pearance of AHF after time had been available for equilibration—22% to 11% in 23 hours. Depending on the extent to which equilibration between plasma and extravascular spaces had actually taken place, the AHF half-life would appear to be at least 23 hours.

Summary. 1. The plasma tide of AHF was measured after subcutaneous and intramuscular injections of bovine AHF fractions into hemophilic dogs. Better results were obtained with intramuscular than with subcutaneous administration; higher plasma AHF levels were maintained for longer times. Citrate in adequate concentration in the AHF solutions appeared to have an adjuvant action. 2. The rate of fall off of plasma AHF was followed after moderate levels of this clotting factor had been maintained for several hours. It is suggested that the biologic half-life of AHF in the dog may be at least 23 hours.

1. Langdell, R. D., Wagner, R. H., and Brinkhous, K. M., PROC. SOC. EXP. BIOL. AND MED., 1955, v88, 212.

2. Brinkhous, K. M., Penick, G. D., Langdell, R. D., Wagner, R. H., and Graham, J. B., *A.M.A. Arch. Path.*, 1956, v61, 6.

3. Macfarlane, R. G., Biggs, R., and Bidwell, E., *Lancet*, 1954, v266, 1316.

4. Kekwick, R. A., and Wolf, P., *ibid.*, 1957, v272, 647.

5. Pohle, F. J., and Taylor, F. H. L., *J. Clin. Invest.*, 1937, v16, 714.

6. ———, *ibid.*, 1938, v17, 779.

7. Lewis, J. H., Tagnon, H. J., Davidson, C. S., Minot, G. R., and Taylor, F. H. L., *Blood*, 1946, v1, 166.

8. Macfarlane, R. G., and Biggs, R., *Med. Res. Counc. Mem.*, London, Her Majesty's Stationery Office, 1955, No. 32.

9. Wagner, R. H., Langdell, R. D., Richardson, B. A., Farrell, R. A., and Brinkhous, K. M., *Fed. Proc.*, 1957, v16, 376.

10. Bidwell, E., *Brit. J. Haemat.*, 1955, v1, 35.

11. Brinkhous, K. M., Graham, J. B., Penick, G D., and Langdell, R. D., *Blood Clotting and Allied Problems*, Trans. 5th Macy Conf., ed. J. E. Flynn, Josiah Macy Jr. Found., 1951, 51.

12. Langdell, R. D., Wagner, R. H., and Brinkhous, K. M., *J. Lab. Clin. Med.*, 1953, v41, 637.

13. Penick, G. D., Wagner, R. H., Roberts, H. P., and Brinkhous, K. M., *Fed. Proc.*, 1955, v14, 415.

Received July 19, 1957. P.S.E.B.M., 1957, v96.

Delayed Nidation in the Rat Induced by Progesterone.* (23418)

ROBERT L. COCHRANE AND ROLAND K. MEYER
Department of Zoology, University of Wisconsin, Madison, Wis.

Delayed implantation of blastocysts occurs in many diverse forms of mammals such as the roe deer, armadillo, bear, and many of the Mustelidae(4), and in the laboratory rat and mouse if the female is nursing a sufficient number of young while pregnant(3,4). Numerous reports have been published describing experiments done in an attempt to prevent or shorten delay under these conditions, but relatively little investigation has been done to artificially delay nidation in non-lactating females. The object of these studies was to determine whether nidation could be delayed by altering the normal hormonal relationship during early pregnancy in the laboratory rat.

Materials and methods. Mature female rats of the Sprague-Dawley strain were caged with mature males and the day of insemination determined by the presence of sperm in vaginal smears taken between 8:00 and 10:30 a.m. daily. The day that sperm were found was designated as Day 1 of pregnancy. The rats were maintained on Rockland rat diet and tap water. Surgical procedures were done under ether anesthesia and semi-sterile conditions. Laparotomies and ovariectomies were performed through dorso-lateral in-

* This investigation was supported by a grant from the N.I.H., Public Health Service, and from funds supplied by The Upjohn Co., and The Wisconsin Alumni Research Foundation.

cisions. The ovaries were removed by slitting the ovarian bursa and by ligating the mesovarium, great care being taken to traumatize the oviduct as little as possible. Complete and partial hysterectomies were done through mid-ventral incisions. Rats were hypophysectomized by the parapharyngeal approach.

