and Griffin of 0.73 ml/min/100 g body weight. The urinary non-creatinine chromogen averaged 7.3% of the total chromogen; under some circumstances this value is sufficiently large to be significant. Correlations of creatinine clearance with body weight, kidney weight, and surface area were approximately the same. Therefore, in this weight range it seems most practical to use body weight as the unit for relating clearances.

I wish to acknowledge the assistance of Dr. Max R. Mickey, Jr. in performing the statistical analyses.

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Received May 5, 1967. P.S.E.B.M., 1967, v125.

Production of Congenital Malformations Using Tissue Antisera III. Placental Antiserum.* (32267)

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The teratogenicity of rat kidney antiserum has been reported(1) and corroborated(2). Rat placental antiserum has also been reported to be teratogenic(3,4) and it is the purpose of this paper to deal with the quantitative and qualitative aspects of the production of congenital malformation in rats utilizing placental antiserum.

Materials and methods. Rat placentae were obtained from rats that were pregnant 21 days. The maternal rats were anesthetized with sodium pentobarbital and heparinized. A laparotomy was performed and a catheter was placed in the abdominal aorta. Immediately following the rapid removal of 8-12 ml of blood, the rat and its pregnant uterus were perfused with aproximately 200 ml of buffered saline. The placentae were then removed from the uterus and a catheter was placed in the umbilical vein. The placentae were perfused with buffered saline until they were a pale tan and the material decidual tissue was white in color. These tissues were lyophilized, ground into a fine powder and stored in a deep freeze.

Adult rabbits were immunized with one of 3 preparations of placentae: whole placenta, fetal placenta or maternal decidual tissue. The rabbits were injected in the lumbosacral muscles weekly for 4 weeks with an emulsion containing 100 mg of dried tissue powder, 1 ml of distilled water and 1 ml of Freund's adjuvant. Forty milliliters of blood were removed by cardiac puncture biweekly and the sera pooled. Three separate pools of rabbit placental antisera were prepared. The preparation of rat kidney antiserum has been described(1,4).

Control groups consisted of litters that were anesthetized and laparotomized after 8 days of pregnancy and another group that underwent the operative procedure and received an injection of pre-immunization normal rabbit serum (Table I). Rats 7, 8, 9, or 10 days pregnant were injected with one of the 3 placental antisera at doses of from 0.25 to 1.5 ml/100 g of pregnant rat. The intravenous or intraperitoneal routes were utilized. Just prior to injection of the antiserum, the pregnant rat was anesthetized and laparotomized. The number, conditions and positions of the embryonic sites were recorded and the incision was then closed with silk sutures and metal wound clips. The fetuses and placentae were removed after 21

* Supported by NIH Grant HD 630.

TABLE I. The Effect of Rabbit-Anti-Rat Whole Placenta When Injected Intraperitoneally into Rats Eight Days Pregnant.

Dose of placental antiserum (ml/100 g of rat)	Dose of control rabbit serum (ml/100 g of rat)	No. pregnant rats	No. of implantation sites	Resorption at term	% Mortality	Term fetal wt	% Malformations
0	0	10	92	3	3.3	4.88 ± .36	1.1
0	2	6	62	2	3.1	4.76 ± .41	.0
.75	0	4	44	14	31.8	4.54 ± .78	46.7
1.0	0	6	65	31	47.9	4.05 ± .73	88.2
1.25	0	4	59	40	67.7	3.73 ± .16	100
1.50	0	6	67	44	65.7	3.69 ± .64	92.5

TABLE II. Teratogenicity of Absorbed Rabbit Anti-Rat Kidney and Placental Antiserum When Injected into 8-Day Pregnant Rats.

