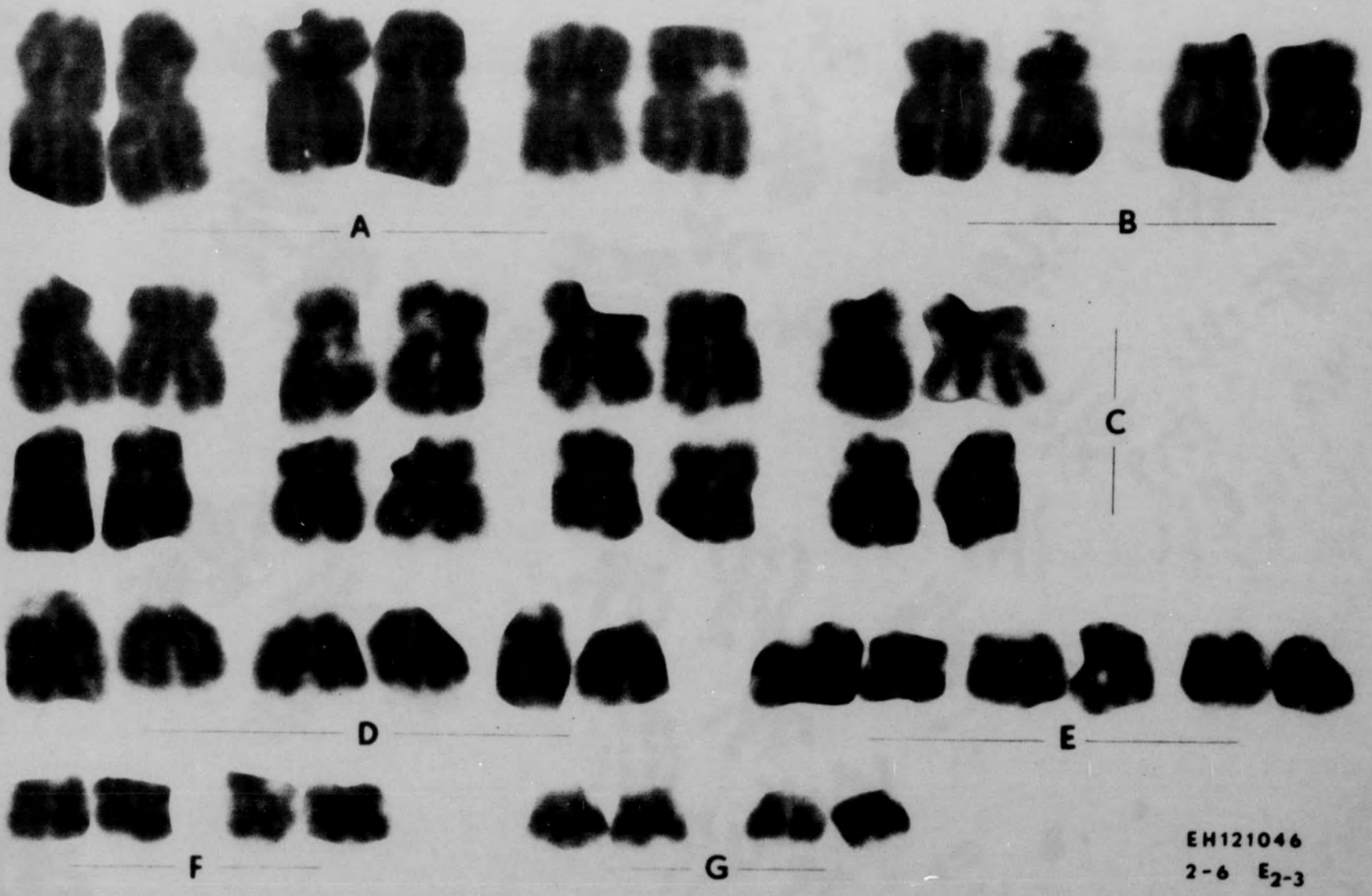


EH 121046

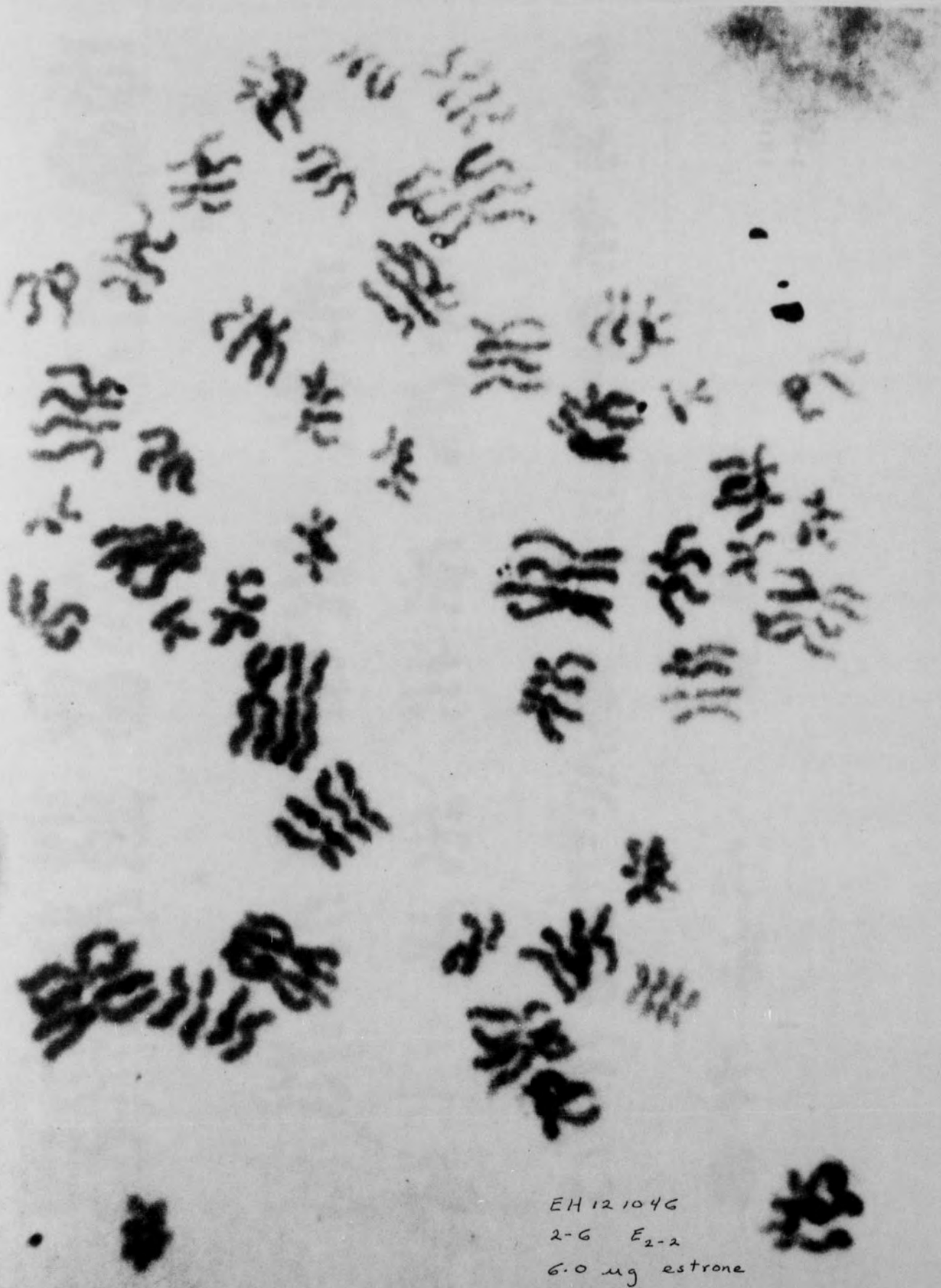
