stimuli have no latent stimulatory effects on the responses measured. However, that there was no apparent decline in any response during the 3-minute interval of sexual quiescence before ejaculation suggested that the effective longevity of sexual preparation stimuli may be greater than we had formerly believed.

Summary. Three intensities of sexual preparation were compared with none using 9 rabbits which each furnished 2 ejaculations every second day for 38 days. The average sperm content of first ejaculations after one false mount (102.9×10^6) was 178% greater than that following no sexual preparation (37.0×10^6) and 3 false mounts resulted in an additional 40% increment in sperm output over one false mount. Sexual preparation also increased average sperm content of second ejaculations, although the increments were not as large as those obtained in first ejaculations. Analyses of seminal volume and total fructose content revealed that sexual preparation stimuli also affected accessory glands, but the magnitude of effect was proportionately smaller than the magnitude of the effect on epididymides. Sexual preparation also reduced the relative magnitude of the within-buck variance of total sperm output.

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Induction of Cleft Palate in Rabbits by Several Glucocorticoids.* (32335)

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Problems in evaluation of the teratogenic effects of drugs were systematically reviewed by Fraser(1). He cited, from the literature on experimental teratology, the many variables relating to genotype, dose, methods of administering the drug and examining the fetuses. Much of the data supported the impression that drug teratogenicity was sporadic, and fortuitous combinations of the previously mentioned variables were necessary to detect teratogenic potential in any one drug. If this is true, then the possibility of developing general principles or extrapolating results to humans would not be great. However, the sporadic results reported may have been due to a sporadic pattern of drug testing rather than to the lack of basic patterns in the teratogenic potential of drugs.

One approach to this problem is to test a group of closely related compounds in animals of several genotypes. Experiments with glucocorticoids have already revealed cleft palate induction by cortisone, hydrocortisone, dexamethasone, prednisolone and triamcinolone in several strains of mice(2,3,4) and by cortisone in rabbits(5). The present experiment concerns the use of 6 glucocorticoids in two strains of rabbits.

Materials and methods. Female rabbits of the New Zealand White or American Dutch strains were placed with a male of the same strain at 5 P.M. and separated the next morning. Ovulation and fertilization were assumed to have occurred 10 hours after coitus. Drugs were administered intramuscularly, starting 13⅓ days postconception, in the doses shown in Tables I to III or listed below. The rabbits were killed with nembutal and chloroform on the 21st day postconception,

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TABLE I. Litter Resorption, Fetal Resorption and Cleft Palate Development in New Zealand White Rabbits After Various Doses of Cortisone.

Dose, mg/day*	No. of litters		No. of fetuses		Palate morphology	
	Treated	Resorbed	Living	Resorbed	Normal	Cleft
25, 25, 25, 25	3	3				
15, 15, 15, 15	2	2				
15, 15, 10, 10	5	2	31	1	22	9
12, 12, 12, 12	2	1	8	0	8	0
10, 10, 12, 12	2	1	10	0	10	0
10, 10, 10, 10	3	1	16	1	14	2
15, 15, 10, 0	1	0	4	2	4	0
10, 10, 12, 0	3	1	8	6	7	1
15, 15, 0, 0	2	0	15	0	15	0

* Daily doses shown from 13⅓ to 16⅓ days postconception.

the uterine horns were dissected out and slit at intervals to facilitate penetration of fixative and then immersed in Bouin's fluid. Subsequent dissection included partial removal of the lower jaw to expose the palatine shelves. No other systematic survey for congenital anomalies was made. The average weight of the New Zealand White females used in this experiment was 4.2 kg and of the American Dutch females was 1.6 kg. This represented a consistent hereditary size difference between the two breeds and they both required about 5 months of growth to reach this weight. There was also a characteristic coat color difference.