Antiserum	Dose of antiserum that yields 50% malformation (ml/100 g of rat wt)	Dose of antiserum administered (ml/100 g of rat wt)	Absorption: Antigen	Absorption: Concentration, mg/ml antiserum	No. of litters	No. of malformed litters	Teratogenic
1. Kidney	.25	.75	Rat serum	75	6	6	Yes
2. "	.25	.75	Kidney	1	4	4	"
3. "	.25	.75	Kidney	5	4	2	"
4. "	.25	.75	Kidney	10	4	0	No
5. "	.25	.50	Placenta	5	4	4	Yes
6. "	.25	.75	Placenta	10	4	4	"
7. "	.25	.75	Placenta	80	4	3	Yes
8. Placenta	.75	1.0	Rat serum	70	4	4	"
9. "	.75	.75	Placenta	10	4	4	"
10. "	.75	.75	Placenta	40	4	0	No
11. "	.75	1.25	Placenta	40	4	4	Yes
12. "	.75	1.0	Kidney	3.75	4	1	"
13. "	.75	1.0	Kidney	10.0	4	0	No

days of pregnancy and were weighed and examined for malformations.

Absorption studies were carried out at 37°C in an Eberbach shaker water bath. Weighed amounts of lyophilized antigen and antisera were resuspended in distilled water in various concentrations and incubated for 30 minutes. These solutions were then utilized in agar diffusion systems or injected into pregnant rats to determine whether the teratogenicity of antisera had been modified (Table II).

The analyses of the precipitin bands formed between the soluble placental antigens and their respective antibodies were performed using an agar diffusion system that consisted of a lucite template with 6 wells in a circle around a center well. All wells were one-half inch from each other, center to center. The wells in the template have a volume of .15 ml and the template is placed on an agar layer 2 millimeters thick. Photographs of the bands were made with darkfield illumination.

Results. The effect of rabbit-anti-rat whole placental serum, rabbit-anti-rat maternal decidual serum and rabbit-anti-fetal placental serum were similar in many respects to the effects of rabbit-anti-rat kidney serum. When injected into pregnant rats at the proper dose, these antisera can produce embryonic death, growth retardation and malformation. Neither control group had any significant alterations in the malformation rate, the resorption rate or the rate of fetal growth (Table I). All the adsorption, LD/50 and immunodiffusion studies were carried out with the antiserum against whole placenta. It was apparent that the immunodiffusion patterns of all 3 antisera were qualitatively identical. Although the rabbits received the same quantity of dried

