12(4) which simulates radiation damage. A preliminary communication(11) has also recently focused attention on the possibility of chromosome breaks in leukocytes of patients with chickenpox, a disease caused by a virus identical with or closely related to HZV. Although chromosome cytology following viral infection is only at its beginning stage of development, it already appears evident that individual viruses may produce specific effects on the nuclei and mitotic cycle of a variety of host cells. Caution is required, however, in interpretation of chromosomal aberrations that are not unique for a specific virus, as a variety of alterations have been reported in actively growing cultures of "normal" cells(12). In the present study, however, parallel control cultures, examined simultaneously, showed none of the marked abnormalities seen in the infected cultures.

Summary. Human embryo lung fibroblasts infected with herpes zoster virus reacted as though they had been treated with colchicine. Most of the infected cells were arrested in metaphase and contained overcontracted chromosomes. Formation of multinucleated cells and micronuclei was also observed. Many chromatid and chromosome breaks were seen during the early stages of infection.

1. Hampar, B., Ellison, S. A., *Nature,* 1961, v192, 145.
2. Nichols, W. W., Levan, A., Hall, B., Östergren, G., *Hereditas,* 1962, v48, 367.
3. Stich, H. F., Hsu, T. C., Rapp, F., *Virology,* 1964, v22, 439.
4. Stich, H. F., Van Hoosier, C. L., Trentin, J. J., *Exp. Cell Res.,* 1964, v34, 400.
5. Hampar, B., Ellison, S. A., *Proc. Nat. Acad. Sci.,* 1961 v49, 474.
6. Koprowski, H., Pontén, J. A., Jensen, F., Ravdin, R. G., Moorhead, P., Saksela, E., *J. Cell. and Comp. Physiol.,* 1962, v59, 281.
7. Shein, H., Enders, J., *Proc. Nat. Acad. Sci.,* 1962, v48, 1164.
8. Moorhead, P. S., Saksela, E., *J. Cell. and Comp. Physiol.,* 1963, v62, 57.
9. Rapp, F., Benyesh-Melnick, M., *Science,* 1963, v141, 433.
10. Rapp, F., Vanderslice, D., *Virology,* 1964, v22, 321.
11. Aula, P., *Hereditas,* 1963, v49, 451.
12. Kleinfeld, R., Melnick, J. L., *J. Exp. Med.,* 1958, v107, 599.

Received June 19, 1964. P.S.E.B.M., 1964, v117.

Pituitary Gonadotropic Inhibitory Activity of Various Steroids in Ovariectomized-Intact Female Rats in Parabiosis. (29634)

FRED A. KINCL, A. J. BIRCH AND RALPH I. DORFMAN
Syntex Research Institute, Palo Alto, Calif., University of Manchester, Manchester, England and Worcester Foundation for Experimental Biology, Shrewsbury, Mass.

The anti-gonadotropic activity of a considerable number of steroids has been evaluated in castrated male-intact female parabiotic pairs(1,2), but few steroids have been studied in the intact female-ovariectomized female parabionts. Meyer and Hertz(3) determined the dose of estrone required to inhibit completely the post-castration hypersecretion of gonadotropins. Biddulph *et al*(4) have studied the relative potency of estradiol-17β, estriol and progesterone in this assay and Byrnes and Meyer(5) reported on the relative pituitary inhibition and uterine stimulation activity of estrone and estradiol-17β. Dorfman and Dorfman(6) evaluated the reproducibility of the assay procedure employing estrone as the reference standard.

The pituitary gonadotropic inhibitory activity of certain synthetic steroids, mainly ring A phenolic compounds, or derivatives thereof, have now been studied with the objective of finding steroids with clear dissociations of pituitary inhibitory activity and estrogenic potencies.

Method and materials. The method has been detailed elsewhere(7) and will only be

TABLE I. Influence of Subcutaneously Injected Estradiol-17β in Parabiotic Rats.