16 mg of progesterone in corn oil were injected daily from the 2nd or 3rd day after breeding to the 9th day in the experiment designed to determine whether large doses of progesterone would delay implantation of embryos in intact normal female rats. All rats were laparotomized on the 8th day and their uteri were examined for the presence of implantation sites. Length of gestation and number of pups born was noted for all rats.

Since the data just described were negative as far as delaying implantation was concerned, a second type of experiment was done in which rats were ovariectomized 45-60 or 69-84 hrs after sperm were found. These rats were injected daily with 4 mg of progesterone in oil for variable periods from the 2nd-50th day, after which time they were injected with 4 mg of progesterone and 1 μg of estrone in oil per day for 10-16 days, and autopsied on the last day that estrone was injected.

All of the rats were laparotomized on the 8th day or on the day before estrone treatment was begun. If nidation had not occurred by that time the animals were laparotomized 5 days later to determine approximate time of implantation. Implantation was considered to have occurred if sites were found. Most of the rats were treated for 5-11 days after implantation had been determined at which time they were killed and autopsied.

Some of the rats were unilaterally hysterectomized or killed and the uteri flushed with either 0.9% saline or modified Krebs' solution and the flushings examined for the presence of blastocysts.

The results of the experiments just described demonstrated that removal of the ovaries followed by progesterone treatment will delay implantation of blastocysts for many days. It then became of interest to determine whether hypophysectomy would substitute for ovariectomy. Rats were hypophysectomized 22-33 or 44-57 hrs after sperm were found in the vagina and injected with 4 mg of progesterone per day from the 2nd-8th or 9th day and thereafter with 4 mg of progesterone and 1 μg of estrone from the 9th or 10th day to the 20th or 24th day. Three types of control animals were used for comparison with the results obtained in the experiments described above. All animals were ovariectomized 45-60 hr after finding sperm and one group of these was not injected, but was autopsied on the 5th and 21st days of pregnancy. Another group of ovariectomized pregnant females was not treated for 8 days but was injected thereafter with 4 mg of progesterone and 1 μg of estrone per day for variable times from the 9th to the 21st day, and autopsied on the last day of treatment. A third group was injected with 4 mg of progesterone and 1 μg of estrone per day from the 2nd-20th day and autopsied late on the 20th day.

Results. Delayed implantation of embryos was not observed in any of the intact rats given 16 mg of progesterone per day from the 2nd or 3rd day to the 9th day after insemination. Apparently the treatment had little or no effect on length of gestation and viability of the fetuses. These results are in agreement with those reported by Sammelwitz, *et al.*(8) who administered 4, 6, or 8 mg of progesterone daily from the day after mating to the 19th day in the rat.

Nidation of blastocysts was not consistently delayed in rats ovariectomized 69-84 hrs after insemination and given progesterone; embryos implanted in some of the females before injection of estrone was begun.

In the rats that were ovariectomized 45 to 60 hrs after breeding and injected with 4 mg of progesterone per day, implantation of blastocysts was delayed as long as the progesterone treatment was continued (Table I). In the majority of these rats implantation occurred within 5 days after administration of 1 μg of estrone per day was given in addition to the 4 mg of progesterone, which is essentially the treatment that Lyons used to maintain pregnancy from the 7th day to the 22nd and 23rd day in hypophysectomized-ovariectomized rats(7). These results are in con-

TABLE I. Effect of Treatment on Nidation.

Treatment	Day estrone begun	No. females failing to implant‖	No. females implanted		Estimated delay in nidation (days)
			Before estrone begun	Within 5 days after estrone treat. begun	
None	— (2*)	2	0	0	—
4 mg progest./day from 9th day	9 (6*)	6	0	0	—
4 mg progest./day from 2nd day	2 (6*)	0	—	6	0
Idem	7 (1*)	0	0	1	1
"	9 (10*)	1	0	9	3
"	10 (1*)	0	0	1	4
"	14 (6*)	3	0	3	8
"	19 (6*)	3	0	3	13
"	24 (2*)	0	0	2	18
"	29 (4*)	3	0	1	23
"	41 (3*)	1	1	1	35
"	51 (1*)	0	0	1	45
"	9 (2†)	0	2	0	0
"	11 (2†)	1	0	1 killed on 10th day; blastocysts recovered	5
"	14 (2†)	0	2	0	0
"	15 (1†)	0	1	0	0
"	9 (2‡)	0	0	2	3
"	10 (1‡)	0	1	0	0
"	9 (9§)	0	3¶	6	3

* No. animals—ovariectomized 45 to 60 hr after breeding.
† " " — " 69 to 84 " " " .
‡ " " —hypophysectomized 44-57 hr " " .
§ " " — " 20-33 " " " .

