nents produced under direction of the viral genome.

Summary. The distribution of incorporated C^{14}-arginine was determined among fractions (acid soluble histones, acid insoluble protein and DNA) of deoxyribonucleoprotein from HeLa cells. During the early stages of infection of cells with poliovirus, the synthesis of the histones was stimulated. This was followed (third hour) by a depressed rate of synthesis of both histones and a markedly reduced incorporation of arginine into DNA. The interrelations of these effects of infection on components of the histone-DNA-RNA synthetic sequence of the host cell are considered.

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Testosterone in the Urine of Castrated Men.* (30364)

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The futility of evaluating the "androgen burden" in women or children or the "effective androgen concentration" in men on the basis of the levels of androgens or 17-ketosteroids in urine and/or blood or on the basis of the individual 17-ketosteroids in these fluids was discussed(1). It was further indicated that titers of testosterone levels in plasma and urine may be a more significant parameter. This has been established now by the finding of a rather large difference between plasma levels of free testosterone for men and women(2-5) and highly significant differences between the urinary titers of testosterone for men and women. The present communication extends these studies

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TABLE I. Urinary Testosterone Levels in Castrated Men.

Age	Age castrated	Testosterone, μg/day	Age	Age castrated	Testosterone, μg/day
32	17	<4	42	20	<2
32	18	<2	43	22	<2
37	12	<1			4.7
37	16	<2	43	25	<3
39	20	<2			<1
39	17	5.9	45	18	<2
40	20	<2	48	31	<1
40	14	<1	48	21	<4
41	11	<2	48	30	<3
		<1	49	28	<2
41	20	<4			
		7.5			

with a consideration of testosterone levels in the urine of castrated men.

Material and method. Urinary testosterone determinations reported here indicate the sum of the free testosterone and that released from the glucuronic acid complex by β-glucuronidase. The method involved the preparation of the ketonic fraction of the urinary extract, thin layer chromatography on Silica Gel G with benzene-ethyl acetate (3:2), followed by gas liquid chromatography using a 6.8% SE-30/Anakrom ABS (110-120 mesh) column(6).

Results and conclusions. Eighteen castrated men of the age range 32 to 49 years, 11 to 31 years after surgery, excreted only minute amounts of testosterone as the free compound and glucuronide (Table I). Fifteen subjects in this class excreted less than 4 μg per day. Five excreted less than 1 μg. One subject had a value of 5.9 μg per day. In one subject a value of <2 and 4.7 μg was found on 2 separate occasions. One subject excreted 7.5 μg in one urine sample, but the second collection indicated a value of <4.

The method was not sufficiently sensitive to delimit precisely the exact testosterone values for the castrated men, but if one assumes the "less than" values to be possible maximum values, the mean for all castrates would be 2.5 μg. Normal men 18-28 years of age had mean values of 171 μg; 30-35 years, 89 μg; and 42-55 years 81 μg. The mean urinary testosterone value for women of 6 μg per day is in the range of that of castrated men.

Pochi *et al*(7) have determined the 17-ketosteroids in the urine of 14 castrated men who were also members of this series. These values were compared by them with those obtained in matched age controls, and they are summarized in Table II. It is clear that no sharp difference is discernible between the 17-ketosteroid values (total or individual) for the castrated and intact men.

In another study, Hamilton *et al*(8) have studied the 17-ketosteroid excretion of this group of castrated men and compared the values to those of intact subjects of essentially the same age. Over the entire age range (28-79), 52 castrated men had a mean 17-ketosteroid excretion of 12.6 ± 6.1 (S.D.) mg per day compared to 15.1 ± 5.0 (S.D.) mg per day for 31 intact subjects (27-81 years). The difference in 17-ketosteroid excretion between the intact and castrate subjects was particularly small, and in some of the age groups no differences were found. This is in contrast to the present finding of a better than a 95% difference in urinary testosterone levels in men without testes.

Summary. The mean urinary testosterone excretion value in castrated men (ages 32-49 years) was estimated to be about 2.5 μg or less per day as compared to a mean of 113 μg/day for normal men (ages 18-55 years) representing a striking 60-fold difference. This emphasizes the meaningfulness of this newly developed parameter in studying the androgenic state as contrasted to the measurement of 17-ketosteroids.

TABLE II. Comparison of 17-Ketosteroids (17-KS) in Urine of Castrated and Normal Men.

Subjects (No.)	Total 17-KS	Dehydroepiandrosterone	Androsterone (A)	Etiocholanolone (E)	11β-Hydroxy A	11-Keto E
Normal men (7)	6.1	1.6	1.5	1.4	.9	.7
Castrated men (14)	5.7	1.0	1.0	1.5	1.2	1.0

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Extracellular pH and Herpes Simplex Virus Multiplication.* (30365)

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It is generally accepted that viruses furnish the infected cell with a template for duplication of their nucleic acid and with information for the synthesis of enzymes and of structural components of the virion. The synthesis of virus-directed nucleic acids and proteins takes place in the milieu of the infected cell and requires the participation of preformed enzymes, ribosomes, and other cellular constituents. Moreover, in both natural and experimental infections the cells are generally maintained under conditions suitable for growth or for survival. It could be expected, therefore, that similar environmental conditions would be required for optimal cell multiplication and for maximum yields of virus. However, this is not the case. Lwoff and Lwoff(1) have shown that the temperature of incubation for optimal poliovirus multiplication is genetically determined by the virus. The purpose of this communication is to report that in human epidermoid carcinoma No. 2 (HEp-2) cells the pH for optimal Herpes simplex virus multiplication is virus strain specific and in one instance substantially below the pH range necessary for growth and maintenance of host cells.

Materials and methods. Approximately 4-8 $\times$ 10^5 HEp-2 cells were exposed in phosphate buffered saline, pH 7.3, to sufficient virus to yield a multiplicity of infection of 10 to 30 plaque forming units per cell. After one hour of incubation at 37°C, the cells were washed twice with buffered saline containing antiviral antibody and once with saline alone. They were then incubated at 37°C in 8 ml of medium consisting of mixture 199 and 1% calf serum and buffered in pH range 5.5 to 8.0. The desired pH was obtained in two ways. In some experiments, media containing a constant amount of $NaHCO_3$ were flushed with 5 to 100% CO_2. In most experiments, media containing varying amounts of bicarbonate were flushed with air containing 10% CO_2. The pH was measured after equilibration of the media with CO_2, both at the beginning and at the end of each experiment. Three virus strains were used in these studies. Strain mP*dk*$^-$ was isolated from a patient with eczema herpeticum; in HEp-2 cell cultures it produces a microplaque consisting of a clump of rounded cells. MP*dk*$^-$ is a mutant derived from mP*dk*$^-$; it produces a macroplaque consisting of polykaryocyte(2). The mP*dk*$^-$ and MP*dk*$^-$ strains do not multiply in dog kidney (DK) cells. Strain MP*dk*$^+$*1p* is a multi-step mutant of MP*dk*$^-$;

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