ingested 12.75 mg of stilbestrol were alive at 20 weeks; of these, 3 have developed tumors, in 20, 24, and 26 weeks.

All 7 tumors were adenocarcinomas, of the same histologic type as the spontaneous tumors in the females of the strain and the tumors in the males following the subcutaneous injection of estrone or stilbestrol.[5]

11643

Effect of Anti-Placenta Serum on Development of Foetus in the Pregnant Rat.

BEATRICE CARRIER SEEGAL AND EMILY NICHOLS LOEB

From the Departments of Bacteriology and Medicine, College of Physicians and Surgeons, Columbia University, New York City.

This paper reports a series of experiments designed to determine the effect of rabbit anti-rat-placenta serum on pregnancy in the rat. The specificity of the results produced by the anti-placenta serum was controlled by the use of normal rabbit serum, rabbit anti-follutein and anti-pituitary serums, rabbit anti-rat-serum serum, and rabbit anti-rat-whole-blood serum. Another group of pregnant animals received no treatment. The experiments were carried out over a period of 3 years in 2 strains of rats.

Preparation of Antiserums. The method of preparing the rabbit anti-rat-placenta serum was as follows: Pregnant rats were sacrificed one day before term, the placentae were washed in saline, weighed, ground with sand and suspended in saline. The average yield of placental tissue from one rat was 4.5 g; this material was divided into 10 parts and injected intraperitoneally into each of 2 rabbits on 5 successive days. After a rest period of one week the animals were given a second, third and fourth course of immunizing injections and were bled out 10 days later. The serum was inactivated at 56°C for 30 minutes and stored without preservative.

Seven rabbit anti-rat-placenta serums were prepared; the last 3 serums were obtained by immunization with placentae from rats which had previously been perfused with 2 liters of saline, in the manner described by Smadel.[1] The results obtained with all 7 serums were identical.

Two rabbit anti-hormone serums were furnished by Dr. S. C.

[1] Smadel, J. E., *J. Exp. Med.*, 1936, **64**, 921.

Werner, one an anti-follutein serum made by immunizing rabbits with Squibb's follutein obtained from the urine of pregnant women, the other an anti-pituitary serum prepared by the injection of rabbits with a pyridine precipitate of the anterior pituitary gland of cattle. An additional rabbit anti-follutein serum was furnished by Dr. H. J. Gegerson.

The anti-rat-serum serum and the anti-rat-whole-blood serum were prepared by injecting rabbits for 5 consecutive days with 0.5 cc either of rat serum or of citrated rat blood. After a rest period of 10 days the injections were repeated for a total of 4 courses. The first course was given intravenously, the remaining 3 intraperitoneally.

Serological Tests. The precipitin content of the serums was determined by diluting the antiserums serially and testing each dilution with antigen diluted 1-20 and 1-100. The presence of lysins was tested for by adding 0.1 cc of increasing dilutions of antiserum to 0.1 cc of 5% rat red blood cells in 0.65 cc of 0.85% saline. After one-half hour at room temperature, 3 units of guinea pig complement in 0.15 cc volume were added. The presence or absence of lysis was noted after 30 minutes at 37°C.

Procedure. The antiserums were injected intravenously into 10-week-old pregnant rats, either of the Sherman strain or of the hooded strain, obtained from Dr. P. E. Smith. The injections were begun routinely on the 11th day of gestation. This was an arbitrarily chosen date since, in a series of 14 pregnant animals in which injections of anti-placenta serums were started variously from the third to the fourteenth day of pregnancy, no difference in results was observed. The injection was repeated on 1 or 2 successive days. In the majority of animals, red blood cell counts were made before injection, 2 days following the last injection and on the 20th day of gestation. The animals were sacrificed on the 20th day of gestation, *i. e.*, one day before the expected date of delivery. At autopsy in each instance the number of normal young as well as the number of placental sites, with or without attached degenerating foetuses and placentae, were noted. Sections of degenerating and of normal placentae, as well as sections of heart, liver, kidney, and uterus, were prepared for histological examination in a representative group of animals.

Results. The series comprises a total of 192 rats. It will be seen from the table that in 68 pregnant rats injected with rabbit-anti-rat-placenta serum, 66% of the developing foetuses were resorbed. Anemia in this group was rare and does not account for the resorption of foetuses observed. At autopsy the only abnormal findings were in

TABLE I.

Effect on Pregnancy in the Rat of the Intravenous Injection of Anti-rat-placenta Serum, Normal Rabbit Serum, Anti-hormone Serum, Anti-rat-serum Serum and Anti-rat-whole-blood Serum.

Type of Rabbit serum	No. of Animals	Avg Dosage, cc	Avg pre-injection RBC count, million per mm³	Avg of lower post-injection RBC count, million per mm³	No. of resorbed young	No. of normal young	% of resorbed young
Anti-rat placenta	68	1.4	7.7	6.4	413	212	66
Controls (uninjected)	27	—	7.0*	6.5*	30	202	13
Normal rabbit serum	15	2.0	8.1†	6.7†	12	121	9
Anti-hormone serums‡	17	2.2	—	—	10	149	6
Anti-rat-serum serum	24	0.9	8.4	5.9	57	170	25
Anti-rat-whole-blood serum	41	0.14	8.1	5.2	279	72	79

*Counts available on 11 of 27 animals. First count taken 11th day of gestation. Second count taken at term.

†Counts available on 5 of 15 animals.