Total dose of estradiol-17β, μg	No. of pairs	Intact female mean ovarian wt, mg ± S.E.	Ovariectomized rat mean uterine wt, mg ± S.E.
0	44	183 ± 8	56 ± 4
.01	47	162 ± 9	60 ± 1
.03	48	142 ± 10	68 ± 4
.09	30	104 ± 10	65 ± 3
.1	15	98 ± 17	79 ± 7
.2	6	67 ± 36	85 ± 9
.3	29	67 ± 8	118 ± 2
.4	6	50 ± 13	123 ± 7
.6	5	39 ± 6	133 ± 10
.9	9	41 ± 5	179 ± 13

outlined here. Intact female-ovariectomized female parabiotic pairs were used. The test material, dissolved in 0.1 ml of sesame oil, was injected subcutaneously once daily for 10 days into the ovariectomized parabiont. Autopsy was performed one day following the last treatment at which time the ovaries and the uterus of the ovariectomized animal were removed and weighed. Most of these assays were conducted at the Endocrine Laboratories, Madison, Wis., under the supervision of Dr. Elva G. Shipley.

Results and conclusions. The influence of graded doses of subcutaneously injected estradiol-17β on the ovarian weight of the intact parabiont and the uterus of the ovariectomized partner is detailed in Table I. These data were used to evaluate the relative potencies of the synthetic steroids by graphic estimation using the steepest portions of the dose-response curves. Activities separation ratio was calculated as the relative gonadotropin inhibition potency divided by the relative uterotrophic potency. A dose of 0.03 μg of estradiol-17β produced a statistically significant pituitary effect but a dose of 0.2 μg was needed for maximum inhibition of gonadotropin secretion by the ovarian weight endpoint. This dose also produced a statistically significant increase in uterine weight in the ovariectomized parabiont. Meyer and Hertz(3) reported a value of 2.0 μg of estrone to achieve a maximum pituitary gonadotropin suppression effect and quite recently Dorfman and Dorfman(6) were able to confirm this finding by the observation that 2.5 μg of estrone produced the same effect. On the basis of these earlier reports, estradiol-17β would be rated some 10 to 12 times more potent than estrone. The data presented here confirm this ratio.

Table II lists the estimated gonadotropin inhibition and estrogenic potency of 20 steroids studied by subcutaneous injection. Ethynylestradiol 3-methylether was 20% as active as estradiol-17β on both parameters. 17α-Ethynylestra-1(10),5-diene-3β,17β-diol was fully active as a pituitary inhibitor at a total dose of 3 μg and relative potency was estimated as 10% that of the standard. The uterotrophic activity was estimated to be 2% of estradiol-17β thus indicating an enhancement of anti-pituitary effect of the order of 5 times. The corresponding 17-keto derivatives, 3β-hydroxyestra-1(10),5-dien-17-one was about 100 times less active than estradiol-17β and the same order of activity was assigned to the corresponding 17β-alcohol, estra-1(10),5-diene-3β,17β-diol. Of interest is the relatively high potency of 2 seven-membered ring A steroids. A-Homoandrosta-1,4,5(10)-triene-3,17-dione was fully active at 10 μg dose level and was considered to possess 4% the activity of estradiol-17β. 17β-Hydroxy-A-homoandrosta-1(10),2,4a-triene-4-one was active at a 100 μg dose level and was assigned 0.1% the relative activity of estradiol-17β.

Four estrene derivatives, lacking either a 3 or 17-oxygen function were studied. 3-Deoxyestrone (estra-1,3,5(10)-trien-17-one) assayed about 0.1% of estradiol-17β and 17-deoxyestrone (estra-1,3,5(10)-trien-3-ol) about 3% the activity of the standard. Whereas, in the case of 3-deoxyestrone no difference was seen between the inhibition of the pituitary and uterotrophic activity, a more than 25-fold separation was apparent in the case of 17-deoxy compounds.

Two 17α-substituted 3-deoxy compounds were tested. The determined values were as follows: 17α-ethynyl-3-deoxyestradiol (17α-ethynylestra-1,3,5(10)-trien-17β-ol) was active as a pituitary suppressor at the 50 μg dose and the relative potency was 0.5% or greater. The corresponding 17α-methyl deriv-

TABLE II. Relative Potency of Various Steroids Assayed by Subcutaneous Injection in Intact Female-Ovariectomized Parabiotic Rats.