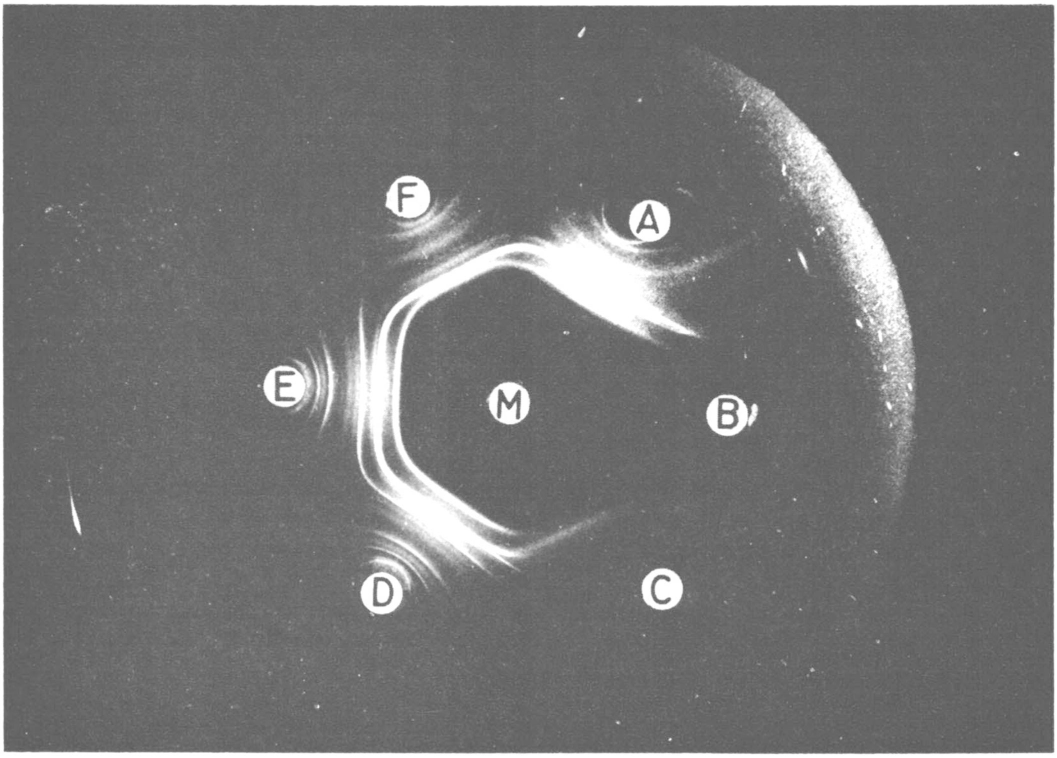

FIG. 1. Immunodiffusion pattern-well contents:
M-Rat kidney antigen (100 mg/ml)
A-F all contain anti-rat-kidney serum. In wells B, C, E and F, the antisera were previously absorbed with various quantities of antigens/ml of kidney antiserum.
B—20 mg kidney/ml of kidney antiserum
C—5 mg kidney/ml of kidney antiserum
E—5 mg placenta/ml of kidney antiserum
F—20 mg placenta/ml of kidney antiserum
It can be observed that the bands between kidney antiserum and kidney antigen are eliminated in reaction B-M by absorption of the antiserum with 20 mg of kidney. Furthermore, the absorbed antiserum in well B is no longer teratogenic when tested *in vivo*. On the other hand, absorption with 20 mg of placenta is not as effective in reducing the precipitin bands and when tested *in vivo* in pregnant rats it is still teratogenic (F-M). In fact 5 mg of kidney/ml of kidney antiserum is more effective in modifying the immunodiffusion pattern and reducing teratogenicity (C-M) than 20 mg of placental antigen (F-M) (Table II).

antigenic material, the pooled antiserum obtained from rabbits immunized with fetal placenta was less potent than the other 2 placental antisera pools.

In the experimental groups receiving the rabbit anti-rat placental antiserum, there was a definite correlation between dose of the antiserum and incidence of malformations, amount of fetal growth retardation and resorption rate (Table I). As the dose of antiserum administered rose from .75 ml to 1.5 ml/100 g of pregnant rat, fetal mortality rose from 31.8% to 65.7%. The average term fetal weights also decreased as the dose of antiserum was increased.

In the group receiving the lowest dose (.75 ml/100 g pregnant rat) the incidence of malformations in the surviving fetuses was 46.7%. Thus, almost half of the surviving fetuses were malformed, in spite of the fact that there was only minimal growth retardation in this group of fetuses. Almost 100% of the surviving fetuses were malformed in the groups receiving higher doses.

The following spectrum of malformations was produced: *Central nervous system* (hydrocephaly, hydranencephaly, microphthalmia and anophthalmia); *Cardiovascular sys-*

