PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 169, 90–94 (1982)

Pregnancy vs Pseudopregnancy in the Induction of Hypertension and Arteriosclerosis in Sprague–Dawley Rats[1] (41313)

J. P. McMURTRY AND B. C. WEXLER[2]

May Institute for Medical Research of the Jewish Hospital, and the Departments of Medicine and Pathology, University of Cincinnati, College of Medicine, Cincinnati, Ohio 45229

Abstract. Sprague–Dawley rats were subjected to either repeated breeding with a minimal amount of rest between pregnancies or to repeated pseudopregnancies induced by electrical stimulation of the uterine cervix. Representative numbers of animals from each of the two groups were killed after two, four, and six pregnancies or pseudopregnancies. The multiparous rats developed progressively increasing blood pressure; the blood pressure of pseudopregnant rats remained normal. The multiparous rats manifested progressively increasing adrenal, heart, and kidney weight accompanied by hyperlipidemia, i.e., triglycerides, free fatty acids, and cholesterol, hyperglycemia, and elevated BUN levels vs elevated free fatty acid levels only in pseudopregnant rats. None of the pseudopregnant rats developed aortic sclerosis, whereas the multiparous developed progressively worsening and virtually ubiquitous arteriosclerosis. It is suggested that although pseudopregnancy, like pregnancy, causes alterations in hypothalamic–pituitary function, the hormonal-metabolic conditions are not the same, e.g., absence of extraadrenal glandular activity. The hormonal–metabolic conditions entrained by pseudopregnancy are not conducive to the development of the cardiovascular degenerative changes associated with repeated pregnancy.

Repeatedly bred rats, without rest between litters, develop naturally occurring hyperglycemia, hyperlipidemia, hyperuricemia, hypertension, arteriosclerosis, and age prematurely (1–5). In the female breeder, the intensity of the spectrum of degenerative changes parallels the number and frequency of pregnancies as well as the number of pups suckled (6). Our investigations indicate that this Cushing's disease-like spectrum of degenerative changes is due to hyperadrenocorticism which develops concomitant with multiple breedings or pregnancies, i.e., due to abnormal activity of the hypothalamic–pituitary–adrenal–gonadal axis (7–9). We have attempted to mimic the hormonal–metabolic conditions which mimic those which occur during pregnancy by implanting mammotrophic tumors (MtT) into virgin rats or by subjecting virgin rats to a great variety of stressful conditions. Although the MtT tumors secrete copious quantities of prolactin, ACTH, and growth hormone leading to hyperlipidemia, the spectrum of spontaneous cardiovascular degenerative changes observed in breeder rats did not appear. Similarly, although chronic stress led to hyperadrenocorticism and hyperglycemia, it did not produce the changes observed in repeatedly bred rats (unpublished observations).

It is now well established that animals may be made pseudopregnant by a variety of agents or stimuli (10–12). The rat is prone to become pseudopregnant following electrical stimulation of the cervix (10–13). In the rat, successful electrically induced pseudopregnancy leads to cessation of cycling, persistent and functional corpora lutea, and the capability of developing decidual reactions characteristic of pregnant rats. Pseudopregnant rats may be made pregnant by implanting fertilized ova. The consensus is that whatever means is used to induce pseudopregnancy, the modus operandi is mediated through cerebral–hypothalamic–pituitary activity (11). Since it is our contention that repeated breeding leads to hyperadrenocorticism because

[1] Supported in part by grants from the National Institute on Aging (AG-585) and the National Heart, Lung, and Blood Diseases Institute (HL-21418).

[2] Send reprint requests to: Dr. Bernard C. Wexler, May Institute for Medical Research, 421 Ridgeway Avenue, Cincinnati, Ohio 45229.

0037-9727/82/010090-05$01.00/0

of abnormal hypothalamic–pituitary function, we postulated that repeated pseudopregnancy may mimic repeated pregnancies. Therefore, we wished to determine if the hormonal changes which accompany successive pseudopregnancies will be conducive to the development of hyperglycemia, hyperlipidemia, hypertension, and arteriosclerosis which is characteristic of multiparous rats.

Materials and Methods. *Animals.* All of the animals were raised in our Research Animal Breeding Colony and were direct descendants of the original Sprague–Dawley strain purchased from Sprague–Dawley Farms (ARS), Madison, Wisconsin, and were maintained by inbreeding techniques, i.e., brother–sister matings. The animals were housed in our air-conditioned (23 ± 2°), humidity (40–50%), and light-controlled (14 hr light–10 hr dark) research colony. The animals were fed a commercial rat chow (Purina) which is relatively low in fat (4%) and were given tap water to drink *ad libitum*.