2-6 E_{2-3}

6.0 ug estrone

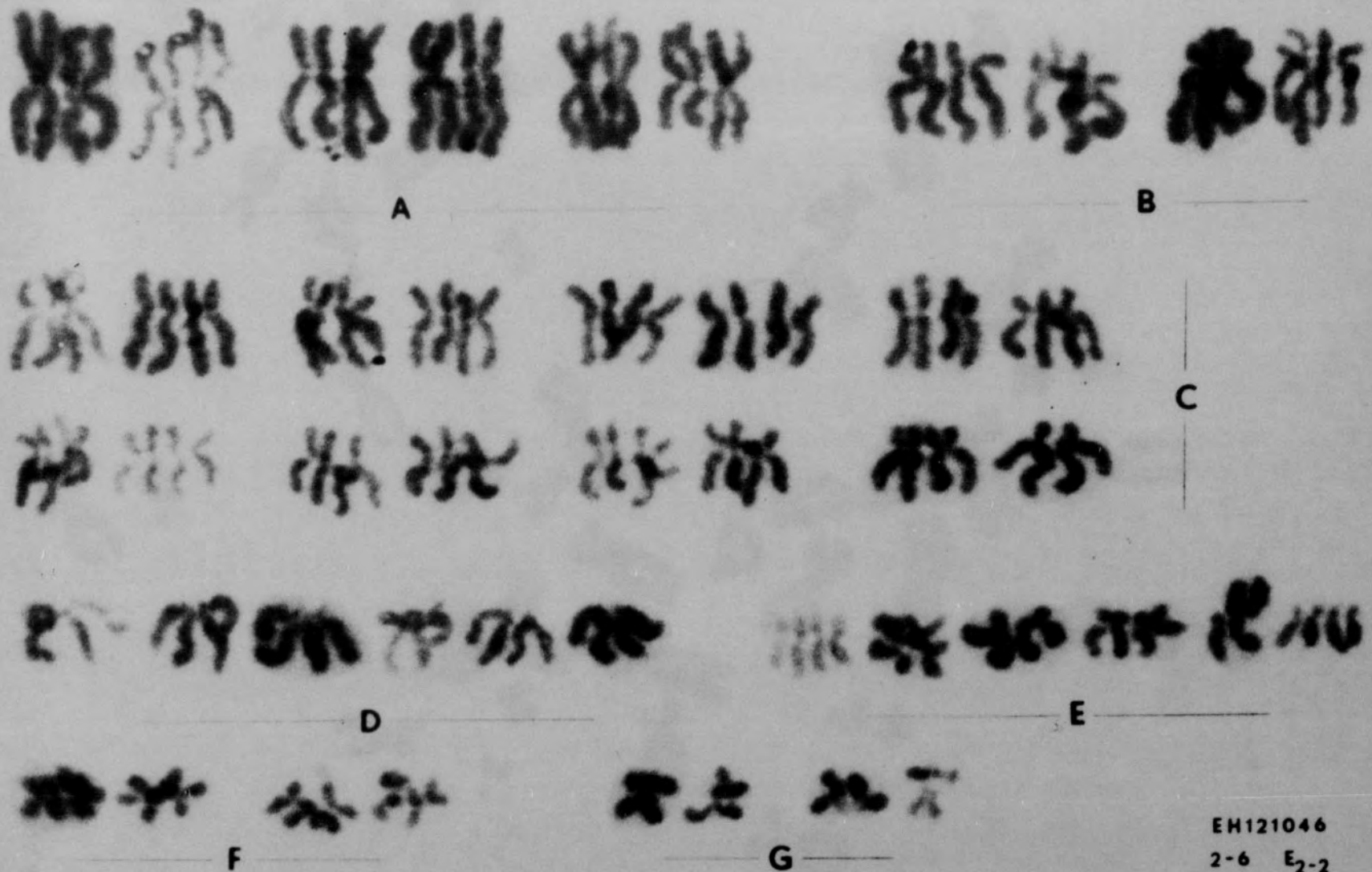