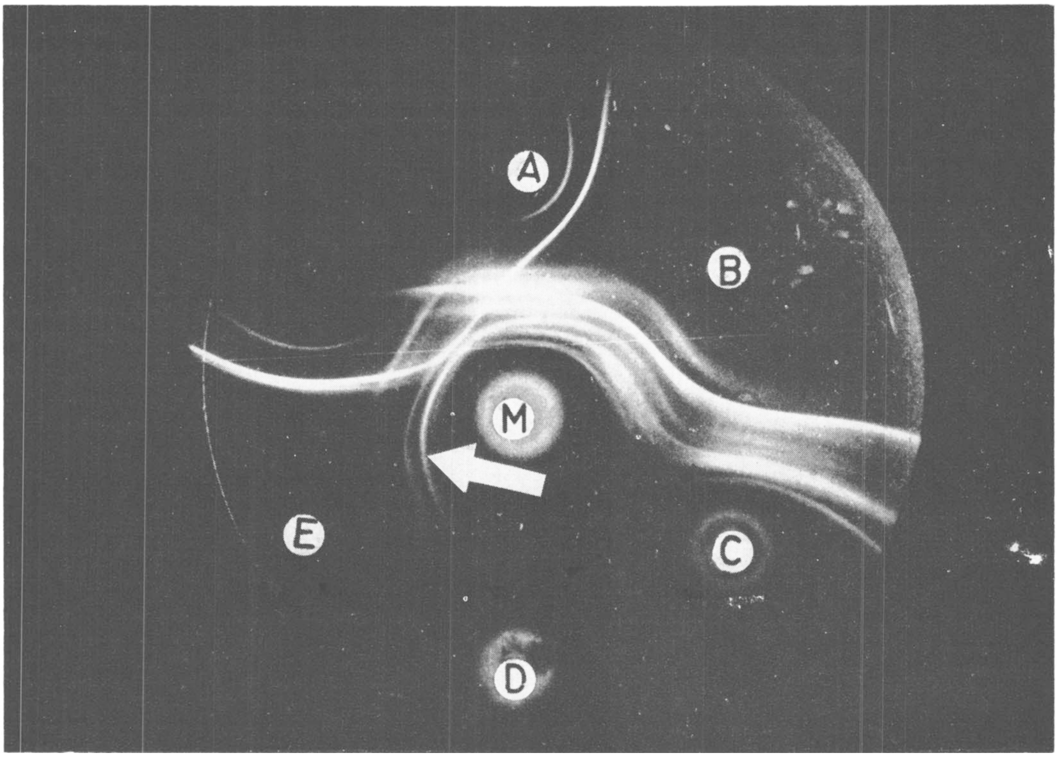

FIG. 2. Immunodiffusion Pattern-Well Contents: M—Rat kidney antigen (100 mg/ml); A—anti-rat-kidney serum. B—Anti-rat-kidney serum absorbed with 80 mg rat serum/ml antiserum. C—Rat kidney antigen (200 mg/ml). E—Anti-rat-placental serum adsorbed with 80 mg rat serum; F—Anti-rat-placental serum. Absorption of anti-kidney and antiplacental serum with rat plasma removes several precipitin bands. Reaction B-M has fewer bands than A-M and reaction E-M has significantly fewer bands than F-M, indicating that placental antiserum contained a higher percent of the plasma antibodies than kidney antisera. The 2 precipitin bands near Wells A and F and between A-B and F-E are due to excess plasma in Wells B and E reacting with plasma antibodies in kidney and placental antiserum. Other studies demonstrated that the remaining bands were not due to red blood cell or remaining plasma antibodies. The bands in B-M, B-C and E-M are due to the reaction of tissue antigens with placental and kidney antiserum. Therefore, there is at least one antibody in placenta and kidney antiserum (arrow) that reacts with kidney antigen.

tem (aortic arch anomalies, situs inversus, atrial and ventricular spetal defects); *Genitourinary system* (renal agenesis and aplasia, gonadal agenesis and malposition); *Face* (cleft lip and palate, nasolacrimal cleft); *Limb and bone* (amelia, hypoplasia). There seemed to be no propensity for the antiserum to affect only one particular organ system, although the central nervous system was the most commonly malformed.