Animals designated to participate in this investigation were subjected to daily vaginal lavage 6 days per week to determine the stages of their estrous cycle. Virgin rats were selected at random to become breeders and were housed in breeding cages when they became 90 days of age. Virgin rats do not develop atherosclerosis spontaneously until they become senile, i.e., 30 to 36 months old (1–4). Female breeders which become pregnant during the first estrous which follows parturition develop the most severe aortic sclerosis. That is, the metabolic stress of nurturing a litter of pups *in utero* while nursing the litter of pups born after a previous pregnancy causes exacerbation of arteriosclerosis. This was achieved in this experiment by joining male and female breeding partners immediately after parturition. Each of the female breeders used in this experiment had nursed five to nine pups following each pregnancy.

Pseudopregnancy was induced in cyclic rats by electric stimulation of the uterine cervix using a Yeda Cervical Stimulator (Rohovot, Israel). Stimulation was performed at 1400 hr on the afternoon of proestrous followed by a second stimulus 24 hr later. A success rate of greater than 90% positive pseudopregnancies was achieved. The first day of vaginal leukocytic infiltration followed by cornification was designated as Day 1 of pseudopregnancy. Daily smears were made to monitor persistence of the pseudopregnant status of each animal. The average length of duration of pseudopregnancy was 12 days. Pseudopregnancies were induced to coincide with completion of pregnancies in multiparous rats. Pseudopregnant and parous rats were the same ages (see below).

Just prior to autopsy, the animals were sedated lightly with seconal and without heat or restraint, the systolic blood pressure was recorded using the Friedman: Freed microphonic manometer and tail cuff procedure. Under deep seconal anesthesia, aortic blood samples were drawn, centrifuged, and stored at −20° until analyzed. Animals were autopsied by random selection after two, four, or six pregnancies or pseudopregnancies and when the average age of these animals was 4.1, 6.5, and 8.1 months old, respectively.[3] Lipids (triglycerides, free fatty acids, and cholesterol), glucose, and blood urea nitrogen (BUN) were determined using the automated procedures described for the Autoanalyzer (Technicon). Key organs were removed, trimmed, weighed, and fixed in 10% buffered neutral formalin for histological analyses and stained with hematoxylin and eosin, alcian and toluidine blue, and Hale stain to detect glycosaminoglycans, Verhoef van Gieson for elastic tissue, von Kossa stain for calcium, and Sudan black B for lipids (frozen sections).

Statistical analyses of the data were performed using analysis of variance and Student's *t* test.

[3] Virgin rats do not develop atherosclerosis unless they survive past 30–36 months of age (1–4). Breeder rats develop atherosclerosis spontaneously based on the frequency of breedings and number of young suckled irrespective of age. Therefore, parity and not age is the key factor in the induction of the spectrum of naturally occurring degenerative diseases in repeatedly bred rats.

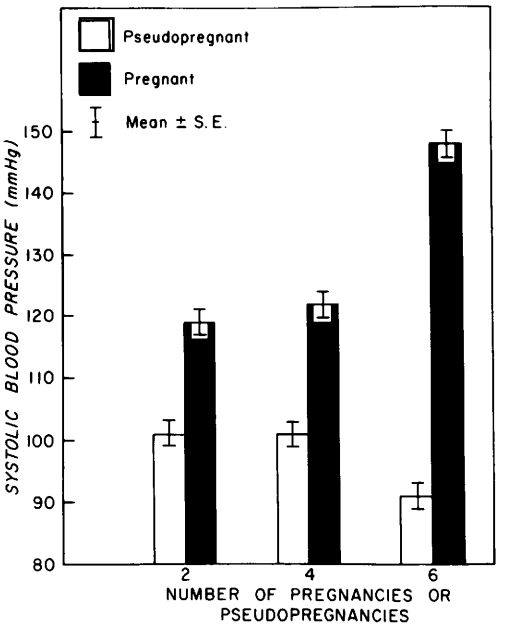

FIG. 1. Barogram depicting the changes in systolic blood pressure of pseudopregnant (n = 12) vs pregnant (n = 12) Sprague–Dawley rats which were bred or made pseudopregnant by electrical stimulation two, four, and six times. In all cases, the blood pressures of the multiparous rats are distinctly higher ($P < 0.001$) than those of the pseudopregnant rats.

Results. *Blood pressure.* The systolic blood pressure of the multiparous rats rose progressively reaching hypertensive levels after six pregnancies (Fig. 1). (A systolic blood pressure over 130 mm Hg is considered abnormal for the Sprague–Dawley strain; normal blood pressure ranges from 90 to 100 mm Hg in virgin rats). The systolic blood pressure of the pseudopregnant rats remained well within the normal range (Fig. 1).