Results. The effect of cortisone on palate morphology in New Zealand White rabbits is shown in Table I. Cleft palates occurred in only 3 litters. Triamcinolone, in daily doses ranging from 0.10 mg to 1 mg, induced cleft palate and in higher doses caused litter resorption (Table II). The teratogenic range for dexamethasone was 0.25 mg to 2.5 mg and for prednisolone was 1.5 mg to 4 mg (Table II). Betamethasone was given in 5 dose levels from 1 to 4 mg per day. Among 24 treated females, 21 had complete resorption of their

TABLE II. Litter Resorption, Fetal Resorption and Cleft Palate Development in New Zealand White Rabbits After Various Doses of Three Glucocorticoids.

Drug	Dose, mg/day*	No. of litters		No. of fetuses		Palate morphology	
		Treated	Resorbed	Living	Resorbed†	Normal	Cleft
Triamcinolone	5.00	1	1				
	2.00	3	3				
	1.00	7	2	32	6	15	17
	.75	2	0	14	2	10	4
	.50	3	0	11	3	9	2
	.30	1	0	4	0	4	0
	.25	4	0	28	0	24	4
	.10	3	0	23	0	19	4
	.01	8	0	55	1	55	0
Dexamethasone	4.00	2	2				
	3.00	2	2				
	2.50	2	0	11	0	3	8
	2.00	2	1	4	0	4	0
	1.00	7	3	26	3	10	16
	.50	3	1	14	3	11	3
	.25	1	0	1	5	0	1
	.10	3	0	25	0	25	0
Prednisolone	8.00	2	2				
	4.00	6	4			11	2
	3.00	4	1	17	10	13	4
	2.00	4	1	16	2	14	2
	1.50	3	1	9	10	8	1
	1.00	5	1	36	0	36	0

* Dose listed was given each of 4 days from 13⅓ to 16⅓ days postconception.
† Resorbed embryos in litters having one or more living embryos.

TABLE III. Fetal Resorption and Cleft Palate Development in American Dutch Rabbits After Various Doses of Triamcinolone.

Dose, mg/day	No. of litters	No. of fetuses		Palate morphology	
		Living	Resorbed	Normal	Cleft
4.00	5	0	32	0	0
2.00	2	5	5	0	5
1.00	3	4	12	0	4
.60	2	9	5	4	5
.30	4	24	4	13	11
.20	7	27	8	26	1
.10	7	32	5	32	0
.01	3	16	1	16	0

litters. Three litters survived, one of which contained 9 fetuses with cleft palate, and the other 2 litters contained a total of 17 fetuses with normal palates. Methylprednisolone was injected at 4 dose levels between 1 mg and 4 mg into 7 females. Litters resorbed in 6 females, and there were 3 fetuses with normal palates in the surviving litter. Only 2 litters out of 13 were resorbed when doses ranging from 0.1 to 0.5 mg (5 levels) were administered. In the 11 surviving litters there were 76 fetuses with normal palates and none with cleft palate.

All of the preceding results were obtained with New Zealand White rabbits. The effect of triamcinolone on American Dutch rabbits is summarized in Table III. Cleft palates developed in fetuses from females receiving daily doses of 0.2 mg to 2 mg. Only 2 litters resorbed completely in this dose range (not listed in Table III), but 4 mg doses consistently produced litter resorptions.

All fetuses with cleft palate produced by any of the 5 effective glucocorticoids had both palatine shelves lying in a transverse plane, but separated throughout their length.

Discussion. Fainstat(5) obtained cleft palates with 4 daily doses of 25 to 30 mg of cortisone in a "local strain" of rabbits with an average weight of 3.7 kg. Daily doses of 10 to 15 mg of cortisone were required to produce cleft palates in the New Zealand White strain, thus indicating a difference in susceptibility between the two breeds of animals. The occurence of cleft palates was sporadic, despite repeated attempts to find a specific teratogenic dose schedule. Regarding the possibility of spontaneously occurring cleft palate, the frequency of this anomaly presumably has not been documented in the various strains of rabbits. However, considering the 240 normal palates seen with low doses of drugs and with methylprednisolone, the spontaneous frequency obviously is not high enough to challenge the significance of the large number of clefts obtained with higher drug doses.

