OBSERVATIONS ON THE PREGNANT RAT INJECTED WITH NEPHROTOXIC RABBIT ANTI-RAT PLACENTA SERUM AND DESOXYCORTICOSTERONE ACETATE*

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In a previous report (1) Seegal and Loeb have described a series of experiments designed to test the idea that a toxemia of pregnancy may result from the action of injurious antibodies evoked by antigenic substances of placental origin. The injection of rabbit anti-rat placenta serum into pregnant rats regularly produced abortion, and, when these animals were observed over a subsequent period of 3 to 14 months, over 50 per cent of them developed chronic glomerulonephritis. However, since non-pregnant females, as well as males, given this serum developed nephritis with equal frequency, the chronic renal lesions could not be considered a manifestation of "toxemia of pregnancy," nor were any acute episodes suggesting this condition observed in the animals.

Subsequent observations by Knowlton *et al.* (2) demonstrated that the administration of desoxycorticosterone acetate (DCA) in the presence of a liberal NaCl intake enhances the renal hypertrophy and nephritis produced in rats by another cytotoxic serum; namely, rabbit anti-rat kidney serum. In addition, the animals so treated developed striking hypertension. In view of the fact that in the work of Seegal and Loeb (1), referred to above, no demonstrable effect of anti-placenta serum, other than abortion, occurred during gestation, the present experiments were planned to determine whether a toxemia of pregnancy might result if the serum were fortified by DCA and NaCl.

EXPERIMENTAL

Fifty-two female rats of the Long Evans strain ranging from 52 to 75 days of age were raised on the following basic diet:

Whole wheat flour	65.5 per cent
Casein	26.0 " "
Wesson oil or butter	4.0 " "
Cod liver oil	2.0 " "
$CaCO_3$	1.5 " "
NaCl	1.0 " "

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The rats were divided into six groups (Table I). Groups I, II, and III were maintained on this basic diet. For groups IV, V, and VI the diet was adjusted to contain 1.5 per cent NaCl and 0.2 per cent NaCl was added to the drinking water in order to enhance the action of the desoxycorticosterone acetate (DCA)[1] which these groups received. Two and one-half mg. of DCA, suspended in peanut oil, 10 mg./cc., were injected daily subcutaneously in different areas over the back throughout the experiment beginning with the 1st day of each rat's gestation period in groups IV and V and throughout equal periods in the non-pregnant animals in group VI. The animals had been bred at estrus and vaginal smears were examined for spermatozoa. One-half of the individuals in each group were sacrificed after 3 weeks of observation. This corresponded to the calculated last day of gestation in the pregnant animals. The remaining half of the animals in each group were observed over a 7 week period.

The rabbit anti-rat placenta serum used was a pool made up of equal amounts of serum obtained from two rabbits immunized according to the method previously described (1). The serum, inactivated by heating to 56°C. for 20 minutes, was given intravenously in doses of 0.4 cc., 0.5 cc., and 0.6 cc., respectively, on 3 successive days 9 days after the initial DCA injection. In pregnant animals, this corresponded to the 9th day of gestation.

TABLE I

Distribution and Treatment of Animals

Group	No. of rats	Condition	NaCl in diet	NaCl in drinking water	DCA	AP serum*
			per cent	*per cent*	*mg. per day*	*cc.*
I	12	Pregnant	1	0	0	0
II	12	Pregnant	1	0	0	1.5
III	6	Not pregnant	1	0	0	1.5
IV	8	Pregnant	1.5	0.2	2.5	0
V	14	Pregnant	1.5	0.2	2.5	1.5
VI	12	Not pregnant	1.5	0.2	2.5	1.5

* AP serum = rabbit anti-rat placenta serum.

Systolic blood pressure measurements, according to the modification of the method of Williams, Harrison, and Grollman (3) described by Sobin (4) were made on all animals except those in groups I and III. Two or three preliminary readings at weekly intervals were made on each animal. Thereafter, weekly readings were recorded throughout the course of the experiment. Each reading is an average of ten consecutive observations. Fig. 1 presents the average of the weekly readings of all animals in groups II, IV, V, and VI as a heavy line, while the weekly individual extremes appear in the upper and lower broken lines.

Preliminary qualitative albumin determinations by the heat and acetic acid method were done on all animals. A quantitative determination of urinary albumin, using the technique of Shevky and Stafford (5), was made on random samples the day before the calculated date of delivery in all pregnant animals sacrificed at term with the exception of those in group I, as well as at a corresponding period after the administration of serum in the non-pregnant rats of group VI. An additional determination of urinary albumin was done at the termination of the experiment in those animals observed over a longer period of time.