The absorption studies detailed in Table II reveal: 1. Large amounts of rat serum proteins (70 mg of serum protein/ml of teratogenic serum) do not protect against the teratogenic effects of either kidney or placental antisera (Table II, Exp. 1, 8). 2. Small amounts of kidney protein (5 and 10 mg/ml of teratogenic serum) protect against the teratogenic effects of both kidney and placental antisera (Table II, Exp. 2, 3, 4, 12, and 13). Furthermore, kidney antigen is more effective than placental antigen in protecting against the teratogenic effects of kidney and placental antiserum (Fig. 1). 3. Placental antigen can protect against the teratogenic effects of placental antisera but relatively larger amounts of placental antigen do not completely abolish the teratogenic effect of kidney antiserum (80 mg of placental antigen/ml of teratogenic serum) (Table II, Exp. 5, 6, 7, 9, 10, and 11).

The immunodiffusion studies indicate that

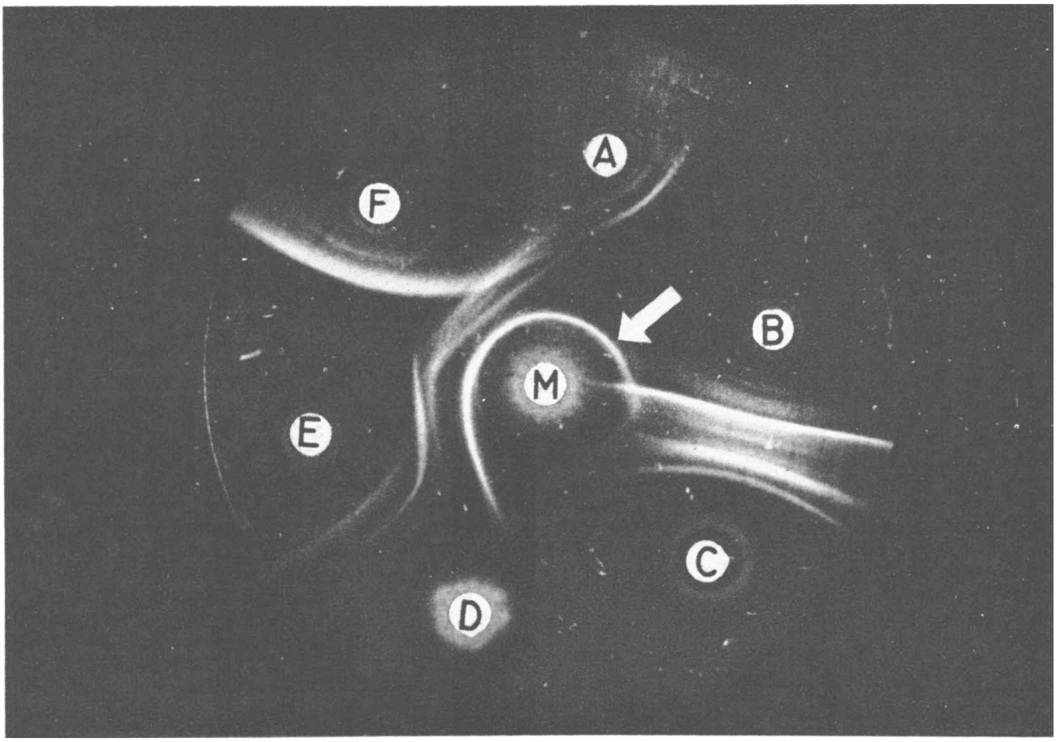

FIG. 3. Immunodiffusion Pattern-Well Contents: M-Rat placental antigen (200 mg/ml) Wells A, B, C, E and F have the same contents as in Fig. 2. Therefore, the only difference between the well contents in Fig. 2 and Fig. 3 is that well M contains placental antigen rather than kidney antigen. Absorbed (with rat serum) and unabsorbed kidney and placental antisera when reacting with placental antigen (E-M, F-M, A-M and B-M) have a precipitin band in common (arrow) which indicates that placental and kidney antiserum contain an antibody in common and placental and kidney tissue have an antigen in common. Other immunodiffusion patterns have demonstrated that the common antigen is not due to red blood cell contamination or unabsorbed plasma antigens. In this particular pattern, the common precipitin band is not continuous with any band in reaction B-C, but other plates have demonstrated that it is continuous with the second band closest to well C. Therefore, the last statement in the description to Fig. 2 can be extended as follows: there is a common antibody in placenta and kidney antisera which reacts with placental antigen.