Compound	Dose range studied, μg	No. of pairs used	Relative potency (Estradiol-17β = 100) Gonadotropin inhibition (A)	Uterotropic potency (B)	Activities separation ratio (A/B)
A-Homoandrosta-1,4,5(10)-triene-3,17-dione	10 –1000	12	≧4	<.03	>120
Estra-1,3,5(10)-trien-3-ol	5 – 150	24	3	>.2	<15
17β-Hydroxy-A-homoandrosta-1(10),2,4a-triene-4-one	5 – 900	33	1	.1	10
17α-Ethynylestra-1(10),5-diene-3β,17β-diol	3 – 450	39	10	2	5
3-Methoxyestra-1,3,5(10)-triene-17β-cyanoethoxy	.1 – 20	32	3	1	3
17α-Ethynyl-17β-hydroxy-5α-estran-3-one	10 –2700	30	> .1	.05	> 2
4-Oxa-17β-acetoxyestr-1,5(10)-dien-3-one	100 –1000	10	.1	.05	2
5,10-Seco-5,19-cycloandrosta-1(10),2,4-triene-3,17β-diol diacetate	10 – 100	23	.5	<.3	> 1.7
Testosterone	50 – 500	28	.02	<.02	> 1
A-Homoandrosta-1(10),2,4-triene-4,17-dione	5 – 125	12	.1	<.1	> 1
1-Methyl-3-acetoxyestra-1,3,5(10),6-tetraene-17-one	10 –1000	15	.01	<.01	> 1
Ethynyl estradiol-3 methylether	.1 – 27	78	20	20	1
3β-Hydroxyestra-1(10),5-dien-17-one	3 – 450	27	1	1	1
Estra-1(10),5-diene-3β,17β-diol	3 – 450	27	1	1	1
17α-Methylestra-1,3,5(10)-trien-17β-ol	.1 –1000	22	.5	.5	1
Estra-1,3,5(10)-trien-17-one	50 – 450	27	.1	.1	1
Estrone	.1 – 4.5	30	10	20	.5
17α-Ethynylester-1,3,5(10)-trien-17β-ol	50 – 500	10	> .2	.6	> .3
Ethynyl estradiol	.02– .9	30	100	300	.3
17α-Ethynylestr-2-en-17β-ol	100 – 900	15	.1	<.5	> .2
17α-Ethynylandrosta-2,4-dien-17β-ol	30 – 270	12	< .03	<.01	—
3-Methyl-5,10-seco-5,10-cycloestr-1(10),2,4-trien-17β-ol	100 –1000	10	< .01	<.01	—
3,6α-Diacetoxy-4-methylacetoxyestra-1,3,5(10)-trien-17-one	100 –1000	8	< .01	<.01	—
3-Acetoxy-4-methyl-acetoxyestra-1,3,5(10)-6-tetraene-17-one	100 –1000	8	< .01	<.01	—
6α-Methylestra-1,3,5(10)-trien-17-one	50 – 150	10	< .05	<.05	—

ative (17α-methylestra-1,3,5(10)-trien-17β-ol) was active at 100 μg but not at 10 μg dose and was considered to be of the same order of activity.

Five steroids were inactive at the dose levels studied as follows: 17α-ethynyl-androsta-2,4-dien-17β-ol (270 μg); 3,6α-diacetoxy-4-methylacetoxyestra-1,3,5(10)-trien-17-one (1000 μg); 3-acetoxy-4-methylacetoxyestra-1,3,5(10),6-tetraene-17-one (1000 μg); 6α-methyl-1,3,5(10)-trien-17-one (150 μg); 3-methyl-5,10-seco-5,10-cycloestra-1(10),2,4,-trien-17β-ol (1000 μg).

Fig. 1 includes the structure of 4 steroids having significantly higher gonadotropin inhibitory activity than estrogenic activity as measured by increase in uterine weight endpoint. An activities separation ratio up to > 120 was observed for compound I (A-homoandrosta-1,4,5(10)-triene-3,17-dione), Compound II (17β-hydroxy-A-homoandrosta-1(10),2,4a-trien-4-one) was 10, Compound III (17α-ethynyl-estra-1(10),5-diene-3β,17β-diol) was 5 and Compound IV (3-methoxy-1,3,5(10)-triene-17β-cyanoethoxy) was 3.

Summary. Steroids of the ring A phenolic group, or derivatives thereof, have been studied for their ability to inhibit biosyntheses and/or secretion of pituitary gonadotropins and uterotropic activity by means of a method utilizing the intact female in parabiosis with an ovariectomized rat.

