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The Effect of Renal Artery Constriction on the Course of Pregnancy in the Laboratory Rat

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THE EFFECT OF RENAL ARTERY CONSTRICTION ON THE COURSE OF PREGNANCY IN THE LABORATORY RAT

by

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INTRODUCTION

Goldblatt and his associates (1934) were the first to show that persistent arterial hypertension could be produced by renal ischemia. They produced renal ischemia by two procedures which involved reduction of the blood supply to one or both kidneys by means of adjustable silver clamps and removal of the non-ischemic kidney where only one kidney was made ischemic. In one procedure the clamps were placed directly on the renal arteries. The second procedure produced renal ischemia indirectly by constriction of the abdominal aorta above and below the site of the origin of both renal arteries (1939). The adjustable silver clamp devised by Goldblatt (1934) and his associates was intended for larger animals such as sheep and dogs, and clamps of smaller scale proved to be cumbersome for rats. Schaffenburg (1959) devised a simplified method for making and applying Goldblatt clips to control the constriction of the renal artery of rats and other small animals.

It has been established that the hypertension which follows renal artery constriction is the result of the release of an enzyme, renin, from the ischemic renal cortex. Corcoran and Page (1942) and Kohlstaedt and Page (1940) have postulated that reduction of pulse pressure in the renal vessels serves as the stimulus for renin release and it has been demonstrated that reduced renal blood flow, renal ischemia itself, or anoxia of renal tissue do not result in renin release. Helmer and Page (1939) found that although renin

itself has no pressor action, it reacts with hypertensinogen in the blood to produce hypertensin (angiotonin) which is an active vasoconstrictor. The presence of one ischemic kidney and one normal kidney does not result in permanent hypertension. Braun-Mendez, et al. (1947) reported that healthy renal tissue produces hypertensinase (angiotonase) which inactivates hypertensin.

Many investigations have been made concerning the relationship between hypertension and pregnancy using various methods of producing renal ischemia. Dill and Erickson (1938) reported the production of an eclampsia-like syndrome in pregnant dogs and rabbits following renal artery constriction. Holm, et al. (1960) observed severe hypertension accompanying renal ischemia in pregnant sheep. It has been proposed by Landesman and his associates (1961) that a segment of the human toxemia syndrome may be identified with the pathological entity, renal artery stenosis.

Conversely, Goldblatt, et al. (1939), Page, et al. (1941) and MacKaness, (1959) found that hypertension fails to develop after renal ischemia in pregnant rats and dogs, and that rats and dogs with renal hypertension prior to pregnancy exhibit a lowering of blood pressure during gestation. This lack of hypertension has been attributed by Goldblatt, et al. (1939) to compensatory action of the fetal kidneys.

The purpose of this study was to determine the effect of renal artery constriction on the course of pregnancy in the laboratory rat. The cross sectional area of the right renal artery was reduced

sufficiently to make the kidney ischemic and cause the release of renin but not to the point of impairing tubular function. The left kidney was removed to prevent the production of hypertensinase and to assure the persistent elevation of blood pressure. An animal which has undergone constriction of one renal artery and excision of the opposite kidney is referred to as a Goldblatt animal since Goldblatt was the first to demonstrate that hypertension could be produced in this manner.

The possibility that the Goldblatt rats in this study would exhibit symptoms of an eclampsia-like syndrome (extreme hypertension, albuminuria, oliguria, tonic and clonic convulsions, and coma) was considered. Edema would have been indicated during gestation by abnormal weight gain and swelling of the extremities, and upon necropsy by general fluid retention and a gelatinous appearance of the pancreas. A simple commercially available protein indicator was employed to test for albuminuria. Coma and convulsions would have been apparent upon superficial observation.

Since the rat has a 4 to 5 day estrus cycle, time of conception was variable and no attempt to compare weights of fetuses was made.

METHODS

To determine the effect of renal artery constriction on the course of pregnancy in the rat, the method employed by Schaffenburg (1959) to produce Goldblatt rats was utilized. Silver clips were made from a strip of annealed, rolled, silver ribbon 2 mm wide,

0.127 mm thick and 6 mm long. Each strip was bent into a U shape over a piece of 22 gauge needle tubing and one end was flared outward from the main axis. Schaffenburg forceps were adjusted to maintain an open space of 0.015 inches (0.038 mm).