‖ As evidenced by laparotomies 1 day before and 5 days after beginning estrone treatment and at autopsy 5 to 68 days after breeding.

¶ Hypophysectomy not complete in 2 of these animals.

trast to those obtained with females which were ovariectomized-untreated and with those which were ovariectomized-untreated to the 9th day then injected with 4 mg of progesterone and 1 μg of estrone per day. As the data in Table I demonstrate implantation did not occur in any of these animals. Although no attempt was made to recover blastocysts from the uteri, it is assumed that the embryos in these rats died before implanting.

Ovariectomized females that were injected daily with 4 mg of progesterone and 1 μg of estrone, beginning on the day before ovariectomy, had embryos apparently implanted at the normal time (assumed to be the 6th day (5)).

Implantation occurred before the 8th or 9th day in only 4 of the 12 hypophysectomized females given 4 mg progesterone per day. In 2 of these 4 animals hypophysectomy was incomplete.

It is obvious from a study of the data in Tables I and II that even with the most successful treatments delayed implantation or recovery of blastocysts is not always accomplished. The reason for the variation is not precisely known, but it seems reasonable that inherent variations in time of ovulation, transport of embryos through the oviduct, and viability of embryos might account for most of the failures.

It is apparent that progesterone in the amounts used in this experiment will prevent nidation of blastocysts for prolonged periods of time up to approximately 45 days, and in such a state that they are capable of implanting if 1 μg of estrone is injected with the progesterone. This delay is, to the best of the authors' knowledge, approximately 28 days greater than the maximum reported for lactating rats(9).

The results obtained with progesterone and estrone in causing nidation are in essential agreement with those of Weichert(9) and

TABLE II. Recovery of Blastocysts.

Day uterus flushed	No. blastocysts recovered	Implant. in remaining uterine horn (day)		No. animals exhibiting implantation	No. animals failing to implant
		After	Before		
6 (1*)	0	6	11	1	0
8 (4*)	8	8	13	3	1
9 (1*)	1	9	14	1	0
13 (2*)	0	13	18	1	1
28 (2*)	3	—	—	0	2
40 (1*)	0	40	45	1	0
§ 8 (1†)	0	—	8	1	0
10 (2†)	5	—	—	0‖	1
8 (1‡)	5	8	13	1	0

Note: All animals inj. with 4 mg progest./day from 2nd day through the day of flushing, after which they were inj. with 4 mg progest. and 1 μg estrone/day.

* No. animals—ovariectomized 45 to 60 hr after breeding.
† " " — " 69 to 84 " " " .
‡ " " —hypophysectomized 20-33 hr " " .
§ Implantation at time of flushing.
‖ One animal killed on 10th day.

Krehbiel(6) for the rat, and those of Whitten in the mouse(10) who shortened delayed implantation by the administration of estrogen to pregnant lactating females.

While our studies were underway Canivenc and Laffargue(1) reported that they could delay implantation in rats ovariectomized on the 4th day after mating by injecting them intramuscularly with 5 and 10 mg of progesterone per day. However, we do not consistently obtain delayed implantation in rats ovariectomized on the 4th day and given 4 mg of progesterone (Table I). Chambon, as reported by Courrier(2), caused delayed implantation in rats ovariectomized 1 day after mating and given 0.25 mg of progesterone per day, but implantation occurred at the normal time if 0.50 mg progesterone per day was given. The delay caused by 0.25 mg progesterone per day treatment could be prevented if 0.16 μg of estradiol-17-beta was administered simultaneously with the progesterone. These data suggest that optimum conditions for implantation of embryos in rats are dependent upon the synergistic action of progesterone and estrogen. It seems probable on the basis of the information available that the quantitative aspects of this relationship must be formulated both in absolute and relative terms, and that many physiological factors participate in determining what this relationship will be.

A comparative study of blastocysts recovered as long as 28 days after mating with those obtained 8 days post-insemination demonstrates that both groups are in the inner cell mass stage, and that the older ones show little, if any, advance in development beyond that seen in the younger ones. Recovery of blastocysts from one horn of the uterus during progesterone treatment and subsequent implantation of the embryos in the other horn induced by progesterone-estrone provides strong circumstantial evidence that the blastocysts are in the uterus in a viable, but resting condition during progesterone treatment.

