but not a single positive was obtained. However a number of biopsies yielded virus when they were grown as explants in tissue culture. Positive biopsy cultures spontaneously developed vacuolating type of degeneration and virus was then readily detected by passage. Kidneys from 5 of 8 green monkeys tested yielded virus by this method 3 months after infection was initiated. This latent infection persisted and a second biopsy 6 to 8 months after infection was positive in 4 of the 5 previously positive monkeys. Only 1 of 6 baboon kidneys yielded virus, again by the biopsy explant procedure.

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Failure of Transient Progesterone Deprivation to Induce Ova Implantation in Ovariectomized Rats.* (27795)

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When nidation is delayed in the ovariectomized rat treated daily with progesterone, the administration of small quantities of estrogen along with progesterone causes prompt implantation of the blastocysts(1,2,3). On the other hand, implantation of ova has also been induced by increasing the amount of progesterone(4,5). The apparent discrepancy existing between these two methods has been explained partially on the basis of time of ovariectomy(6). More recently implantation has been reported to occur when progesterone is temporarily discontinued for one day during the 12th to 15th day of pregnancy(7). Data to be presented here do not support the conclusion that transient progesterone deprivation initiates nidation.

Methods. Mature virgin female rats weighing 190-230 g were caged with adult males in the evening and insemination confirmed the next morning by the presence of spermatozoa in vaginal smears. The day spermatozoa were found was designated as day 1 of pregnancy. The inseminated animals were ovariectomized on the third day of pregnancy according to the method of Cochrane and Meyer(2) and placed in 3 experi-

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TABLE I. Effect of Progesterone Withdrawal during Delayed Nidation on Subsequent Implantation and Embryo Survival. (Day refers to day of pregnancy. Day 1 is day of insemination. Daily injections of 4 mg progesterone day 3 through day of second laparotomy except for period of withdrawal indicated; then 4 mg progesterone and 1 μg estrone until day of autopsy.)

Exp & period of progesterone withdrawal	No. of rats	Day of 1st lap.*	Day of 2nd lap.	Day of 3rd lap.	Total No. of sites	Day of autopsy	Total No. of live fetuses	Avg body wt of live fetuses (g)
				†			‡	
A. Day 9	7	8	13	18 (5)	40	29	22 (55.0)§	2.59
None‖	8	8	13	18 (6)	38	29	30 (78.9)	2.05
B. Day 9 and 10	9	8	13	18 (7)	37	29	18 (48.6)	2.3
None‖	10	8	13	18 (5)	28	29	18 (64.3)	2.2
C. Day 14	7	13	18	23 (4)	16	34	5 (31.2)	1.61
None‖	8	13	18	23 (5)	16	34	7 (43.8)	2.42

* Laparotomy. † No. of rats with implantation sites. ‡ % embryo survival calculated from ratio of number of live fetuses to number of implantation sites. § Significant difference, $P = 0.05$. ‖ Control.

ments designated A, B, and C. Each experiment consisted of an experimental group and a concurrently run control group. The control animals were injected daily with 4 mg progesterone in 0.25 cc corn oil from the day of ovariectomy through the 13th day (Exp. A and B) or the 18th day (Exp. C), and each day thereafter with 1 μg estrone in combination with 4 mg progesterone per 0.25 cc corn oil until autopsy. The experimental groups were injected according to the same schedule, except progesterone was replaced with corn oil on day 9 (Group A), day 9 and 10 (Group B) or day 14 (Group C). All injections were by the subcutaneous route. The animals used came from 2 different sources but had similar genetic backgrounds. The Sprague-Dawley (Holtzman Co.) strain of rats was used in Group A and C, and the Sprague-Dawley (Badger Research Corp.) strain in Group B. Approximate times of implantation and number of implantation sites were determined by a series of 3 laparotomies performed at 5-day intervals beginning on the 8th day (Group A and B) or 13th day (Group C). The first laparotomy was done on the day before withdrawing progesterone. On the 11th day after the third laparotomy (day 29 or 34 of pregnancy), animals were killed and autopsied. The day of autopsy corresponded to approximately the 19th day of a normal pregnancy, as estimated from time of implantation and stage of fetal development. At autopsy the numbers of live fetuses, dead or resorbing fetuses, and placental scars were counted and recorded along with weights of the live fetuses.

A comparison of numbers of living and nonliving embryos between experimental and control groups of each experiment, as well as number of rats with and without implantation sites, was made with a 4-fold contingency table chi-square analysis(8). A Rank-Sum test(9) was used to compare the number of implantation sites observed at time of laparotomy. All animals were continued on experiment until the scheduled day of autopsy, whether or not implantation was observed during the series of laparotomies.

Results. The data and schedule of operations are summarized in Table I. Ovariectomy followed by continuous treatment with progesterone prevented nidation in all animals. Withdrawal of progesterone on days 9, 9 and 10, or 13 of pregnancy failed to induce implantation of ova. The absence of implantation following these treatments was verified by exploratory laparotomies performed on the day before withdrawal of progesterone (first laparotomy) as well as 4 to 5 days afterwards (second laparotomy). Injection of 1 μg estrone in combination with 4 mg progesterone induced implantation within 5 days, as verified by a third laparotomy, in both experimental and control animals in all 3 experiments. Although injections of progesterone and estrone were continued until autopsy, no additional implantations occurred after the third laparotomy. Comparing control and experimental groups in each experi-

ment, there were no significant differences in the number of rats exhibiting implantation, or in total number of implantation sites.