Thirty-five Wistar-Upjohn female rats were subjected to one stage surgery involving a left nephrectomy and placement of a silver clip on the right renal artery adjacent to the aorta. Ethyl ether anesthesia was used. The kidneys were located by palpation and exteriorized via separate costo-lumbar incisions. The left ureter, renal vein and artery were exposed and clamped with a hemostat; the kidney was excised; and a single ligature was applied. The right renal artery was separated from the surrounding tissue by blunt dissection with a small glass probe made from drawn-out glass rod. The clip was placed in the Schaffenburg forceps and applied to the renal artery at the point nearest to the aorta so as to assure partial occulsion of the entire blood supply to the kidney. Incisions were closed with interrupted sutures of 4-0 size chromic gut and wound clips were applied to the skin. Upon recovery from the anesthesia, the rats were placed 7 to a cage (16 x 10 x 7 inches) and given tap water and their normal diet which contained 24 per cent crude protein. A control group of 35 rats of the same age and strain was maintained under identical conditions. No surgery was performed on the control group. All rats were weighed and numbered at the start of the experiment and individual records were kept for each animal.

Prior to this study, 100 Wistar-Upjohn female rats, which had undergone identical surgery to that described above for the Goldblatt rats, were intubated according to the method developed by Weeks and Jones (1960). This intubation of a polyethylene cannula into the aorta makes it possible to measure directly the arterial pressure in unanesthetized rats. Ninety-six per cent of these rats developed non-malignant hypertension in about one week, i.e., 96 per cent had a mean arterial blood pressure greater than 146 mm Hg. Analysis of variance of 132 direct blood pressure measurements of 44 normal Wistar-Upjohn female rats showed that the probability that a normal Wistar-Upjohn female rat would have a mean arterial pressure of greater than 146 mm is 0.05.

After allowing ten days for complete recovery from the surgery and development of the non-malignant hypertension, one Wistar-Upjohn male rat (2.5 months old) was placed in each cage of both the control and Goldblatt series and kept there 5 days. Each female rat was checked daily and any indication of illness was noted. All females were weighed on days 2, 5, 7, 11, 14, 17, 19, 20 and 21, with day 1 being considered the day of the first possible conception. On days 17, 19, 20 and 21, determinations of protein in the urine were made by means of "Albustix" (Ames Co.). On day 21 all animals were sacrificed. On post-mortem examination, the number of successful implantations and unsuccessful ones (abortions) was noted and an assessment of the condition of the various organs was made with particular attention paid to that of the liver, kidney(s), pancreas and lungs.

RESULTS

All animals remained in good health during the course of the experiment. Pregnant rats in both groups exhibited no signs of weakness, lassitude, convulsions, edema, or hematuria. These symptoms were observed in dogs and rabbits in a similar study by Dill and Erickson (1938). Results of tests for albuminuria during the last 4 days of gestation were inconclusive. Post-mortem examinations revealed no gross pathological conditions. Weight gains in both groups during gestation were parallel (Tables 1 and 2).

Seven of 13 pregnant Goldblatt rats parturated 1 to 4 days earlier than expected (depending on the time of conception) but no control rat parturated before day 21.

The incidence of pregnancy (number of pregnant females/total number of females) in the control group was 60.0 per cent compared to 35.2 per cent in the Goldblatt group.

The total number of successful implantations in the 21 pregnant rats of the control group was 212, this is an average of 10.09 per rat. In the Goldblatt group of 13 rats, the average was 8.00 per rat (Table 3).

The number of abortions, as judged from implantation scars, in the Goldblatt group (average of 1.54 per rat) was approximately 3 times higher than that for the control group (average of 0.43 per rat).

Table 1. Weights (grams), number of successful implantations (Imp), and number of abortions (Ab) for each of the Goldblatt Rats, for the days indicated before and after conception.