48 hours



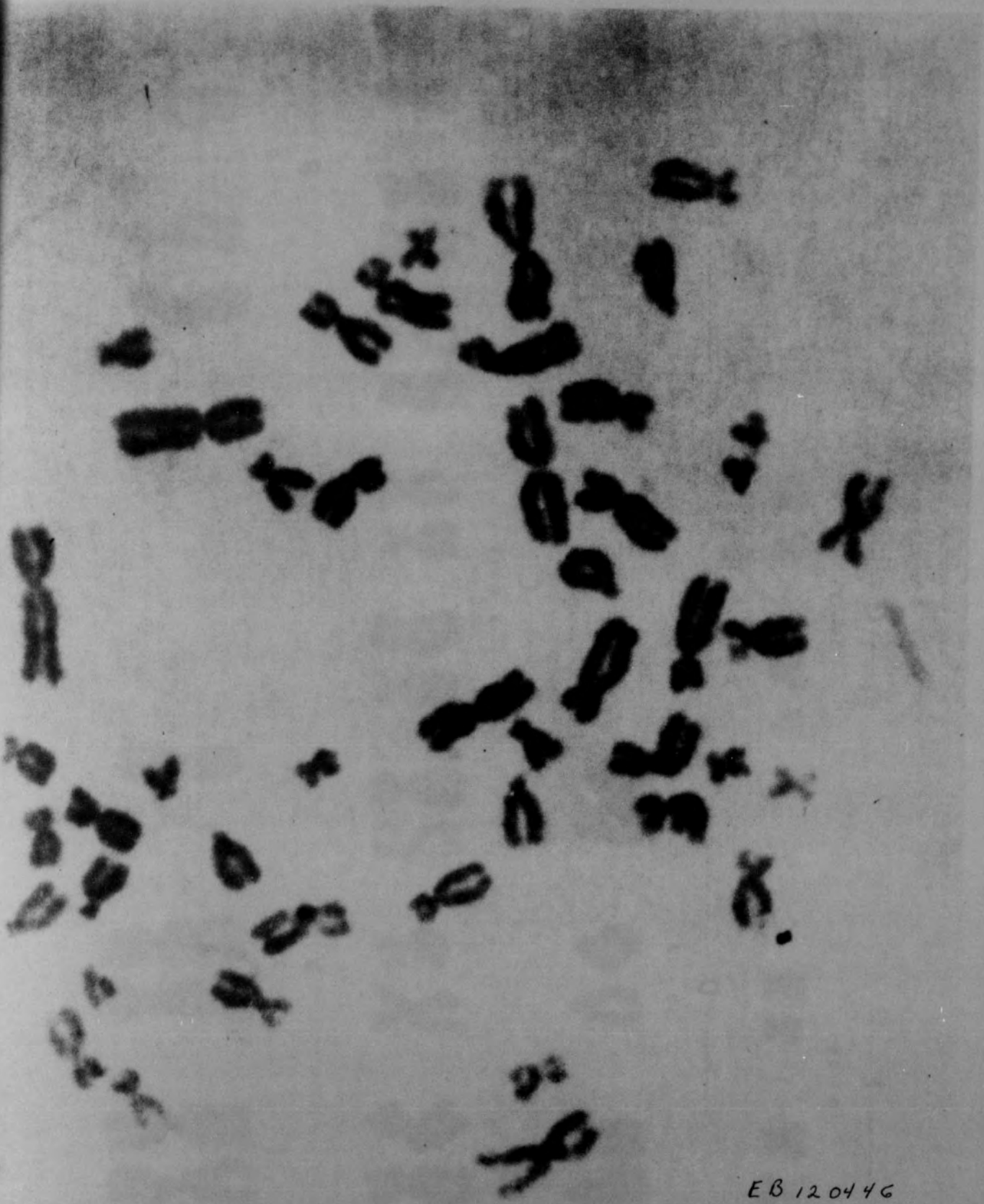
EH121046
2-6 E_{2-3}