In every case the summation of live fetuses, dead fetuses, and placental scars corresponded to the number of sites observed for that animal during the third laparotomy. The percentage embryo survival (Table I), serves to express the distribution of the living and non-living embryos between control and experimental groups. Embryo survival in Exp. A, B, and C was consistently lower in the progesterone-deprived animals than in the non-deprived ones. However, the lower rate of survival was statistically significant only in Exp. A. There was no difference in weights of fetuses, confirming the fact that nidation occurred at the same time in both experimental and control animals.

Discussion. Implantation of ova was consistently prevented in ovariectomized animals treated with progesterone either daily or daily except for one or two days. Nidation was subsequently observed in these same animals 5 days after daily injections of progesterone and estrone were begun. Thus, it is apparent that viable blastocysts were in the reproductive tract during the period of withdrawal treatment. Regardless of the method used for imposing transient progesterone deprivation, the subsequent early stages of implantation were not affected as indicated by the fact that nidation occurred with equal success in both experimental and corresponding control groups. Contrary to Johnson and Shelesnyak(7), our study demonstrates that withdrawal of progesterone does not cause implantation.

In contrast to the absence of any effect on initiation of nidation, temporary withdrawal of progesterone appeared to have a deleterious effect on embryonic survival. In each experiment, temporary cessation of progesterone therapy was associated with an increase in embryonic mortality after implantation, although this was statistically significant only when progesterone was not given on the 9th day. A similar increase in post-nidation embryonic mortality was observed following pre-nidation treatment of inseminated ovariectomized rats with small doses of progesterone (3). Thus, acute or chronic progesterone deficiencies occurring before implantation may adversely affect the ability of blastocysts to survive beyond the early stages of implantation.

During this discussion we have assumed that the withdrawal treatment effectively reduced the amount of progesterone available to the animal. Although there is no evidence to the contrary, attention should be called to the possibility of progesterone stores accumulating and serving as a source of the hormone after withdrawal. Evidence for storage in the fatty tissue has been reported for the rabbit (10). On the other hand, storage resulting from an accumulation of oil appears unlikely since it has been shown that progesterone is rapidly removed from the oil solvent(11,12).

It is apparent that an adequate uterine environment can be experimentally produced with progesterone and estrogens which will cause implantation. However, in spite of much speculation, the mechanism by which these hormones mediate their effects on the endometrium, on the blastocyst, or on both remains to be demonstrated.

Summary. Daily injections of 4 mg progesterone caused a delay in implantation of ova in 50 rats ovariectomized on the third day of pregnancy. The day sperm were found in the vaginal smear was designated day 1 of pregnancy. This response was not altered by temporary withdrawal of progesterone on day 9, 9 and 10, or 13 of pregnancy. Nidation was subsequently induced in the same animals shortly after beginning daily injections of 1 μg estrone in combination with 4 mg progesterone. The number of rats with implantation sites and total number of sites in the experimental groups was not significantly different from corresponding control values. At autopsy 16 days after beginning estrone-progesterone treatment, embryo survival was significantly lower only when progesterone was deprived on day 9 of pregnancy.

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The Immune Response in Cold-Acclimated Mice.* (27796)

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Recent studies of the effects of cold exposure upon animals have indicated that changes in the immune response may occur. Rats exposed to cold demonstrate an increase in the alpha and beta globulin fractions of the blood and a corresponding decrease in the gamma globulin fraction(1). Similar results have been reported for cold-acclimated rabbits(2). In guinea pigs exposed to low temperature, there is an increase in the complement-titer and serum bactericidal activity for *Staphylococcus pyogenes*(3).

The rate of decay of passively administered antibodies is greater in depilated rabbits exposed to −15°C(4,5) than in controls maintained at room temperature (20-25°C). Cold exposure is also accompanied by an increased rate of decay of actively produced antibodies(6), and a slower rate of increase in the level of circulating antibodies(5).

It is well established that bacteriophages are good antigens; coliphage has been shown to serve as suitable antigen in rabbits(7) and actinophage as a good antigen in mice(8). One definite advantage in using phage as an antigen is that it allows for very early detection of antibody production(8) which can be accurately and rapidly determined from the decrease in plaque formation.

In this study the effect of cold-acclimation upon production of active coliphage antibodies in mice was investigated. The temperature of acclimation was kept above zero (4°C) and the pelage left intact to reduce stress.

Materials and methods. One hundred and twenty 8-week-old albino Swiss mice were divided into 4 groups. Two were control groups and were kept at room temperature (23°C). The other 2 groups were cold-acclimated at 4°C for 4 weeks. After the 4-week acclimation period, immunization with coliphage was begun on one group. The remaining acclimated group was then placed at room temperature for an additional 4 weeks of de-acclimation before being immunized. A control group was immunized at the same time as each experimental group. All animals were given Purina Chow pellets and water *ad libitum.*

Immunization and collection of blood samples. All animals were injected every other day for 6 days intraperitoneally with 1 ml of 5×10^{10} plaque-forming units of coliphage strain T_2r^+.‡ Blood samples were obtained from mice killed on the 2nd, 6th, and 10th days after the final immunization injection.

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