only the accumulation in the kidney tissue. Cortisone did not influence the diuresis caused by the parathyroid extract.

Summary. 1) Parathyroid extract increased the urinary excretion of calcium and strontium as well as increased deposition of these minerals in the kidney tissue. 2) Cortisone prevents accumulation of strontium and calcium in kidney tissue but does not influence urinary excretion or diuresis.

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Deoxyribonucleic Acid Content and Mitotic Activity of Vagino-Cervical Epithelium in Colchicine-Treated Mice During Estrus.* (22754)

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The estrous cycle of the mouse is characterized by alternating periods of rapid cell growth and involution of genital tissues and affords an excellent opportunity for study of fundamental chemical aspects of cellular growth in response to hormonal stimulation. Quantitative cytochemical studies of estrus have been limited largely to the study of resting cells of uterine glands, to surface epithelial cells of rat uteri(1) and to total nucleic acid content of uteri. Greater accuracy of microspectrophotometric analysis of individual cells in elucidating their chemical status over chemical analysis of tissues containing heterogeneous cell types has been shown. The value of exfoliative cytology in following physiological and pathological events in the female genital tract has been well established(3-5). The present work was undertaken to study the deoxyribonucleic acid (DNA) content of exfoliated cervical and vaginal cells during the estrous cycle and to correlate these findings with DNA content and mitotic index of basal cells in tissue sections of vaginal fornix.

Materials and methods. Fourteen female C3H mice, 2 to 3 months of age and weighing 18 to 22 g, were divided into 6 groups. Groups 1 and 2 consisted of 3 animals each, Groups 3, 4, 5 and 6 of 2 animals each. Animals of Groups 5 and 6 were ovariectomized 10 days before start of experiment. Animals were kept in air-conditioned room (68-72° F), fed Purina Dog Checkers supplemented by daily addition of fresh bread and carrots. Daily vaginal smears were taken and animals sacrificed at various stages of estrous cycle. Animals of Group 6 were injected subcutaneously with 25 RU of theelin in peanut oil and sacrificed 10 and 20 hours later. Animals of Group 5 served as ovariectomized controls. Mitoses in basal cells of vagina and cervix were arrested in metaphase by the colchicine method of Bullough(6) in which period of treatment was limited to 5 hours to prevent interference with normal mitotic rate. Each animal received 0.1 mg of drug in 0.25 ml of normal saline subcutaneously and sacrificed by cervical dislocation 5 hours later. Smears were prepared immediately and stained by method of Papanicolaou(7). Vaginas and uteri were removed *in toto*, fixed in 10% buf-

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TABLE I. Nuclear DNA of 585 Nuclei and Mitotic Index of Basal Cells in Various Stages of Estrus following Colchicine Treatment, 5 Yr.

Group	No. of animals	Stage of estrus	Total nuclei counted	Diploid cells		% intermediate cells	Tetraploid cells		Mitotic index
				%	DNA, rel. units		%	DNA, rel. units	
1	3	Proestrus	150	82	1.03 ± .09	12.6	5.3	1.98 ± .21	40.2 ± .41
2	3	Estrus	85	67.3	1.07 ± .11	22.2	10.5	2.04 ± .30	223.8 ±10.2
			20*	100	1.01 ± .27	0	0		
			15†		.53 ± .31	0			
3	2	Metestrus	80	100	1.11 ± .22	0	0		9.5 ± 1.11
4	2	Diestrus	75	98.7	1.10 ± .16	1	0		7.3 ± .92
5	2	Castrates	75	100	1.08 ± .13	0	0		11.8 ± 1.23
6	1	Castrate + theelin, 10 hr	45	71	1.05 ± .17	22.2	6.7	2.10 ± .18	28.7 ± 3.2
	1	Castrate + theelin, 20 hr	50	64	1.06 ± .11	26	10	1.90 ± .22	48.3 ± 6.4

* Pyknotic nuclei. † Advanced pyknotic nuclei.

