production of adenovirus and did not suppress production of poliovirus. The fact that it also did not suppress production of either VSV or measles virus suggests that these are both RNA viruses.

Addendum. Since this manuscript was submitted for publication, two other reports on the failure of halogenated deoxyuridines to inhibit measles virus have come to our attention: Lam, K. S. K. and Atherton, J. C., *Nature,* 1963, v197, 820; Sultanian, J. V. and Gordon, I., *Bact. Proc.,* 1963, 158.

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Role of Relaxin in Pregnancy Maintenance and Termination in Mice.* (28447)

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It has been shown that relaxin inhibits uterine motility in rats, mice and guinea pigs *in vitro*(1,2,3,4,5) and *in vivo*(2,6). Quiescence of the uterus and symphysial relaxation would facilitate maintenance of pregnancy and parturition. Relaxin has been shown to stimulate uterine growth or hypertrophy(7,8, 9,10,11) and cervical dilation(3,12,13,14,15).

Hall(16,17) reported on an extensive study in mice ovariectomized on day 14 of pregnancy. Progesterone, estradiol, and relaxin separately and in combination were administered. Smithberg and Runner(18,19) have also studied the problem of the role of relaxin during pregnancy. A study of the effect of relaxin on the placentomata reaction in mice has been reported(20). Kroc *et al.*(3) suggested that relaxin may exert an effect on placental detachment.

In the study to be reported, the mice were ovariectomized either on day 7 or 13, then various combinations of progesterone, estradiol benzoate and relaxin were administered to day 18. In addition, the effect of relaxin on placental detachment in mice pregnant 12 days was studied.

Methods and Materials. Mice kept in individual cages and fed Purina Lab. Chow and water *ad libitum,* were ovariectomized on day 7 or 13 of their first pregnancy. Various levels of progesterone, estradiol benzoate, and relaxin alone or in combination were injected daily following operation to day 18 of gestation unless otherwise stated (Table I).

The relaxin used was in preparation 1164A-66‡, assayed 150 GPU/mg, and was administered in 0.05 ml of beeswax carrier or 0.1 ml of normal saline. The steroids were injected in 0.05 ml of olive oil. Animals were autopsied after abortion or parturition or on days 22 to 24 when parturition had not taken

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‡ Relaxin preparation kindly supplied by Drs. R. L. Kroc and B. G. Steinetz, Chilcott Laboratories, Morris Plains, N. J.

TABLE I. Effects of Ovarian Hormones on Maintaining Pregnancy of Mice Ovariectomized on Day 7 or 13 of Gestation.

Group	Dose of hormone injected			Ovariectomized, days of gestation	Duration of inj, days		No. of mice	Unsuccessful pregnancy				Successful pregnancy		
	Progesterone, mg	Estradiol benzoate, μg	Relaxin, GPU		From	To		No. of mice	Abortion	No. parturition, dead fetuses *in utero*	Spontaneous parturition, delivered dead fetuses	No. of mice	Litters all living	Litters living and dead
1	—	—	—	7	—	—	8	8	8	—	—	0	—	—
2	.5	—	—	"	7	18	13	13	13	—	—	0	—	—
3	.5	.5	—	"	"	"	11	10	10	—	—	1	1	—
4	.75	—	—	"	"	"	10	9	2	3	4	1	—	1
5	.75	.1	5	"	"	"	8	8	8	—	—	0	—	—
6	.75	.1	5*	"	"	"	4	2	2	—	—	2	1	1
7	1.0	—	—	"	"	"	7	7	3	3	1	0	—	—
8	1.0	—	—	13	13	18	8	7	1	2	4	1	—	1
9	1.0	.75	—	7	7	18	16	16	4	3	9	0	—	—
10	1.0	.75	5	"	"	"	4	4	4	—	—	0	—	—
11	1.0	.75	20	"	"	"	10	10	10	—	—	0	—	—
12	1.0	.75	30	"	"	"	9	9	9	—	—	0	—	—
13	1.0	.75	30	13	13	18	10	5	3	—	2	5	4	1
14	.75	25.0	—	7	7	18	11	11	11	—	—	0	—	—
15	10.0	.1	—	"	"	"	10	10	9	1	—	0	—	—
16	10.0	.1	5	"	"	"	11	11	9	2	—	0	—	—
17	.75	.75	5	13	13	18	4	3	2	1	—	1	—	1
18	—	.75	10	"	"	"	4	4	4	—	—	0	—	—
19	—	.1	30	"	"	"	4	4	4	—	—	0	—	—
20	—	—	5	7	7	13	10	10	10	—	—	0	—	—

Relaxin was injected concurrently with ovarian steroid hormones unless otherwise stated.

* Relaxin was injected from 13th to 18th day of gestation.

place, in order to check contents of the uterus.

To study the effect of relaxin on placental detachment, mice pregnant 12 days were opened by an incision in the right flank, and checked for the presence of fetuses. Then a dose of 10 or 50 GPU of relaxin in 0.025 ml of normal saline was injected into the lumen of the right uterine horn. A second group of mice was injected with the same dose of relaxin solution heated for 1 hour at 100°C, and a control group was injected with normal saline.

Animals were sacrificed 24, 48, 72, or 96 hours following the treatment, and the injected horns were checked for number of living and dead fetuses and the state of the placentae in comparison with control horn.