Triamcinolone, dexamethasone and prednisolone all produced cleft palates in a systematic fashion. Frequency of cleft palate increased with increasing dose levels until the point of complete litter resorption was reached. The teratogenic activity of these 3 compounds was equally evident in mice(3,4). In contrast, methylprednisolone did not induce cleft palate at any of the doses used. Had this drug been used alone, an investigator would not have been alerted to the teratogenic potential of glucocorticoids, whereas in the broader screening program utilized in this experiment, the potency of the glucocorticoids for production of cleft palate is very striking.

Regarding the effects of genotype, the American Dutch rabbits required twice as much triamcinolone to produce cleft palates as did the other strain, even though they weighed less than half as much. This dose difference between breeds of rabbits is as great as the difference between rabbits and mice(4), if compared by dividing dose by weight. A similar ratio comparison with humans indicates that the teratogenic dose of triamcinolone in rabbits falls within the range of therapeutic doses used in humans.

The variable to negative results obtained with some glucocorticoids used in this experiment contrasts with the clear evidence of a specific teratogenic effect when glucocorticoids are tested as a group. This is consistent with the proposal that a group of closely related drugs should be tested together to evaluate teratogenic potential. The important question of whether this relatively specific teratogenic property of glucocorticoids as a group applies to a wide variety of mammalian genotypes still remains to be answered. Consistent results with the several strains and species tested so far indicates that this

evaluation of the genetic factor should be pursued further.

Summary. Six glucocorticoids were administered separately to pregnant rabbits of one breed over a 4-day period preceding the time of normal palate closure. Triamcinolone, dexamethasone and prednisolone regularly produced cleft palates at several dose levels below that causing litter resorption. Cortisone and betamethasone induced cleft palate sporadically and methylprednisolone did not cause cleft palate formation at any of the dose levels tested. In a second breed of rabbit higher doses of triamcinolone were required to produce cleft palates than in the first breed.

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Patterns of Antibodies to SV_{40} in Children Following the Last Booster with Inactivated Poliomyelitis Vaccines. (32336)

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The presence of infectious SV_{40} in many preparations of inactivated poliomyelitis and adenovirus vaccines(1,2), the demonstration of oncogenic properties of this virus in newborn laboratory animals(3) and its ability to multiply in, and initiate transformation of human cells *in vitro*(4) have raised the question of its possible oncogenicity in man. Two epidemiological studies designed to evaluate cancer mortality in children, in relation to SV_{40} in poliomyelitis vaccines have failed to reveal any differences in trends between vaccinated and control groups(5,6).

The availability of sequential serum samples of children following their last booster dose with formalin-inactivated poliomyelitis vaccines, afforded an opportunity to study the patterns of antibodies to SV_{40} in relation to persistence of antibodies to poliomyelitis virus. The findings of this study are the subject of this report.

Materials and methods. Serum samples. Retained sera from trials prior to the recognition of SV_{40} were available of 40 subjects inoculated with three 1.0 ml doses of formalin-inactivated poliomyelitis vaccines. The sera were collected following the last booster dose, during a period of 3-6 years before 1961.

Serum neutralization test. Sera were inactivated at 56°C for 30 minutes. Equal volumes of serum and approximately 200 $TCID_{50}$ of SV_{40}, strain 777, diluted in Eagle's basal medium, were incubated for 3 hours at 37°C, then held overnight at 4°C. Tube cultures of African green monkey kidney cells were inoculated with 0.2 ml of the serum-virus mixtures, using 4 cultures per dilution. The cells were maintained with Eagle's basal medium and 2% fetal calf serum, at 35°C, and were observed for a period of 14 days. The 50% serum neutralization endpoint was determined by the method of Reed and Muench(7). For the purpose of this study, a serum neutralizing titer of 1/10 or greater, was considered as significant.

Metabolic-inhibition test (MIT). Antibody levels to Type II poliovirus were determined by the MIT(8), modified as described previously*(9).

Complement-fixation tests (CF). Complement-fixation tests for antibodies to SV_{40} tumor antigen were performed by the microtiter technique(10). The antigen was prepared from cell packs of a virus-free hamster SV_{40}

* These tests were carried out by Mrs. Eva R. Brown, Serology Section, DBS.