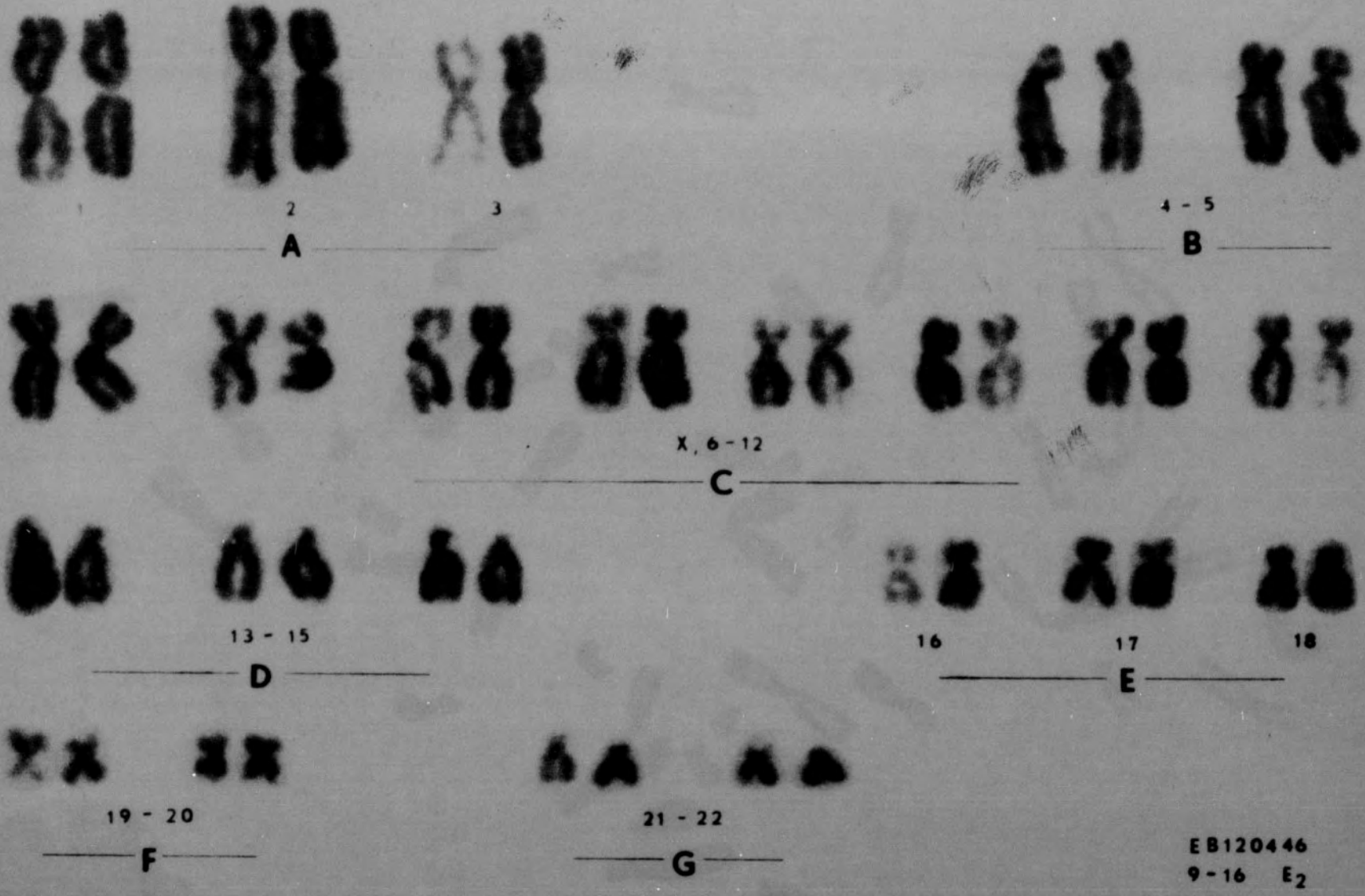
EH 12 104G
2-6 E_{2-2}
6.0 ug estrone
48 hours