some of the precipitin bands observed in the reaction between rabbit anti-rat kidney and placental sera and kidney and placental antigen are due to the reaction of rat serum antigens in the placental and kidney antigen preparations and rat serum antibodies in the placental and kidney antisera (Fig. 2). Absorption of these antisera with rat serum removes many of these bands but not the teratogenicity of these antisera (Table II, Exp. 1, 8; Fig. 2). Furthermore, when all the serum protein bands have been removed, there still is a common precipitin band between the reaction of kidney antigen and placental and kidney antiserum (Fig. 2, 3).

Discussion. The similarity between the pathologic effects of placental and kidney antiserum has long been known. Both antisera have been reported to produce a nephritis(5). The globulin fraction of each antiserum, when labeled with fluorescein, will stain the basement membrane and vascular structures of both kidney and placenta(6). Placental antiserum and kidney antiserum have been known as abortogenic agents. And finally, it is now apparent that both kidney and placental antisera are teratogenic agents (1,3,4).

Pressman and Korngold believe that the abortogenic and nephritogenic factors in placental antiserum are not the same(7). Steblay's *in vitro* studies have demonstrated that fluorescein labeled placental antiserum specifically stains the kidney and similarly labeled kidney antiserum specifically stains the placenta(6). Studies from our laboratory, utilizing *in vivo* fluorescein labeling techniques, have demonstrated that the specific fluorescence of teratogenic kidney and placental antiserum were quite similar in the kidney, adrenal, liver and spleen and that, furthermore, both antisera specifically localized in the yolk sac membrane of young rat embryos(8).

It is, therefore, evident from the literature and this paper that placenta and kidney have antigens in common and, furthermore, that antiserum prepared against either tissue cross reacts with the other tissue. This in itself is not proof that the identical immunoproteins from kidney and placental antiserum are involved in the teratogenesis, although there is no question that both kidney and placenta contain antigens which can neutralize the teratogenic effects of their respective antiserum. Furthermore, it is evident that dried lyophilized kidney contains a higher concentration of this neutralizing antigen (Table II). This can be indirectly deduced from the fact that less kidney and more placenta was necessary to neutralize the teratogenic effects of placental antisera (Table II) and that the same amount of kidney antigen produced a much more potent antiserum than did the injection of a similar amount of placental antigen. The fact that placental antigens were not able to neutralize completely the effect of teratogenic kidney antiserum might be due to the fact that the concentration of these antigens was much lower in the placenta than in the kidney or it might indicate that the spectrum of placental antigens which induce teratogenic antibodies are completely represented in the kidney but the spectrum of antigens in the kidney which induce teratogenic antibodies are not completely represented in the placenta. In either event there are one or more identical teratogenic antibodies in kidney and placental antiserum.

Summary. Rabbit anti-rat placental serum is a potent teratogenic agent when injected into pregnant rats. It produces fetal growth retardation, fetal death and malformations. In these respects, it cannot be differentiated from rabbit anti-rat-kidney antiserum. Kidney and placental antisera have precipitin bands in common and the tissue antigens of kidney can protect against the effects of either antiserum. Although this cannot be stated with absolute certainty, it seems likely that there are one or more identical antibodies responsible for teratogenesis in kidney and placental antisera.

I would like to thank Miss Myrma Spoerl, Miss Joan Garaguso and Mr. Albert Johnson for assistance with this project and Mrs. Elizabeth Ward for typing the manuscript. This research was supported by NIH Research Grants HD 630 and 1 T1 HD 141.

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Received September 2, 1966. P.S.E.B.M., 1967, v125.