Blood was withdrawn from the heart for the determination of urea nitrogen by the micro-

[1] The authors wish to acknowledge their indebtedness to Dr. Kenneth Thompson of Roche Organon, Inc., Nutley, New Jersey, for supplying the DCA used in these experiments.

Kjeldahl technique at the termination of the experiment, when the animals were weighed and sacrificed with ether. The uterus was examined for degenerating placentae, placental sites, or viable young. The heart, kidneys, and adrenals were weighed and sections removed for histological study. Sections of lungs, liver, spleen, and ovaries were also examined microscopically.

RESULTS

Abortion.—Abortion occurred in 100 per cent of pregnant animals given anti-placenta serum. The placentae had been completely resorbed or extruded in these animals examined at term. Placental sites were noted as proof that the animals had been pregnant. No instance of abortion occurred among the un-

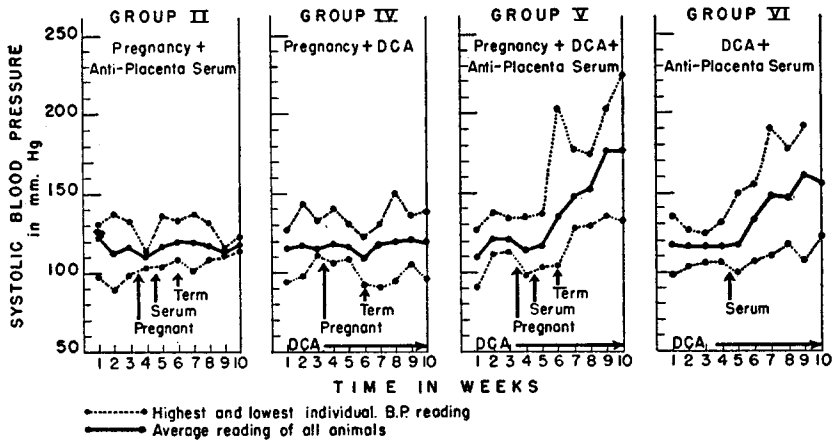

FIG. 1. The results of weekly blood pressure determinations on rats in designated groups.

treated pregnant animals of group I. One pregnant rat of group IV treated with DCA alone aborted.

Blood Pressure.—Fig. 1 presents the systolic blood pressure readings of all animals in groups II, IV, V, and VI. No determinations were made on rats in groups I and III. Hypertension occurred only in those animals receiving DCA in addition to rabbit anti-rat placenta serum; *i.e.*, groups V and VI. A definite trend toward the development of hypertension is apparent at the time of the calculated date of delivery in group V and after a similar time interval among animals in group VI. Among the 7 animals of group V and the 6 animals of group VI, where observations were continued over an additional 4 week period, the blood pressure continued to rise, the average reading being slightly higher in the group V animals. Whether this is a significant difference, in view of the small number of rats observed, is questionable.

Albuminuria.—As has been previously stated, no albumin determinations were done in group I. In the remaining groups the results of the qualitative

determination of urinary albumin at the beginning of the experiment varied from 0 to ++, which is normal for this strain of rats. The results of quanti-

TABLE II

Compilation of Significant Data: Body and Organ Weights; Distribution and Extent of Nephritic Lesions; Quantitative Urine Albumin