It is not known whether failure of the blastocysts to implant when progesterone alone is given is due to a defect in the embryo or in the uterus. Preliminary data which we have obtained, however, demonstrate that the endometria of the sterile horns of these rats do respond to artificial trauma by production of placentoma at a time blastocysts in the other horns are not implanted. The results of this and other experiments concerned with hormonal regulation of early rat development will be described later.

Summary. Nidation of embryos was delayed from 1 to 45 days in rats ovariectomized on the 3rd day after mating, by subcutaneous injection of 4 mg of progesterone per day. Implantation was delayed until such time as 1 μg of estrone per day was given in addition

to the progesterone. Recovery of blastocysts from one horn of the uterus during progesterone treatment and subsequent implantation of the embryos in the other horn induced by the progesterone-estrogen indicates that the embryos are maintained in the uterus in a viable, but resting condition during progesterone treatment. If rats were ovariectomized on the 3rd day and untreated for 8 days, nidation of embryos was not observed when progesterone and estrogen was initiated on the 9th day and given for the next 5 to 12 days. Rats ovariectomized on the 4th day after mating were variable in their response to 4 mg of progesterone, some exhibited delay others implanted at the normal time. Delayed nidation up to at least 3 days was obtained in hypophysectomized rats when they were injected only with 4 mg progesterone per day.

1. Canivenc, R., and Laffargue, M., *Compt. Rend. Acad. Sci.*, 1956, v242, 2857.
2. Courrier, R., *Vitam. and Horm.*, 1950, v8, 179.
3. Enzmann, E. V., Saphir, N. R., and Pincus, G., *Anat. Rec.*, 1932, v54, 325.
4. Hamlett, G. W. D., *Quart. Rev. Biol.*, 1935, v10, 432.
5. Krehbiel, R. H., *Anat. Rec.*, 1941, v81, 43.
6. ———, *ibid.*, 1941, v81, 381.
7. Lyons, W. R., PROC. SOC. EXP. BIOL. AND MED., 1943, v54, 65.
8. Sammelwitz, P. H., Dziuk, P. J., and Nalbandov, A. V., *J. Anim. Sci.*, 1956, v15, 1211.
9. Weichert, C. K., *Anat. Rec.*, 1942, v83, 1.
10. Whitten, W. K., *J. Endocrin.*, 1955, v13, 1.

Received July 22, 1957. P.S.E.B.M., 1957, v96.

Effect of Starvation on Sulfide Oxidizing System in Rat Liver.* (23419)

CLAUDE F. BAXTER,† ROBERT VAN REEN‡ AND CESIA ROSENBERG
(Introduced by P. B. Pearson)
McCollum-Pratt Institute, Johns Hopkins University, Baltimore, Md.

In mammals, hydrogen sulfide is produced both by the microorganisms of the intestine (1) and by the body tissues from cysteine (2). The ability of mammalian tissues to oxidize small quantities of sulfide to thiosulfate and sulfate has been repeatedly demonstrated(3,4,5) and may under normal physiological conditions protect the tissue from sulfide poisoning.

In a recent study designed to elucidate the mechanism of this oxidation and the nature of the catalytic system involved(6), our research was initially complicated because of the great differences in ability of rat liver extracts from different animals to oxidize sulfide to thiosulfate. These differences were not eliminated by rigidly standardizing preparatory technics, age, sex, the commercial source of the rats, and method of sacrificing. It was noted, however, that rats sacrificed immediately after arrival from commercial sources (and in transit for some time with little food), and those used in experiments immediately following weekends (during which the feed in the rat colony had run low) almost always contained livers whose sulfide oxidizing capacity was high. Consequently, the effect of inanition on sulfide oxidation in rat liver was investigated.

Materials and methods. Young adult male rats of the Wistar strain ranging in weight from 200 to 250 g were used. They were maintained (unless otherwise specified) on Purina rat chow. They were sacrificed by decapitation, their livers exsanguinated *in situ* and an extract of the livers prepared. These procedures are described elsewhere in greater detail(6). The extracts were incu-

* Contribution of the McCollum-Pratt Institute. Aided by a grant from the Nutrition Foundation.
A preliminary report of a part of this paper was presented at the Federation Meeting in Atlantic City, 1956.

† McCollum-Pratt Inst., Postdoctoral Research Fellow. Present address: Medical Research Institute, City of Hope Medical Center, Duarte, Calif.
‡ Present address: N.M.R.I., Nat. Naval Med. Center, Bethesda, Md.