Rat	Day												Imp	Ab
	-13	-4	-2	2	5	7	11	14	17	19	20	21		
37	170	190	185	195	205	215	235	255	260	285	305	265*	5	2
39	165	185	190	205	215	225	250	265	280	305	310	270*	2	4
40	175	190	195	205	215	225	240	255	275	280	295	310	12	0
43	185	200	200	230	235	240	250	270	280	295	305	325	8	2
51	190	200	205	215	225	235	245	260	275	295	305	315	9	2
52	195	210	210	230	240	250	265	285	300	330	345	350	12	1
56	195	205	205	215	225	230	245	270	285	305	315	265*	5	3
58	180	200	195	215	220	225	250	270	285	310	330	270*	11	0
59	185	195	200	210	225	235	260	280	295	315	340	345	12	1
61	185	200	200	210	220	230	250	275	265	280	295	240*	4	2
63	175	200	195	210	215	220	240	275	295	325	345	275*	13	0
66	170	195	200	210	220	230	245	265	275	300	305	320	7	1
67	170	195	195	210	220	225	250	265	270	295	325	260*	4	2

*Terminated gestation before day 21

Table 2. Weights (grams), number of successful implantations (Imp), and number of abortions (Ab) for each of the Control Rats, on the days indicated before and after conception.

Rat	Day												Imp	Ab
	-13	-4	-2	2	5	7	11	14	17	19	20	21		
2	175	210	220	220	230	235	265	285	300	325	335	350	11	1
3	145	170	170	190	200	205	215	240	245	260	265	280	12	1
5	165	190	185	205	215	225	235	240	240	245	245	250	4	0
6	160	175	180	195	204	210	225	235	240	255	260	270	6	0
7	170	185	190	195	205	210	230	250	260	280	285	300	5	0
9	155	175	180	195	200	210	230	245	260	270	275	280	11	0
12	185	200	205	215	225	240	250	280	285	310	335	345	13	1
13	190	200	205	220	230	235	255	275	280	305	320	325	8	0
14	180	205	200	220	235	240	260	280	310	335	355	370	13	0
15	165	185	190	210	225	225	245	265	290	320	335	340	11	1
17	180	195	190	205	215	225	240	255	265	280	295	305	9	0
20	175	180	175	190	200	210	230	250	260	285	300	315	11	1
23	170	175	160	170	180	195	210	235	250	275	285	295	5	0
24	170	185	200	205	210	210	225	230	230	245	255	270	8	0
26	175	185	195	210	220	235	250	275	285	305	325	345	12	0
27	190	205	205	215	225	230	250	270	290	315	330	340	13	0
28	175	195	205	210	220	230	255	275	290	315	335	345	12	1
29	180	190	200	220	230	240	250	265	280	305	320	335	11	1
30	180	205	200	210	225	235	260	280	295	325	240	255	11	1
32	190	200	195	220	225	235	265	290	305	335	345	360	12	0
34	170	210	210	200	205	225	240	260	280	305	324	340	11	1

Table 3. Average weights (grams), number of successful implantations (Imp), and number of abortions (Ab) for Goldblatt and Control Groups on the days indicated before and after conception.

Day	Goldblatt	Control
-13	180.0	173.1
-4	197.3	191.9
-2	198.1	193.6
2	212.3	205.7
5	221.5	215.5
7	229.6	224.0
11	248.5	242.1
14	268.5	261.0
17	280.0	273.3
19	301.5	295.0
20	317.0	307.9
21	-----	319.7
successful implantations	8.0	10.1
abortions	1.54	0.43

DISCUSSION

Although both the Goldblatt and control groups of pregnant rats remained in good health throughout the course of the experiment, there were significant differences between the two groups in duration of gestation, numbers of abortion, incidence of pregnancy, and number of successful implantations. Impaired renal function or perhaps an endocrine imbalance due to renal ischemia is probably responsible for both the shorter length of gestation and the higher rate of abortion in the Goldblatt group as compared to the control group which had normal renal function and endocrine balance. Chi square analysis of variance revealed that the possibility for the threefold increase in fetal mortality in the Goldblatt group to be attributed to chance distribution was less than 0.5 per cent (significance at the 0.5 per cent level). In a study of pregnancy of rats after unilateral nephrectomy by Lahtinen, et al. (1962) abortion occurred in 20 per cent of the cases. Dieckmann (1952) noted that the usual result of renal or vascular disease in human pregnancy is an increase in maternal and fetal mortality.

The lower incidence of pregnancy (significance at the 10 per cent level) and the lower number of implantations (successful plus unsuccessful implantations) in the Goldblatt group may be due to decreases ovulation as a result of the hypertension or renal ischemia present before mating, to the presence of adhesions which interfered

with the passage of the ova from the ovary to the oviduct, or to the physical presence of the silver clip. Although no gross adhesions were apparent at necropsy, it is possible that in the course of the surgery on the kidneys trauma in that area resulted in adhesions.