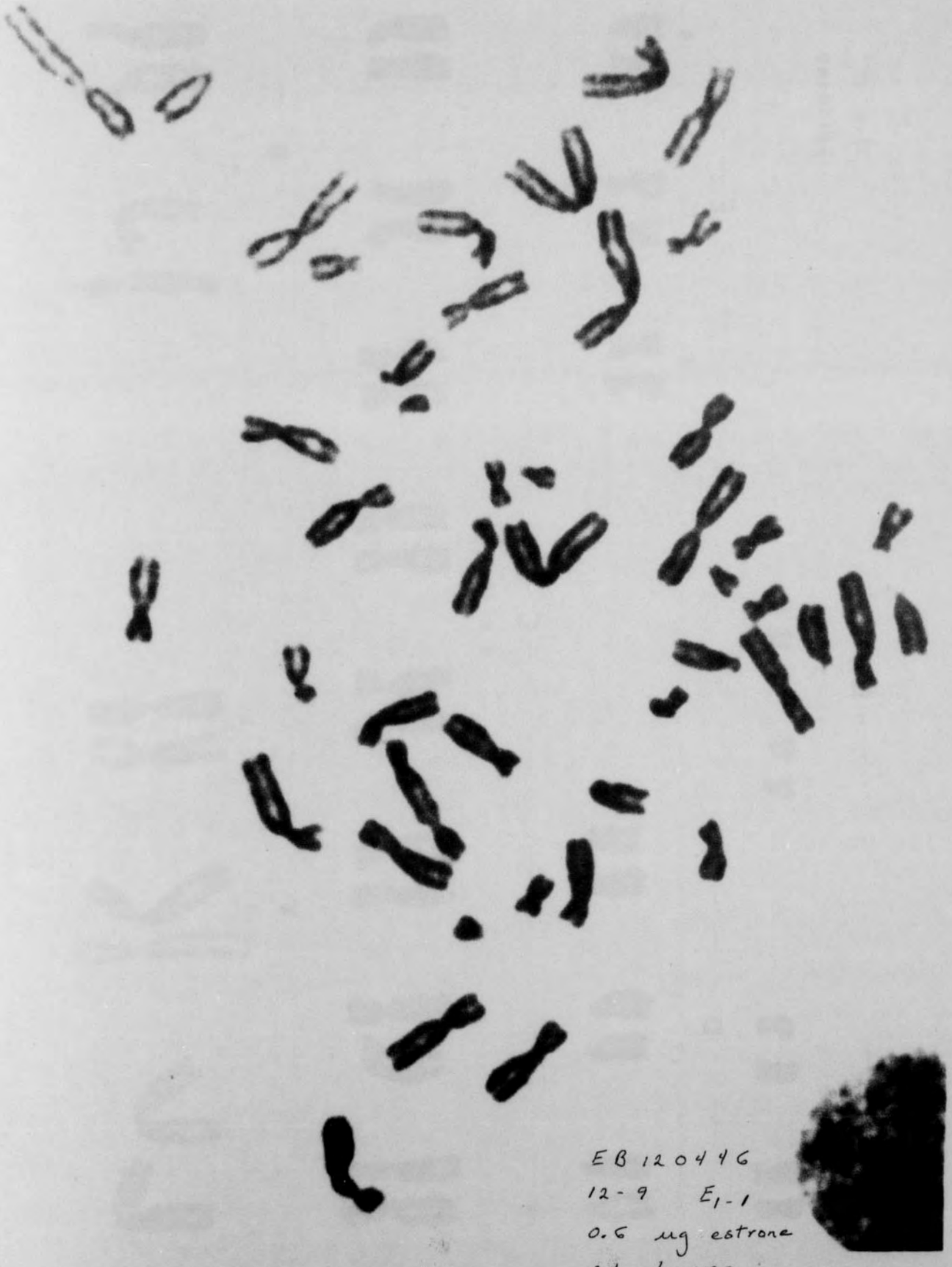


EH121046
2-6 E_{2-2}

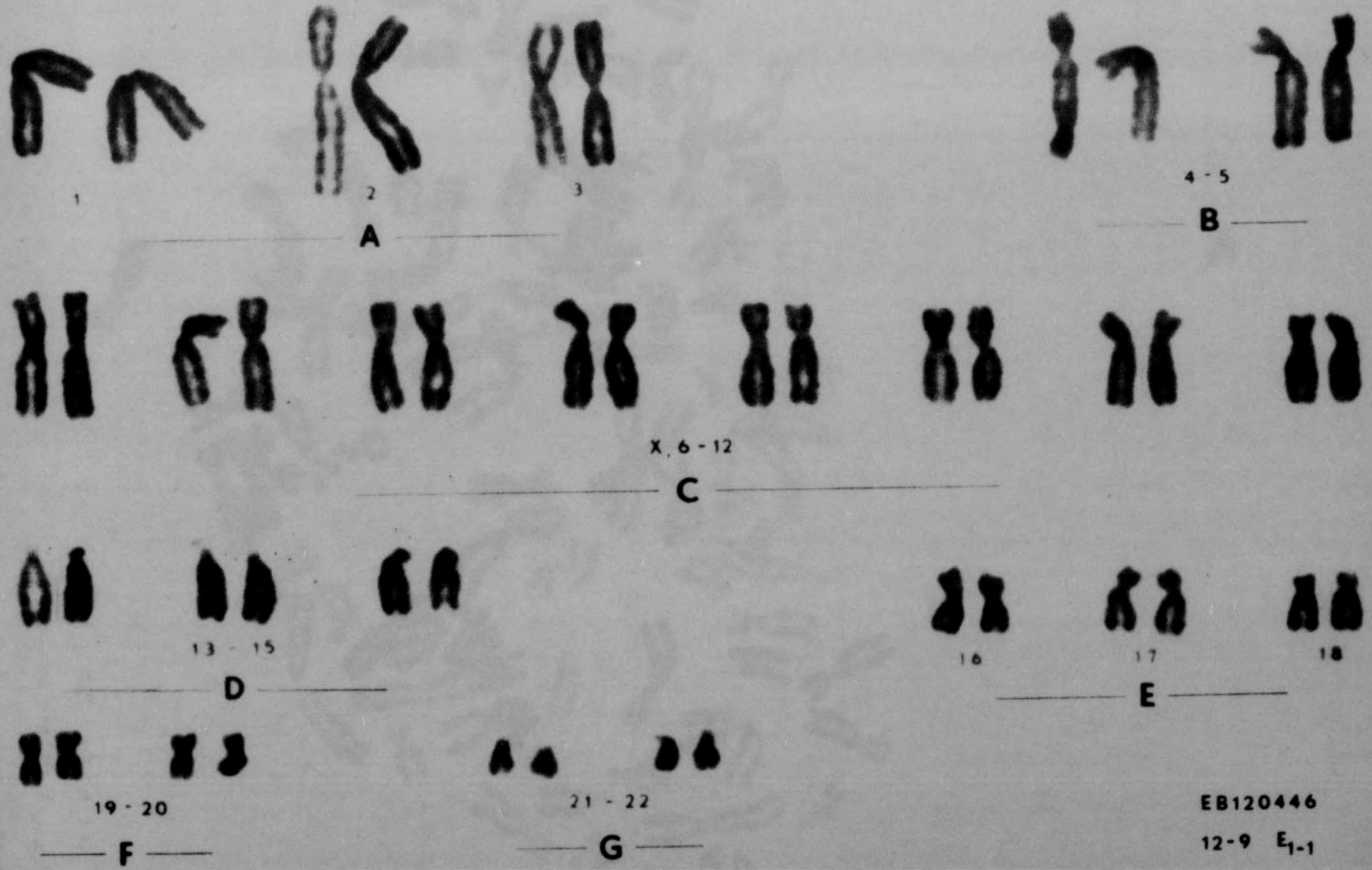


EB 12044G
9-16 E_2
6.0 ug estrone
72 hours


1
2
3
A
4 - 5
B
X, 6 - 12
C
13 - 15
D
16
17
18
E
19 - 20
F
21 - 22
G
EB120446
9-16 E2



EB 120446
12-9 E_1-1
0.6 ug estrone
24 hours


1
2
3
A
4 - 5
B
X, 6 - 12
C
13 - 15
D
16
17
18
E
19 - 20
F
21 - 22
G
EB120446
12-9 E1-1

EB 12044G
12-9 E_{2-2}
0.6 mg estrone
48 hours

LITERATURE CITED

Allen, J. M. 1962. The molecular control of cellular activity. McGraw-Hill Book Co. New York.

Arey, Leslie Brainerd. 1965. Developmental anatomy. W. B. Saunders Co. Philedelphia and London.

Bennett, N. B., P. N. Bennett, H.W. Fullerton, C. M. Ogston and D. Ogston. 1966. Effect of estriol on platelet adhesiveness and fibrinolysis in men. Lancet. 7469. 881-882.

Boccabella, Anthony V., and Elizabeth A. Alger. 1964. Influence of estradiol on thyroid: serum radioiodide concentration ratios of gonadectomized and hypophysectomized rats. Endocrinology. 74(5): 680-688.