	Group I		Group II		Group III		Group IV		Group V		Group VI	
	Pregnant		AP serum* Pregnant		AP serum* Non-pregnant		Pregnant DCA		AP serum* Pregnant DCA		AP serum* Non-pregnant DCA	
Duration of experiment, *wks.*.....	3‡	7	3‡	7	3‡	7	3‡	7	3‡	7	3‡	7
No. of animals..	6	6	6	6	3	3	4	4	7	7	6	6
Average body weight, *gm.*...	188	190	179	194	168	190	165	198	170	194	163	184
Range, *gm.*.....	160–220	170–220	150–200	165–205	160–180	180–200	150–173	175–210	150–185	170–215	135–190	162–203
Average heart weight, *mg.*...	629	618	628	674	643	646	610	780	694	930	690	834
Range, *mg.*.....	587–712	524–705	501–709	646–782	629–663	559–711	563–694	653–897	583–819	795–1186	563–823	679–987
Average adrenal weight, *mg.*...	50	46	54	49	60	47	47	43	51	42	42	40
Range, *mg.*.....	44–57	38–53	44–68	43–61	51–67	44–52	40–52	35–48	47–63	35–50	35–52	30–47
Average kidney weight, *mg.*...	1191	1232	1503	1415	1169	1285	1572	1774	2142	2054	1721	1917
Range, *mg.*.....	1128–1308	1129–1397	1168–2035	1211–1716	1156–1197	1242–1366	1391–1785	1562–2150	1624–2970	1781–2303	1400–2005	1465–2307
Quantitative urine albumin, average gm. *per cent.*.....	—	—	3.2	0.8	1.1	0.6	0.1§	0.1	4.0	0.5	1.8	0.6
Range, *gm.*.....			0.4–5.9	0.2–1.9	0.6–1.7	0.3–0.9	0.1–0.1	0–0.2	1.0–7.2	0.1–1.0	1.0–4.7	0.3–1.1
Intensity 0	IIIIII‖	IIIIII	III	IIIII	III	III	IIII	IIII	II	—	III	III
and dis- +	—	—	II	I	—	—	—	—	III	II	II	II
tribu- ++	—	—	I	—	—	—	—	—	I	—	I	I
tion +++	—	—	—	—	—	—	—	—	I	IIII	—	—
of ++++	—	—	—	—	—	—	—	—	—	I	—	—
nephritic lesions												

* Rabbit anti-rat-placenta serum.

‡ End of gestation period, or at an equivalent point in time in non-pregnant animals.

§ Average of 3 rats; see text.

‖ Each "I" = 1 animal.

tative albumin determinations are given in Table II. It will be seen that the group IV rats, which received DCA but no cytotoxic serum, continued to have normal amounts of urinary albumin when quantitative determinations were made at term as well as 4 weeks later, with one exception. This animal pre-

sented significant albuminuria which was, however, unassociated with histological evidence of renal pathology and this value is omitted from the table. On the other hand, all serum-injected animals, *i.e.* those of groups II, III, V, and VI were observed to have significant albuminuria when examined at "term," 9 days after serum administration. This was far in excess of the amount attributable to rabbit protein in the injected immune serum and was conspicuously more marked in serum-treated pregnant animals both with and without DCA (groups V and II, respectively) than in the similarly treated non-pregnant animals (groups VI and III, respectively). The heavy albuminuria observed in serum-treated animals decreased in intensity and subsequent determinations made at 7 weeks showed consistently lower values. A comparison was made between the lesions seen histologically and the extent of the albuminuria in individual animals and no consistent correlation between the two could be found.

Blood Urea Nitrogen.—With two exceptions, the values for blood urea nitrogen were normal for all animals in each group. One value of 37 mg./100 cc. occurred in an animal of group IV and remains unexplained. The other was a value of 39 mg./100 cc. in an animal of group V which was found at autopsy to have nephritis and generalized arteritis.

Organ Weights.—Body and organ weights are presented in Table II and it will be seen that the body weights are comparable in all six groups.

Progressive cardiac enlargement had occurred in all groups receiving DCA, *i.e.* groups IV, V, and VI, when observed at the end of a 7 week period. As can be seen in the table, this was greatest in the hypertensive animals of groups V and VI, which received nephrotoxic antiplacenta serum in addition to daily DCA administration. The difference in magnitude between these two groups is of doubtful significance. These findings parallel previous observations (2) on the cardiac hypertrophy occurring in non-pregnant female rats as well as in male rats treated with DCA or with DCA plus anti-kidney serum. The cardiac enlargement in animals receiving serum unfortified by DCA, *i.e.* groups II and III, is of questionable significance.

The observed adrenal weights (Table II) suggest that DCA administration may have partially counteracted the anticipated hypertrophy due to pregnancy.

The administration of anti-placenta serum by itself did not result in renal enlargement during the period of observation (see group III in Table II), the weights recorded here being within the normal range for rats of this age, sex, and strain (unpublished data). However, the injection of anti-placenta serum in pregnant rats was followed by enlargement of the kidneys during gestation (see group II, Table II).

The continuous administration of DCA with adequate NaCl (see groups IV, V, and VI, Table II) resulted in marked renal enlargement in all animals. As in the groups which received no steroid injections this enlargement was most

striking in those rats given anti-placenta serum during pregnancy (group V, Table II).

