intestinal mucosa also sedimented as 7S globulin and were inactivated by mercaptoethanol. 7S antibody to bovine serum albumin was mercaptoethanol resistant.

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Altered Embryonic Effects of Uterine Vascular Clamping in the Pregnant Rat by Uterine Temperature Control.* (31717)

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Brent and Franklin demonstrated that congenital malformations are produced by temporarily clamping the uterine blood supply in the pregnant rat(1). The results of this procedure vary with the duration of clamping and the stage of gestation. It is presumed that the malformations result from an altered metabolic environment within the clamped uterus. If this is true, the temperature of the tissue might influence the results of the uterine vascular clamping procedure.

This paper describes the modifying effect of controlling the temperature of the uterus of the pregnant rat upon the growth retarding, lethal, and malforming effects of uterine vascular clamping.

Materials and methods. Rats 8 days pregnant were anesthetized with pentobarbital taped in a supine position. Pregnancy had been determined by vaginal smear; 9 a.m. on the day sperm were seen was considered to be 0 hours and 0 days of gestation. After an abdominal incision, the uterine horns were brought to the exterior. The position and condition of the implantation sites were recorded. By incising the avascular areas of the mesometrium a wedge of insulating plastic could be inserted under the uterine horns between the ovarian and cervical ends. Thermister probes were placed in the rectum, between the scapulae, and beneath the clamped uterine horn. An electronic thermometer was used to record the temperature (Fig. 1).

Control of the uterine temperature was achieved by means of a thin polyethylene bag connected by tubing to a pump and a constant temperature bath. When the uterus reached the desired experimental temperature, the covering bag was removed to permit clamping of the cervical and ovarian ends of one horn. Following application of the clamps, the bag was replaced over both uterine horns. The distention of the polyethylene bag was controlled by raising or lowering a reservoir bottle outfitted with a discharge tube. The bag was sufficiently flexible to cover both

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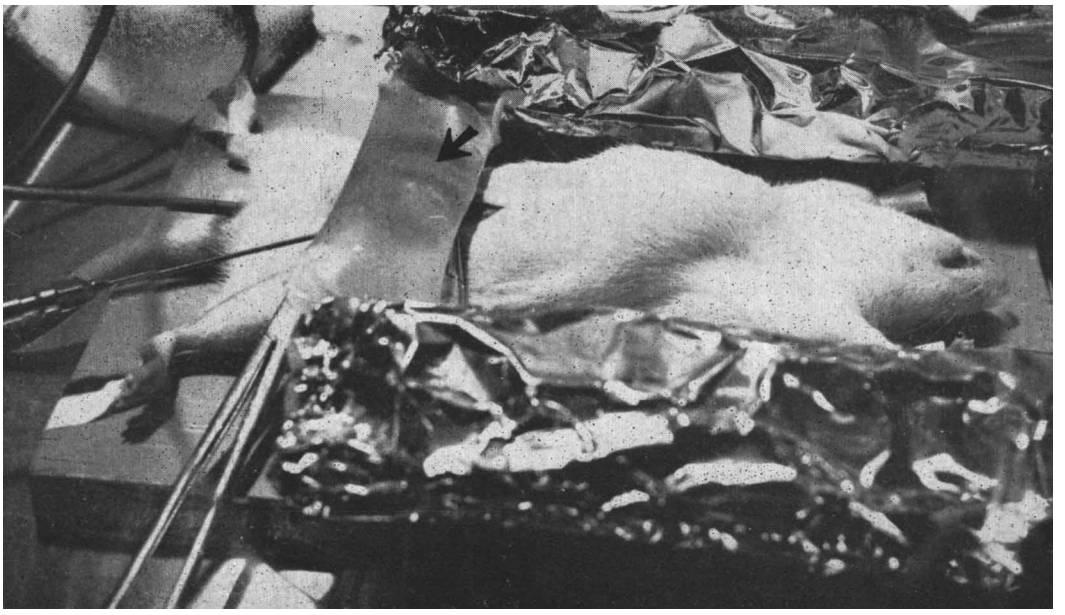

FIG. 1. Anesthetized pregnant rat with exteriorized uterine horns. A temperature controlled plastic bag is placed over the uterine horns (Arrow). A thermister probe continuously records the temperature of clamped or unclamped uterine horns.

uterine horns adequately, and it contained enough water to limit the temperature drift to 0.5°C. All experimentation was carried out in a high humidity incubator maintained 28-30°C.