Organ and body weight changes. The multiparous rats manifested their typical progressive increase in body weight with successive breedings, whereas the pseudopregnant rats reached a plateau of normal weight (Table I). The pituitary glands of pregnant and pseudopregnant rats increased in size and weight with increasing age but there were no statistically significant differences between the two groups. The adrenal glands of the pregnant rats manifested a statistically significant ($P < 0.001$) increase with repeated breeding (Table I). The thymi became involuted as the animals aged. The hearts and kidneys were much heavier in the multiparous rats ($P < 0.001$) concomitant with their abnormally elevated blood pressure (Fig. 1). The weights of the ovaries were similar in both groups (Table I).

Triglyceride levels were slightly higher (but not statistically significant) in breeder vs pseudopregnant rats. After six breedings, hypertriglyceridemia, which is characteristic of breeder rats, became well established ($P < 0.001$, Table II). Although multiparous rats show progressively greater levels of circulating free fatty acids, the pseudopregnant rats also manifested progressively rising free fatty acid levels eventually exceeding the levels observed in six times mated breeders ($P < 0.001$, Table II). Circulating cholesterol levels became

TABLE I. COMPARISON OF ORGAN AND BODY WEIGHT OF SPRAGUE-DAWLEY RATS AFTER TWO, FOUR, AND SIX PREGNANCIES OR PSEUDOPREGNANCIES

	Group	Number of animals	Final body wt (g)	Organ wt (mg)					
				Pit	Adr	Thymus	Heart	Kidney	Ovary
2×	Pseudopregnancy	14	256 ± 9	10.3 ± 0.6	34 ± 1	152 ± 15	869 ± 24	846 ± 22	43 ± 3
	Pregnancy	19	275 ± 7	10.3 ± 0.7	38 ± 3	239 ± 20[a]	1111 ± 35[a]	1022 ± 29[a]	48 ± 2
4×	Pseudopregnancy	12	270 ± 6	12.3 ± 0.7	33 ± 1	125 ± 17	909 ± 17	912 ± 27	45 ± 4
	Pregnancy	10	295 ± 5[a]	11.8 ± 0.9	48 ± 4[a]	224 ± 22[a]	1123 ± 34[a]	1099 ± 19[a]	58 ± 3[b]
6×	Pseudopregnancy	11	272 ± 4	15.6 ± 0.8	35 ± 1	138 ± 12	908 ± 24	965 ± 35	47 ± 4
	Pregnancy	15	326 ± 6[a]	14.9 ± 0.9	52 ± 3[a]	129 ± 6	1080 ± 30[a]	1115 ± 25[a]	58 ± 5

Note. Results are means ± SE.
[a] $P < 0.001$.
[b] $P < 0.05$.

TABLE 2. COMPARISON OF CIRCULATING LIPIDS, GLUCOSE, AND BUN OF SPRAGUE–DAWLEY RATS AFTER TWO, FOUR, AND SIX PREGNANCIES OR PSEUDOPREGNANCIES

	Group	Number of samples	Free fatty acid (meq/liter)	Mg%			
				Triglyceride	Cholesterol	Glucose	BUN
2×	Pseudopregnancy	14	530 ± 54	59 ± 8	71 ± 5	148 ± 7	25 ± 1
	Pregnancy	19	653 ± 18[a]	67 ± 14	78 ± 6	148 ± 6	28 ± 1
4×	Pseudopregnancy	12	706 ± 69	44 ± 9	72 ± 3	151 ± 6	21 ± 1
	Pregnancy	12	628 ± 70	67 ± 11	146 ± 7[a]	165 ± 5	21 ± 1[b]
6×	Pseudopregnancy	9	839 ± 77	44 ± 9	73 ± 3	131 ± 3	26 ± 2
	Pregnancy	15	627 ± 28[a]	146 ± 9[a]	185 ± 13[a]	196 ± 13[a]	37 ± 3[a]

Note. Results are means ± SE.
[a] $P < 0.001$.
[b] $P < 0.05$.

significantly elevated ($P < 0.001$) in multiparous but not in pseudopregnant rats (Table II). Both the pseudopregnant and pregnant rats exhibited mild hyperglycemia with the pseudopregnant rats eventually manifesting normal blood glucose levels vs definite hyperglycemia in multiparous rats (Table II). Multiparous rats showed progressively increasing BUN levels (Table II).