Bonner, J. 1965. The molecular biology of development. Oxford University Press. New York and Oxford.

Chicago Conference: standardization in human cytogenetics. 1966. In: Daniel Bergsma. Birth defects: original article series. 2(2).

Cutts, J. Harry and R. L. Noble. 1964. Estrone-induced mammary tumors in the rat. I. Induction and behavior of tumors. Cancer Res. 24(7): 1116-1123.

Daly, D. W., J. A. Dossett and J. W. Jull. 1963. An estrogen-secreting sex-chromatin positive teratoma of the testis, associated with gynecomastia. Brit. Jour. Surg. 50(226): 816-819.

Desoille, H., M. Philbert and C. Albahary. 1961. Hormonal incidences on chronic benzene poisoning in the rabbit. I. Influence of natural estrogens on the red and white blood cells. Arch. Mal. Profess. Med. Travail et Sec. Soc. 22(12): 681-693.

Engel, Lewis L. 1959. Mechanisms of action of estrogens. In: Robert S. Harris, G. F. Marrian and Kenneth V. Thimann [ed.] Vitamins and hormones(17). Academic Press. New York and London.

Fisher, D.A., and T.H. Oddie. 1963. Thyroxine rate during infancy: Effect of estrogen. Jour. Clin. Endocrinol. and Metab. 23(8): 811-819.

Gorski, Jack and Nira J. Nelson. 1964. Ribonucleic acid synthesis in the rat uterus and its early response to estrogen. Arch. Biochem. Biophys. 110. 284-290.

Harris, Robert S., Ira G. Wool and John A. Loraine. 1965. Vitamins and hormones(23). Academic Press. New York and London.

Hechter, O. and I. D. K. Halkerston. 1965. Effects of steroid hormones on gene regulation and cell metabolism. Ann. Rev. Physiol. 27.

Hermreck, Arlo S. and Gilbert S. Greenwald. 1966. Response of the vagina of the spayed guinea pig to low or high doses of estrogen. Amer. J. Anat. 118(3): 861-872.

Jarzyna, Augustyn and Marian Wroblewski. 1966. The effect of oestrogens on blood clotting. Pol. Tyg. Lek. 21(36): 1363-1366.

Kim, Untae, Jacob Furth and Kaity Yannopoulos. 1963. Observations on hormonal control of mammary cancer. I. Estrogen and mammotropes. Jour. Natl. Cancer Inst. 31(2): 233-259.

Kuchler, Robert J. and Robert C. Grauer. Effects of natural estrogens on L strain fibroblasts in tissue cells.