The uterus was clamped for 2½ hours. Following this procedure, the uterine horns were replaced, and the abdomen was closed with 3-0 silk suture and metal wound clips. The pregnancy was allowed to continue for 21 days. The fetuses were delivered by Cesarean section.

There were 6 experimental and six control groups. One experimental and one control group followed the original method with no temperature control. The remaining groups were maintained at 35, 30, 25, and 4°C.

Results. Alterations in the temperature of the clamped pregnant uterus significantly affected fetal survival, fetal growth and incidence of fetal malformations (Table I).

The highest mortality in control (unclamped) animals occurred at 30°C, the lowest at 4°C. The range of control mortality was 4.2-23.8%.

In the experimental embryos, there was a direct relationship between the temperature of the clamped horn and the percentage of resorptions (Fig. 2). Those embryos clamped at 35°C had a mortality of 100%; at 4°C the mortality was 16.7%. Thus, at 4°C there was a significant reduction in the lethal effects of vascular clamping.

Uterine vascular clamping produced growth retardation of 8 day embryos clamped for more than one hour(2). Analysis of variance of the data of this experiment showed a significant effect (p less than .001) of both clamping and temperature change. At 30°C and 25°C the mean weight of the clamped

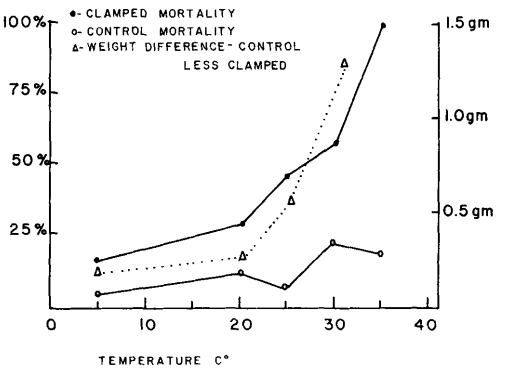

FIG. 2

TABLE I. Modifying Effect of Uterine Temperature Control upon Lethal, Growth Retarding and Teratogenic Effects of Uterine Vascular Clamping.

	Temp, °C	No. embryos	% Mortality	Term No.	Fetuses wt, g	No. malformations	
Unclamped	35	15	20.0	12	4.75		
	Without temp control*	33	15.1	28	5.02		
	30	21	23.8	16	4.72	6/125	4.8%
	25	27	7.4	25	4.84		
	20	24	12.5	21	4.37		
	4	24	4.2	23	4.64		
Clamped	35	21	100				
	Without temp control*	54	68.5	17	3.77	13	76.4%
	30	19	57.9	8	3.34	4	50.0%
	25	32	46.9	17	4.24	5	29.4%
	20	30	30.0	21	4.13	5	23.8%
	4	30	16.7	25	4.42	1	4.0%

* Approximately 33 C in operated animal without temperature control.

embryos followed the pattern of the experiments with uncontrolled temperature: there was significant weight reduction (t test p less than .05). At 20°C and 4°C no significant difference was found between unclamped and clamped weights. The decreasing difference between the weights of clamped and unclamped embryos with the lower temperatures is shown in Fig. 2.

The incidence of malformations in the unclamped control embryos was 4.88% (6/125). As we previously reported, spontaneous malformations are found in the strain of rats used in this laboratory; among 946 embryos from unclamped horns studied in other clamping experiments, 25 fetuses were found to be malformed, an incidence of 2.8%(2). Twenty-eight embryos from the surviving group of 88 clamped embryos were malformed. Most of the malformations were found in the group without temperature control. The incidence of malformations decreased as the temperature was lowered; at 4°C only 4.0% of the survivors (1/25) were malformed (Table I). The types of malformations found paralleled those reported previously; they included hydrocephalus, anophthalmia, abdominal wall defects and renal agenesis.