fered formalin, embedded in paraffin and sagittal serial sections cut at 7 and 15 μ thickness. 7 μ sections were stained with hematoxylin and eosin for mitotic counts. Sections cut at 15 μ were used for demonstration of DNA. They were subjected to the Feulgen reaction(8) carried out on smears and tissue sections after hydrolysis with N HCl at 60°C for exactly 12 minutes. Intact interphase basal, parabasal and keratinized cell nuclei selected at random in each vaginal smear stained by the Feulgen method were measured by a microspectrophotometer similar in design to that described by Pollister(9) and satisfying the requirements set by Pollister and Ornstein(10). A wavelength of 560 mμ was used, supplied by Bausch and Lomb grating monochromator. This wavelength represents maximum absorption peak of stain. To minimize light scattering, slides of smears were mounted in oil with a refractive index of 1.572, which matched that of unstained mucus. Relative amounts of DNA of individual nuclei in smears were obtained by multiplying extinction values by the nuclear area since all the nuclei were flattened(11). The major (a) and minor (b) axes of nuclei were measured with an optical micrometer and the area determined by the formula $A = \pi \frac{1}{2}a \times \frac{1}{2}b$. To include basal cells from both ectocervical and vaginal epithelium interphase basal cell nuclei were measured in cervical and vaginal portions of the vaginal fornix. Since care was taken to select uncut nuclei with no overlap, this sharply limited the number of nuclei measured, as cells were crowded especially during estrus. Relative amounts of nuclear DNA in tissue sections were obtained by the method of Swift(12).

Results. The results of DNA measurements in 550 basal and parabasal cell nuclei, 20 keratinized cells containing pyknotic nuclei, and 15 keratinized cells showing advanced pyknosis, karyolysis or karyorrhexis are presented in Table I. Representative histograms were prepared showing the DNA content of basal, parabasal and keratinized cells during various stages of estrus. Basal cells containing intermediate and tetraploid amounts of nuclear DNA were in vaginal smears and tissue sections during early proestrus, where keratinization was not of sufficient thickness to impair basal cell exfoliation. During estrus basal cells containing increased nuclear DNA, *i.e.* intermediates and tetraploids, were absent in vaginal smears while a considerable number were present in the tissues. Twenty hours following subcutaneous injection of theelin in ovariectomized mice the number of exfoliated basal cells containing increased nuclear DNA in smears had decreased, while basal cells containing increased nuclear DNA in the tissues had increased. Appearance of cells showing increased amounts of DNA paralleled the increased mitotic activity of vaginal and cervi-

cal basal cells during proestrus and estrus. During periods of quiescence (metestrus, diestrus and castrates), when mitotic activity was low, a constant diploid value of nuclear DNA was present (Table I). Even though some of the superficial cells exfoliated during estrus showed a marked degree of nuclear enlargement and early keratinization, the DNA content remained at a diploid value. A significant decrease in nuclear DNA occurred only in keratinized cells showing advanced pyknosis, karyolysis or karyorrhexis (Fig. 2).