In the study by Dill and Erickson (1938), Goldblatt clamps were applied bilaterally to the renal arteries of dogs and rabbits in the latter stages of pregnancy and the ensuing renal ischemia precipitated an eclampsia-like syndrome and rapid death. In this study, although renal ischemia was present and hypertension was well developed before the onset of pregnancy, no symptoms of the syndrome were observed. If the renal ischemia or the hypertension were in some way responsible for the lack of ovulation (which would account for the lack of pregnancy) or the decreased ovulation (which would account for the fewer number of implantations), this may be compensatory action for the renal ischemia. If the primary cause of the eclampsia is a generalized vasoconstriction caused by an ischemic kidney or an ischemic placenta, the lack of the eclampsia-like syndrome may be attributed to the compensatory hypotensive action of the fetal kidneys as postulated by Goldblatt, et al. (1939). Also, the shorter gestational period, fewer number of implantations, and numerous abortions would tend to lighten the demands made on the ischemic kidney and may account for the lack of the eclampsia-like syndrome.

SUMMARY

Although the Goldblatt rats in this study had sufficient renal function for the non-gravid state, gravidism apparently put a strain

on the ischemic kidney. The overall effect of the renal artery constriction appeared to be a lowering of the reproductive capacity of the rat indicated by a higher rate of abortion, lower incidence of pregnancy, fewer number of implantations among pregnant rats and shorter gestational period.

LITERATURE CITED

Braun-Mendez, E., J. C. Fasciolo, L. F. Leloir, and J. M. Munoz. 1947. The substance causing renal hypertension. J. Physiol., 98: 283-298.

Corcoran, A. C. and I. H. Page. 1942. Renal blood flow in experimental hypertension. American J. Physiol., 135: 361-371.

Dieckman, William J. 1952. Toxemias of pregnancy. C. V. Mosby Co., St. Louis, 710 pp.

Dill, L. V. and C. C. Erickson. 1938. Eclampsia-like syndrome occurring in pregnant dogs and rabbits following renal artery constriction. Proc. Soc. Exptl. Biol. & Med., 39: 362-365.

Goldblatt, H., J. Lynch, R. T. Hanzel, and W. W. Summerville. 1934. The production of persistent elevation of systloic blood pressure by means of renal ischemia. J. Exp. Med., 59: 347-380.

Goldblatt, H., J. R. Kahn, and R. T. Hanzel. 1939. The effect on blood pressure of constriction of the abdominal aorta above and below the site of the origin of both main renal arteries. J. Exp. Med., 69: 649-674.

Helmer, O. M. and I. H. Page. 1939. Purification and some properties of renin. J. Biol. Chem., 172: 757-763.

Holm, L. W., Y. W. Katz, H. R. Parker, L. C. Chesley, and N. S. Assali. 1960. Experimental hypertension in pregnant sheep. American J. Physiol., 199 (4): 633-636.

Kohlstaedt, K. G. and I. H. Page. 1940. The liberation of renin by perfusion of kidneys following reduction of pulse pressure. J. Exp. Med., 72: 201-216.

Lahtinen, Kalevi, Esko Naatanen, and Marja Kajenen. 1962. A study of pregnancy of rats after unilateral nephrectomy. Ann. Med. Exptl. et Biol. Fenniae, 40 (1): 13-19.

Landesman, Robert, Mordecai Halpern, and Robert Knapp. 1961. Renal artery lesions associated with the toxemias of pregnancy. Obst. & Gynec., 18 (6): 645-652.

MacKaness, G. B. 1959. The effect of pregnancy and of renin on the blood content of hypertensinogen in rats. British J. of Pathol., 40 (5): 424-431.

Page, Ernest, Henry Patton and Eric Ogden. 1941. The effect of pregnancy on experimental hypertension. American J. Obst. & Gynec., 41: 53-60.

Schaffenburg, Carlos. 1959. Device to control constriction of main renal artery for production of hypertension in small animals. Proc. Soc. Exptl. Biol. and Med., 101: 767-677.

Weeks, J. R., and J. A. Jones. 1960. Routine direct measurement of arterial pressure in unanesthetized rats. Proc. Soc. Exptl. Biol. and Med., 104: 646-648.