Results. All mice ovariectomized on day 7 of pregnancy aborted within a few days. Replacement therapy of 0.5 mg/day of progesterone (P) alone was unsuccessful. On P 0.75 mg/day, 2 mice aborted within a few days; of the 8 remaining animals, 3 delivered dead fetuses between days 21 to 23, 4 maintained pregnancy but failed to parturate because of the closed pelves and 1 dropped 4 weak young which died within 12 hours. This level of progesterone appeared capable of carrying fetuses to full term, but relaxation of the symphysis pubis was abnormal and parturition was difficult.

Increasing progesterone to 1.0 mg/day after day 7 of ovariectomy, resulted in 3 abortions, 3 with dead fetuses, and 1 parturition with dead fetuses. On overiectomy at day 13, one aborted, 2 had dead fetuses, and 4 parturated with dead fetuses, and 1 delivered dead and living fetuses.

Of 11 mice ovariectomized on day 7 and given 0.5 mg P and 0.5 μg estradiol benzoate (EB), 10 aborted at various times. One mouse delivered 9 young on day 20 but the pups died without nursing.

When P was increased to 0.75 mg, EB was reduced to 0.1 μg and 5 GPU of relaxin was given to 8 mice, all aborted. When relaxin was given from the 13th to 18th days to 4 mice, 2 mice aborted, 1 mouse gave birth to living young, and 1 mouse delivered both live and dead fetuses.

A group of 16 mice was administered 1 mg P and 0.75 μg EB. Of these, 4 aborted, 3 had retained fetuses, while 9 delivered dead fetuses. When relaxin was added at levels of 5, 20, and 30 GPU, all of 23 mice aborted. In a group of 10 mice ovariectomized on day 13 and injected with above levels of steroid hormones plus 30 GPU of relaxin, 3 aborted and 2 delivered dead fetuses on day 17 and 19. In 4 mice normal parturition occurred on day 20 and living young were delivered. One mouse delivered living fetuses with one dead.

A group of 11 mice was given 0.75 mg P and a high level of EB (25 μg). All mice aborted. When P was increased to 10 mg and EB reduced to 0.1 μg in a group of 10 mice, 9 mice aborted and 1 carried fetuses to term without delivery. When 5 GPU of relaxin was added in 11 mice, the results were not improved. Nine mice aborted and 2 carried to term but failed to deliver. When 5 GPU of relaxin was administered alone, all of 10 mice aborted.

In the final series, mice were ovariectomized on day 13 and injected from day 13 to 18. A group of 4 mice received 0.75 mg P, 0.75 μg EB and 5 GPU relaxin. Two aborted, 1 showed dead fetuses, and 1 gave birth to living and dead fetuses. In 2 other groups of 4 mice on 0.75 μg EB and 10 GPU relaxin and on 0.1 μg EB and 30 GPU relaxin all animals aborted.

In the experiment on the influence of relaxin on placental detachment 60 mice were used. In no case did abortion occur. Examination at 48, 72, or 96 hours after relaxin injection at levels of 10 or 50 GPU showed the fetuses living and normal except in some cases 1 or 2 dead near the site of injection in both control and experimental groups. No significant differences were observed between the controls and injected horn in individual animals. Death of the fetuses appeared to be due to damage of the uteroplacental junction caused by the injection. These observations suggest that relaxin in these amounts at this stage of pregnancy does not cause separation of the maternal and fetal placentae (detachment effect).

Discussion. The need of ovarian hormones

throughout pregnancy is reaffirmed. It is suggested that insufficient ovarian hormones are secreted by the placenta of the mouse at 7, 13, 14 or 16 days of pregnancy. In the present study with ovariectomy performed on day 7, administration of P alone at a level of 0.5 mg was unsuccessful; however, at 0.75 mg most of the animals carried fetuses to full term but relaxation was abnormal and parturition difficult. No improvement was observed at 1.0 mg level. Hall(17) reported maintenance of pregnancy to term in 83% of mice spayed on day 14, but a high proportion were stillborn and the symphysis pubis remained closed. Kroc *et al.*(3) who spayed mice on day 16, reported maintenance of pregnancy but high fetal mortality *in utero*. These data suggest that 0.75 to 1.0 mg P/day is sufficient to maintain pregnancy but relaxation of the symphysis pubis is not produced.

In trials with P and EB 0.5 mg P and 0.5 μg EB were ineffective, only 1 of 11 mice carried to term. All mice aborted on P, 0.75 mg, EB 0.1 μg plus 5 GPU of relaxin except when relaxin was injected from days 13 to 18. On 1 mg P and 0.75 μg EB maintenance of pregnancy was partially successful but fetuses were dead on delivery. Addition of 5 to 30 GPU of relaxin resulted in abortion in all cases. These experiments indicate that physiological levels of ovarian hormones and relaxin for maintenance of pregnancy, relaxation of the symphysis and normal parturition in mice ovariectomized on day 7 have not been determined. In mice ovariectomized on day 13, one group on P 1.0 μg, EB 0.75 μg and R 30 GPU was partially successful with 50% of the mice delivering normal young.

Relaxin at levels of 10 or 50 GPU on day 12 of pregnancy injected into one horn of uterus failed to cause abortion or a detachment effect on the maternal and fetal placenta.

Summary. Various levels and combinations of progesterone, estradiol benzoate, and relaxin were administered to pregnant mice ovariectomized on day 7 or 13. P alone at level of 0.75 mg was successful in carrying many fetuses to term but relaxation was abnormal and parturition difficult. In mice ovariectomized on day 7 of pregnancy various combinations of EB, P, and R were generally unsuccessful in the maintenance of pregnancy, relaxation, and normal parturition. Ovariectomy on day 13 of pregnancy and treatment with these hormones had a favorable effect on parturition and 50% of the mice delivered normal young.

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