Kusuda, Masahiko and Michio Matsumoto. 1963. Effects of oestriol on the female genital tract and its clinical applications. Kyushu Jour. Med. Sci. 14(1): 1-5.

Lagova, N. D. 1964. Mechanism of the anticancerous effect of thioTEPA combined with estrogens in mammary gland cancer in rats. Byul. Eksp. Biol. Med. 57(5): 70-73.

Lancet. 1966. Annotations: cancer research. April 16: 866-867.

Lasnitzki, Ilse. 1965. The action of hormones on cell and organ cultures. In: E. N. Willmer. Cells and tissues in culture (1). Academic Press. New York and London.

Levier, R. R. and E. Spaziani. 1965. The effects of estradiol on the occurrence of mast cells in the rat uterus. International Society for Cell Biology. Exper. Cell Res. 41(2).

Maisel, Albert Q. 1965. The hormone quest. Random House. New York.

Mansour, A. Maher and M. C. Nui. 1965. Functional studies with uterine RNA. Proc. Nat. Acad. Sci. 53: 764-770.

Mellman, W. J. 1965. Human peripheral blood leucocyte cultures. In: Jorge J. Yunis [ed.] Human chromosome methodology. Academic Press. New York and London.

Min-Hsin, Li, and Ts'ai Hai-Ying. 1964 recd. 1965 . Influence of hormones on carcinogenesis of uterine cervix of mice induced by methylcholanthrene. Acta. Unio. Int. Contra. Cancer. 20(6/7). 1514-1518.

Musa, Byron U. 1965. Elevation of certain plasma proteins in man following estrogen administration: a dose-response relationship. J. Clin. Endocrinol. Metab. 25(9): 1163-1166.

Nillson, Ove. 1966. Estrogen-induced increase of adhesiveness in uterine epithelium of mouse and rat. Exp. Cell Res. 43(1): 239-241.

Rauramo, Lauri and Matti Gronroos. 1963. Estrogen metabolism in postmenopausal women with genital cancer. Ann. Chir. et Gynaecol. Fenn. 52(3): 351-355.

Sandberg, Avery A. 1966. Cytogenetic studies of normal and neoplastic cells in vitro. 21st Symposium on Fundamental Cancer Research.

Schitkova, T. A. 1964. Histochemical changes in the anterior lobe of the pituitary following transplantation of mammary carcinoma and estrogen therapy. Probl. Endokrinol. Gormonoterap. 10(1): 66-69.

Science. 1966. Liver cancer: neonatal estrogen enhances induction by a carcinogen. 154(3749).

Segal, S. J. 1966. Role of RNA in the regulatory action of estrogen. (Lecture at the University of North Carolina at Chapel Hill).

Slebodzinski, Andrzej and Kazimierz Wojcik. 1962. Protein level and the trend of protein fractions in the serum of sheep treated with oestrone. Acta. Physiol. Polon. 13(4): 501-509.

Sobotka, Harry. 1938. The chemistry of the sterids. The Williams and Wilkins Co. Baltimore.

Svendsen, Reiner and Borge Sorensen. 1964. The plasma concentration of unconjugated oestrone and 17- - oestradiol during the normal menstrual cycle. 47(2): 245-254.

Timonen, Sakari and Leo Tervila. 1963. Uterine carcinoma and oestrogen reaction in vaginal smear. Ann. Chir. et Gynaecol. Fenn. 52(2): 143-147.

Tips' Micro Procedure for Peripheral Leucocyte Culture. (Communication from the Wistar Institute, Chicago).

Tuttle, W. W. and Byron A. Schottelius. 1961. Reproduction. Textbook of physiology. Fourteenth edition. C. V. Mosby Co. St. Louis.

Villee, Claude A. 1962. The role of steroid hormones in the control of metabolic activity. In: J. M. Allen. The molecular control of cellular activity. McGraw-Hill Book Co. New York.

Watterson, Ray L. 1959. Symposium on endocrines in development, Shelter Island, New York (1956). Bull. Univ. of Chicago Press. Chicago.

Yamada, Takashi, Yoshihiro Takemura, Isao Kobayashi and Kojiro Smichijo. 1966. Re-evaluation of the effect of estrogen on thyroid activity in the rat and its mechanism. Endocrinology. 79(5): 849-857.

Zumoff, Barnett, Jack Fishman, Joseph Cassouto, Leon Hellman and T. F. Gallagher. 1966. Estradiol transformation in men with breast cancer. J. Clin. Endocrinol. Metab. 26(9): 960-966.