Discussion. The stimulating effect of estrogenic hormones on growth of genital epithelium is well established, although the bulk of uterine growth following estrogen treatment is largely due to increase in cell volume rather than to cell multiplication(13). Allen *et al.*(14) utilizing colchicine to arrest mitoses have shown that estrogens actually markedly increase the rate of mitosis in vaginal and cervical epithelium of the mouse. The presence of numerous mitoses and interphase basal cells containing intermediate and tetraploid values of nuclear DNA in vaginal and cervical tissue during proestrus and estrus is in accord with this observation. This is in contrast to previous reports of constancy of nuclear DNA in hormonally stimulated uteri (1). However, several reasons may account for this difference. First, cells of vaginal and cervical epithelium where highest mitotic activity is seen, were not studied; secondly, due to the fact that vaginal and cervical epithelium represents but a small fraction of total uterine weights, a chemical determination of nucleic acids of the total uterus does not reflect the high degree of DNA synthesis occurring there. Bloch(15) found that although colchicine arrests cells in mitosis it does not interfere with DNA synthesis and that nuclei falling into higher DNA classes began to appear 10 hours after colchicine treatment. The period of colchicine treatment used in this study was of insufficient length to impair the rate of mitosis or to result in polyploidy due to DNA synthesis without subsequent mitotic division. The increase in nuclear DNA is probably due to a premitotic synthesis of DNA, as has been shown to occur in rapidly growing tissues by Patau and Swift(16). Furthermore, since Howard and Pelc(17) found that synthesis of DNA in the interphase nucleus occurs within a relatively short time is completed some time prior to mitosis, it is not surprising to find resting basal cells with increased amounts of nuclear DNA during periods of augmented growth. The preponderance of basal cells showing intermediate DNA values, and the relatively small tetraploid population, may indicate that once DNA synthesis has been completed division quickly ensues. This may explain the low mitotic index of uterine tissue following hormonal stimulation reported by Drasher and Salvatore(2,13). Basal cells containing increased amounts of DNA were present in vaginal smears taken during proestrus. The paucity of basal cells in smears during late proestrus and throughout estrus is due to the thick layer of keratin which prevents their exfoliation and is not a true reflection of the high degree of DNA synthesis present in the tissues during these periods.

Although cells exfoliated into the vaginal pool are more prone to cellular death and pyknosis, microspectrophotometric studies revealed that the majority of nuclei contained the normal diploid amount of DNA. This was true even though nuclear size was increased and early keratinized cells showing advanced pyknosis. This is in agreement with Leuchtenberger's report(18) on nuclear DNA changes during pyknosis.

It would appear from these experiments that the DNA content of exfoliated basal and parabasal cells in the vaginal pool is quite stable and within limits, corresponds to the DNA content of these cells in the cervical and vaginal mucosa during various physiological states. These findings indicate that except during high estrus it is possible to study DNA synthesis of epithelial cells during the estrous cycle by microspectrophotometric analysis of representative vaginal smears.

Summary. 1. Increased amounts of nuclear DNA were found in basal cells during periods of augmented growth in the estrous cycle. 2. These changes in nuclear DNA parallel the mitotic activity of the basal cells. 3. Exfoliated basal cells containing intermediate

and tetraploid amounts of DNA were found only during early proestrus. 4. The paucity of a tetraploid nuclear population during estrogenic stimulation may indicate rapid cellular division once DNA synthesis has occurred.

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Bioenergetic Basis of Light-induced Fat Deposition in the White-crowned Sparrow.* (22755)

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It has been clearly established in at least 6 species of migratory birds that vernal premigratory deposition of fat in visceral and subcutaneous depots can be simulated by subjecting such birds to artificially prolonged daily photoperiods(1-6). That this phenomenon is the consequence of temporary hyperphagia and not the result of increased time for feeding, as daylength increases, appears evident from the experiments of Odum and Major(6) with white-throated sparrows (*Zonotrichia albicollis*) and Koch and de Bont(5) with a single chaffinch (*Fringilla coelebs*). These investigators, and Wallgren(7) also, have proposed that this response must involve the hypothalamus, a suggestion which is consistent with known effects of light on avian hypothalamo-hypophyseal system(8) and the role of hypothalamic subcenters in regulation of food intake in laboratory mammals(9-11). This investigation provides a more extensive and more quantitative basis for understanding this interesting phenomenon by *1.* definition of temporal course of fat deposition in wild populations of a typical migratory passerine bird (*Zonotrichia leucophrys gambelii*), *2.* measurement of energy intake during premigratory period in captive birds under natural conditions of light and temperature, and *3.* examination of bioenergetics of fat deposition obtained experimentally with prolonged daily photoperiods.

Material and methods. Animals: White-crowned sparrows (*Z. l. gambelii*) were captured from migrant and wintering populations in the vicinity of Pullman, Wash. Specimens for extraction of fat were weighed and killed immediately and kept frozen until extractions

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