‡Two anti-follutein serums; one anti-pituitary serum.

the placentae and foetuses. In those pregnancies which failed to proceed to term the foetus was either completely autolysed and unidentifiable or it was markedly degenerated so that neither grossly nor microscopically could individual organs be recognized. The placentae in such pregnancies were one-half or less the expected size, were brown rather than red and firmer than normal on section. The placentae which showed the more marked gross changes microscopically presented necrosis of chorionic villi, with hyaline thrombi in the sinusoids, and degeneration of syncytial cells with leukocytic infiltration. In placentae less profoundly affected the syncytial cells appeared similar to those in normal placentae at term but there was atrophy of the villi and hyaline deposits. The picture was similar to that which might be expected coincident with the detachment of the placenta.

In contradistinction to the above group, normal rabbit serum and the 3 anti-hormone serums resulted in no interference with pregnancy. The percentage of normally developing foetuses in these animals was actually somewhat better than that in the control uninjected group. The probable reason for this may lie in the fact that among our control group were 4 animals with a spontaneous anemia with red blood cell counts ranging from 5.6 to 6.0 million cells per cubic millimeter. In one of these 4 animals all of the 8 foetuses had been resorbed. In another only 2 of 7 foetuses had developed. The

remaining 2 anemic animals had normal young. No explanation for the anemia was found. Smears from the spleen were negative for *Bartonella muris*. A fifth animal of this group which had resorbed all of its 6 foetuses had not had a red blood cell count. Rats whose initial red blood cell counts were lower than 6 million per cubic millimeter were not used for injection of any of the antiserums.

Increasing doses of injected rabbit anti-rat-serum serum produced an anemia which may account for the interference with the normal development of the foetus found in some of the animals injected with this antiserum. On the whole, however, this serum, except in large doses, produced very little interference with pregnancy. Only 25% of the young were resorbed. This is of particular interest since it was found that the titer of precipitins for rat serum was approximately 30 times as great in the anti-serum serum as in the anti-placenta serum. In the former the antiserum diluted 1-30 gave a precipitate with rat serum, whereas the anti-placenta serum failed to precipitate rat serum at any dilution and gave only a trace when undiluted.

The rabbit anti-rat-whole-blood serum injected into 41 pregnant rats proved extremely damaging, inasmuch as it often produced a severe anemia and also resulted in resorption of the foetuses. Seventy-nine per cent of the young were resorbed. As little as 0.1 cc sometimes was capable of producing an anemia of 2.5 million red blood cells. This anemia alone, however, was not accountable for the interference with the development of the foetuses, since 12 animals with lowest counts of 6 million or better had resorbed all their foetuses. The precipitin titer of this serum was comparable to that of the anti-rat-serum serum. This serum lysed rat red blood cells at a dilution of 1-64, whereas anti-placenta serum lysed only at a dilution of 1-2 and no lysins were demonstrable in the anti-serum serum.

Discussion. The experiments have shown that degeneration of the placenta and foetus of the pregnant rat follows intravenous injection of rabbit anti-rat-placenta serum. This observation is in agreement with the early work on the rabbit and guinea pig by Dobrowalski,[2] and with the more recent findings of Cohen and Nedzel[3] in the guinea pig. The specificity of the action of the antiserum on the placenta is in doubt, however, since a rabbit anti-whole-blood serum is many times more effective in producing the same gross result. The part which antibodies against rat red blood cells may play in the reaction is as yet undetermined. Five absorptions of the rabbit anti-

[2] Dobrowalski, M. S., *Bull. Int. d. l'Acad. d. Sci. de Cracovie*, 1903, No. 5, 256.

[3] Cohen, H. R., and Nedzel, A. J., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 249.

rat-placenta serums with washed rat red blood cells have failed to influence the potency of the serum in producing resorption of the placentae. The anti-placenta serums have all been very weak in their content of antibodies for rat red blood cells even before absorption. Furthermore, anti-placenta serums obtained by immunizing rabbits with well perfused rat placentae were not any less capable of causing degeneration of rat placentae than were serums from animals immunized with placentae containing gross blood. Six absorptions of rabbit anti-rat-whole-blood serum with rat red blood cells reduced but did not eliminate their content of antibodies against rat red blood cells, yet the ability of this serum to cause degeneration of placentae and foetuses was apparently reduced by a disproportionately small degree. Furthermore, this anti-whole-blood serum may be given in such small amounts that the effect on the red cells, as determined by red blood cell counts, is absent, while its ability to cause degeneration of the placentae persists. This problem is under investigation.

The effect of anti-whole-blood serum on pregnancy in the rat appears to be independent of its content of precipitins for rat serum. Anti-rat-serum antibodies have not been injurious to pregnancy in the absence of a serious anemia.

The failure of anti-follutein and anti-pituitary serum to affect the course of pregnancy in the rat agrees with the findings of Zondek and Sulman,[4] who tested anti-gonadotropic serums derived from the goat and were unable to interrupt pregnancy in the rat by this method.

Conclusion. (1) Rabbit-anti-rat-placenta serum effectively interferes with the normal development of the placenta and foetus in the pregnant rat. (2) The same result is obtained with much smaller injections of rabbit anti-rat-whole-blood serum. (3) Rabbit anti-rat-serum serum fails to influence pregnancy unless injected in sufficient volume to induce anemia. (4) Anti-hormone serum and normal rabbit serum are without effect on pregnancy in the rat. (5) The immunological factor or factors responsible for the resorption of the foetuses following the injection of anti-placental serum and anti-whole-blood serum are as yet unknown.

[4] Zondek, B., Hochman, A., and Sulman, F., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 338.