Discussion. The demonstration that the effects of uterine vascular clamping are temperature dependent supports the thesis that the previously reported results might be effected by controlling the uterine temperature. On the basis of studies with uncontrolled groups in these experiments, the temperature in the exposed horns during a 2½ hour experiment is between 30°C and 35°C. It should be noted therefore, that the mortality from clamping in experiments without temperature control is more likely to be lower than the mortality at 35°C or more.

Since the clamping technique has been used to provide control embryos for experiments affecting maternal fetal relationships, the protection of the embryos from the untoward effects of clamping by cooling extends the usefulness of the procedure, because the cooled clamped horn can be isolated from maternal circulation for longer periods. By the use of clamping alone, embryos could be isolated from the maternal circulation for periods of 30-90 minutes without appreciable mortality. Cooling the clamped horn to 4°C protects the embryos even after 2½ hours of clamping.

The effect of cooling on the developing embryo has not been extensively studied in mammals, although lower animal development is easily modified by alterations in temperature(3,4). The incidence of cleft plate following administration of vitamin A to pregnant mice can be increased by raising the environmental temperature from the normal 23°C to 34°C(5). Tissue oxygen levels have been observed to fall in mouse testes and spleen cooled to 1C(6). Since vascular clamping is accompanied by hypoxia, it is clear that cooling the tissues is of sufficient benefit to offset the possible additive effects of reduced oxygen tension.

On the other hand, the present experiments do not favor any one hypothesis for the mechanism of vascular clamping: a) hypoxia b) acidosis c) accumulation of toxic metabolities e) formation of new breakdown products under extreme conditions. They do suggest that the clamping procedure has a primary metabolic effect, since reduction of metabolic needs is protective.

Summary. Vascular clamping of the pregnant rat uterus results in congenital malformations, fetal growth retardation, and embryonic death. Varying the temperature of the clamped uterus can modify the effects of the clamping procedure. Two and one-half hours of clamping the 8 day pregnant uterus maintained at 35°C resulted in fetal mortality of 100%. With the same clamping procedure and a uterine temperature of 4°C the fetal mortality was 16.7%. Weight reduction and malformation rates were also reduced by lowering the temperature of the clamped horn. These results substantiate the concept that uterine vascular clamping results in congenital malformations *via* a disturbance in some metabolic pathway. They also show that the uterine vascular clamping can be utilized to isolate one uterine horn for extended periods for the study of fetal drug effects and maternal fetal relationships.

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Comparison of the Effect of Hypothalamic and Pituitary Implants of Estrogen and Testosterone on Reproductive System and Adrenal of Female Rats. (31718)

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In an earlier report(1), we were able to demonstrate that implants of either testosterone or estradiol in the median eminence (ME) could decrease the weights of the accessory sex organs in the male rat. These same implants frequently evoked adrenal enlargement and the implants of estradiol produced an enlargement of the anterior pituitary gland. Some of these effects could also be obtained by implantation of the steroids in the anterior pituitary itself or in the mammillary bodies, but implants in other hypothalamic areas were usually ineffective. The conclusion was drawn that these gonadal steroids acted locally in the hypothalamo-hypophyseal region to inhibit gonadotrophin secretion and to stimulate the release of ACTH. It appeared of interest to determine if similar relationships existed in the female, and the present report describes the results of experiments in which implants of estradiol or testosterone were located in various hypothalamic areas or in the pituitary gland of female rats.

Methods. The experimental animals in this study were female, Sherman rats which weighed 220-300 g. The animals were divided into 3 groups. The first was a group of normal controls sacrificed at 4 stages of the cycle. The other 2 groups of rats were implanted in various loci of the hypothalamo-pituitary region with either gonadal steroids or cholesterol to serve as a control on the possible non-specific effects of the implantation procedure and the implants themselves. For each group implanted with testosterone or estradiol-17B, a group of animals from the same colony was implanted on the same day