Three hundredth μg of estradiol-17β was the smallest total dose to produce pituitary inhibition and 0.2 μg was the minimum dose to produce a maximum inhibition. The cor-

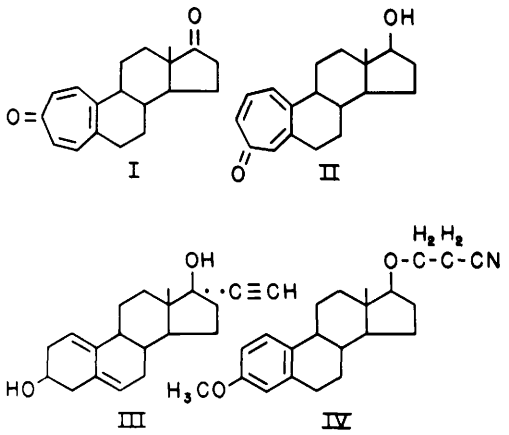

FIG. 1. Compounds with activities separation ratio (ASR) of 3 or greater: I—(A-Homoandrosta-1,4,5(10)-triene-3,17-dione) ASR = >120. II—(17β-Hydroxy-A-homoandrosta-1(10),2,4a-trien-4-one) ASR = 10. III—(17α-Ethynylestra-1(10),5-diene-3β,17β-diol) ASR = 5. IV—(3-Methoxy-1,3,5(10)-triene-17β-cyanoethoxy) ASR = 3.

responding total doses of 0.2 and 0.9 μg of estradiol-17β were required to produce minimum uterine weight stimulation and the minimum dose to produce a maximum response, respectively. Four steroids showed significantly greater pituitary inhibition than uterotrophic activity; 17α-ethynylestra-1(10),5-diene-3β,17β-diol, A-homoandrosta-1,4,5(10)-triene-3,17-dione, 3-methoxyestra-1,3,5(10)-triene-17β-cyanethoxy and 17β-hydroxy-A-homoandrosta-1(10),2,4a-trien-4-one.

1. Kincl, F. A., Ringold, H. J., Dorfman, R. I., *Acta Endocrinol.*, 1961, v36, 83.

2. Kincl, F. A., Dorfman, R. I., *Methods in Hormone Research,* Ralph I. Dorfman, Ed., Academic Press, Inc., New York, vIV, in press.

3. Meyer, R. K., Hertz, R., *Am. J. Physiol.,* 1937, v120, 232.

4. Biddulph, C., Meyer, R. K., Lumbreck, L. G., *Endocrinology,* 1940, v26, 280.

5. Byrnes, W. W., Meyers, R. K., *ibid.,* 1951, v48, 133.

6. Dorfman, R. I., Dorfman, A. S., *ibid.,* 1963, v72, 115.

7. Shipley, E. G., *Methods in Hormone Research,* Ralph I. Dorfman, Ed., Academic Press, Inc., New York, 1962, vII, 179.

Received June 26, 1964. P.S.E.B.M., 1964, v117.

Lipid Composition of *Trypanosoma ranarum.* (29635)

S. HALEVY AND ORNA GISRY (Introduced by Chloe Tal)

Laboratory of Nutrition, Department of Biochemistry, Hebrew University-Hadassah Medical School, Jerusalem, Israel

Very little is known of the lipid biochemistry of trypanosomes(1), although it has been shown that some trypanosomes contain considerable amounts of lipid material(2). A basic knowledge of the lipid composition of trypanosomes is important for studies of lipid metabolism and immunological investigations. While most trypanosomes require the presence of serum or blood in their medium *Trypanosoma ranarum* can be grown for several generations in absence of serum. This makes possible the study of its lipid synthesizing capacities without the interference of serum lipids. The lipid composition of *T. ranarum* is described in this paper.

Materials and methods. A strain of *Trypanosoma ranarum* F-13 was used in this study (by courtesy of Dr. F. G. Wallace, Dept. of Zoology, Univ. of Minnesota). Stock cultures of this organism were maintained in a medium composed of lactalbumin hydrolysate (Nutritional Biochemical Co., Cleveland, Ohio) 2%, yeast extract (Difco Lab., Detroit, Mich.) 0.2%, bovine albumin powder 0.05%, hemin 1 mg%, and triethanolamine 0.25%. The ingredients were dissolved in Hanks' balanced salt solution without sodium bicarbonate, and the pH was adjusted to 7.8. 5% rabbit serum was added to the medium, which was dispensed in 10 ml portions into 4 oz rectangular bottles. This medium without serum, (designed "serumless" medium) was used in our experiments.

An aliquot of cells grown in the presence of serum was centrifuged, washed with physiological saline and inoculated into "serum-