Histopathology.—Both kidneys of each animal were examined histologically and the presence of cytotoxic serum nephritis was graded, as in previous studies (2), 1 to 4 plus according to the extent and severity of the lesions. The results can be seen in Table II. Animals receiving no cytotoxic serum, groups I and IV, had no evidence of nephritis. Of the remaining groups, all of which were serum-treated, 4 instances of nephritis occurred among the 12 pregnant rats of group II in contrast to the absence of renal lesions in all 6 non-pregnant rats of group III. Similarly, when the action of the serum was reinforced by DCA, 12 of 14 pregnant rats of group V developed nephritis in contrast to 6 instances of nephritis among 12 non-pregnant rats of group VI. Moreover the more severe lesions occurred in the former group.

One pregnant rat in group V whose blood pressure reached 204 mm. Hg showed, in addition to marked nephritis, a generalized arteritis at autopsy. All animals receiving DCA showed the previously described (2) tubular lesions characteristic of the action of this steroid in the dosage employed.

All other organs examined showed no significant abnormalities.

COMMENT

Previous studies (1) have demonstrated that rabbit anti-rat-placenta serum injected into rats produces a chronic progressive nephritis that is ultimately indistinguishable from that induced by the injection of specific rabbit anti-rat-kidney serum. Subsequent observations (2, 6, 7) have established the fact that renal and cardiac hypertrophy occur in rats maintained on a liberal NaCl intake and injected daily with DCA. It has also been shown that when this regimen is imposed upon rats whose kidneys have been previously injured with anti-kidney serum both the cardiac and renal hypertrophy are increased, the nephritic process is intensified, and the rats become strikingly hypertensive (2).

The present results confirm the fortifying effect of DCA upon the action of cytotoxic serum, in this instance, anti-placenta serum. In addition, evidence is presented which indicates that the kidney of the pregnant rat is more susceptible than is that of the non-pregnant rat to injury through the action of nephrotoxic anti-placenta serum. This is demonstrated by the higher incidence as well as greater intensity of renal lesions occurring among the serum-treated pregnant animals of groups II and V than among the similarly treated non-pregnant rats of groups III and VI. Thus, it seems probable that pregnancy itself, like DCA, increases the vulnerability of the rat's kidney to injury with anti-placenta serum. Hypertension, which was observed in pregnant animals on adequate NaCl intake, treated with DCA and anti-placenta serum, is not dependent upon pregnancy, inasmuch as a commensurate rise in blood

pressure was observed in non-pregnant animals similarly treated. It appears, furthermore, that the cardiac hypertrophy described is independent of pregnancy.

The question arises whether the effects produced by DCA and anti-placenta serum in pregnant rats constitute a toxemia of pregnancy. The animals aborted, developed hypertension, and renal damage. However, unlike the generally beneficial effects of emptying the uterus in cases of human toxemia, no decline in hypertension occurred following abortion and the renal lesions progressed. For this reason the observed increased susceptibility of the kidney of the pregnant rat to the action of nephrotoxic serum and DCA is perhaps more analogous to the acceleration of chronic nephritis in the human being, which is frequently observed during or following intercurrent pregnancy.

CONCLUSIONS

1. Pregnancy enhances the susceptibility of the rat to intercurrent renal damage produced by anti-placenta serum. This is manifested by the development of renal hypertrophy and nephritis in a number of these animals. Both renal hypertrophy and nephritis are consistently intensified by the concomitant administration of DCA.

2. Hypertension develops in both pregnant and non-pregnant rats treated with the anti-placenta serum employed together with the daily administration of DCA.

3. Termination of pregnancy, in the face of continued DCA administration, fails to lower the blood pressure or to arrest the nephritic process.

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BIBLIOGRAPHY

1. Seegal, B. C., and Loeb, E. N., *J. Exp. Med.*, 1946, **84**, 211.
2. Knowlton, A. I., Loeb, E. N., Stoerk, H. C., and Seegal, B. C., *J. Exp. Med.*, 1946, **85**, 188.
3. Williams, J. R., Harrison, T. R., and Grollman, A., *J. Clin. Inv.*, 1939, **18**, 373.
4. Sobin, S. S., *Am. J. Physisol.*, 1946, **146**, 179.
5. Shevky, M. C., and Stafford, D. D., in Laboratory Methods of the United States Army, (J. S. Simmons, editor), Philadelphia, Lea and Febiger, 4th edition, 1935, 290.
6. Selye, H., *J. Clin. Endrocrinol.*, 1946, **6**, 117.
7. Knowlton, A. I., Stoerk, H. C., Seegal, B. C., and Loeb, E. N., *Endocrinology*, 1946, **38**, 315.