Gross and microscopic pathology. No gross or microscopic lesions were found in any of the pseudopregnant animals. Although none of the two-times breeders displayed grossly visible aortic sclerosis, 40% had microscopic aortic lesions; 31% of the four-times breeders had grossly visible lesions, 69% had microscopic lesions; 49% of the six-times breeders had grossly visible lesions, 96% had microscopic lesions. The morphology of the spontaneously occurring lesions in repeatedly bred rats has been described in detail (1–5) and will not be repeated here. The livers of the multiparous rats showed severe fatty infiltration, whereas the livers of the pseudopregnant rats were normal. Similarly, the insulin-producing β cells of the islets of Langerhans of breeder rats were severely degranulated, whereas the islets of pseudopregnancy rats were normal.

Discussion. These findings demonstrate that in contrast to pregnancy, the hormonal changes induced by several successive pseudopregnancies do not provide the proper endogenous hormonal milieu conducive to the induction of hypertension and arteriosclerosis. Although the pseudopregnant rat may simulate some of the physiologic conditions of pregnancy, e.g., persistent and functional corpora lutea, decidua formation, etc., the two conditions are not the same. For example, in pseudopregnant rats there is a gradual decline in progesterone secretion and hyperprolactinemia is not sustained but occurs in episodic bursts (14, 15). It is apparent from this investigation and ancillary experiments that only multiparous rats secrete extra ACTH (16) and develop enlarged adrenal glands which secrete extra quantities of adrenocorticoids (7, 8). We believe that repeated breeding leads to an enzymatic defect in adrenal steroidogenesis which leads to extra production of the aldosterone precursor 18-OH-deoxycorticosterone and its companion mineralocorticoid, deoxycorticosterone (7, 8). The progressive rise in mineralocorticoid production in repeatedly bred rats is most likely responsible for the progressive rise in blood pressure. The increased heart and kidney weights observed in multiparous rats only is most likely a reflection of their hypertension, i.e., increased heart and kidney weight is a reliable index of high blood pressure.

Multiparous rats become progressively obese, hyperlipidemic, and hyperglycemic in parallel with the number and spacings of their breedings (1–5). The breeder rats in this experiment emulated these metabolic conditions, e.g., fatty livers and hyperlipidemia, but the distinctive rise in free fatty acids in the pseudopregnant rats with normal livers was unexpected. It is sug-

gested that the pulsating hyperprolactinemia and elevated blood progesterone levels which occurs in pseudopregnant rats would best explain the elevated free fatty acid levels.

In conclusion, it is our hypothesis that closely spaced, repeated pregnancies and lactation leads to abnormal function of the hypothalamic–pituitary–adrenal–gonadal axis which favors the development of an abnormal hormonal–metabolic milieu which is conducive to the development of diabetes, abnormal lipid metabolism, high blood pressure, arteriosclerosis, and accelerated aging. Although repeated pseudopregnancies are also associated with abnormal hypothalamic–pituitary–gonadal function (11), the same spectrum of hormonal–metabolic conditions prevalent during pregnancy does not obtain, perhaps due to the absence of adrenocortical involvement, which obviates the pathogenesis of cardiovascular degenerative changes.

The authors are grateful for the technical expertise of K. Kern, G. Williamson, D. Conatser, and E. Domingo.

1. Wexler BC, True CW. Circ Res **12**:659, 1963.
2. Wexler BC. J Atheroscler Res **4**:57, 1964.
3. Wexler BC. Circ Res **14**:32, 1964.
4. Wexler BC. Circ Res **15**:485, 1964.
5. Wexler BC, Greenberg BP. Circ Res **42**:126, 1978.
6. Judd JT, Wexler BC. J Atheroscler Res **10**:153, 1969.
7. Wexler BC, Kittinger GW. J Atheroscler Res **5**: 317, 1965.
8. Iams SG, Wexler BC. Atherosclerosis **24**:63, 1976.
9. Iams SG, Wexler BC. Atherosclerosis **27**:311, 1977.
10. Greep RO, Hisaw FL. Proc Soc Exp Biol Med **39**: 359, 1938.
11. Everett JW. Physiol Rev **44**:373, 1964.
12. Banik UK, Ketchel MM. J Reprod Fertil **10**:85, 1965.
13. Shelesnyak MC. Anat Rec **49**:179, 1931.
14. Freeman ME, Neill JD. Endocrinology **90**:1292, 1972.
15. Voogt JL. Endocrinology **106**:1670, 1980.
16. Wexler BC, Lutmer RF. Atherosclerosis **22**:199, 1975.

Received June 5, 1981. P.S.E.B.M. 1982, Vol. 169.