Rat Virus (RV) Infection in Fetal and Pregnant Hamsters.* (28121)

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The fact that rat virus (RV) will proliferate to a greater extent in pregnant as compared to non-pregnant hamsters and has a predilection for placental, uterine and fetal tissues, has formed the basis of this report which is part of an investigation of *in utero* virus infections and their possible relationship to congenital malformations. Rat virus (1) was chosen for study for two reasons. First, it can produce a syndrome in suckling hamsters suggestive of human mongolism, consisting of stunted growth and shortened facies(2), and a similar type of peridontal disease(3,4). Secondly, as a possible relative of the Papova group of viruses(1), RV may have oncogenic properties as yet unrevealed. This communication is concerned largely with the relation of placental and uterine infection to fetal infection. This problem has been approached by means of intravenous inoculation of RV on known days of gestation in timed pregnancies and subsequent titrations of virus in various maternal and fetal tissues.

Materials and methods. Breeding of hamsters: Syrian hamsters purchased from the Lakeview Hamster Colony, Newfield, N. J., were bred in the early evening hours under direct observation, the males being left with the females overnight. Animals thus bred were placed in individual cages after mating and maintained on Purina lab chow for the duration of the experiment.

Tissue cultures. Rat embryo tissue cultures were prepared from embryos approximately 15 days of age. These cultures were re-trypsinized prior to use and maintained on medium 199 containing penicillin and streptomycin as described elsewhere(1).

Virus. Stock preparations of RV, although propagated in rat embryo tissue cultures, represented a strain which has been carried through 41-42 passages in suckling hamsters. The hemagglutination titers of the preparations ranged from 1:64 to 1:256.

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Intravenous inoculations. Intraperitoneal Nembutal anesthesia (6.5 mg/100 g) was given to pregnant and non-pregnant control hamsters prior to making a 1 cm incision over the femoral vein. 1.5 ml of tissue culture virus was then injected directly into the vein and the wound was closed with cotton sutures.

Intraembryonic inoculations. Certain litters were chosen for direct embryonic injection of RV on either the 12th or 13th day of gestation. Under Nembutal anesthesia the gravid uteri were delivered through midline abdominal incisions onto saline gauze pads, and up to 0.1 ml of virus was injected into each fetus by means of a #30 gauge hypodermic needle.

Harvesting of tissues. Hamsters were anesthetized and cardiac blood withdrawn into a heparinized syringe on day of sacrifice. Deaths resulted from exsanguination. The abdomens of the freshly killed hamsters were then opened under sterile conditions and the various tissues harvested and placed into sterile vials. All specimens were stored at −40°C. It was possible to separate yolk sac from chorio-allantoic placentas in some of the animals. In all cases, fetuses belonging to the placentas removed were carried through 2 washes in sterile saline to remove traces of amniotic fluid and maternal blood which might have been present.

Tissues from non-pregnant control hamsters were taken by comparable methods and portions of tissues harvested, as well as other organs, were placed into Bouin's fixative prior to histological examination. Embryos were either placed in alcohol-formalin-acetic acid fixative for gross examination or fixed in 95% alcohol. The alcohol-fixed embryos, 2 from each litter, were then cleared and the skeletons stained with Alizarin red according to the Dawson method(5).

Titration. Tissues tested were made up as 10% suspensions, using medium 199, to which 10% calf serum, penicillin and strepto-

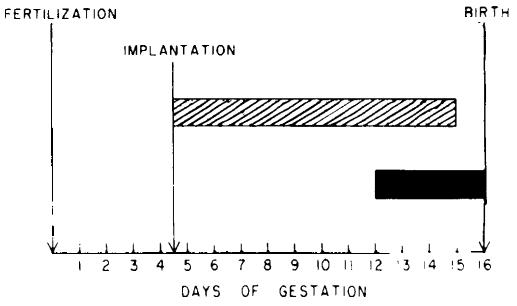

FIG. 1. Schematic representation of events in gestation period of hamsters in relation to inoculations with rat virus. Durations of experiments involving intravenous injections are denoted by cross-hatch line; those involving intraembryonic inoculation by solid line.

mycin had been added. These preparations were titrated for the presence of hemagglutinins by means of guinea pig erythrocytes and for infectivity by intracerebral inoculation of suckling hamsters, 24 hours or less in age, using death as an end point. The inoculated animals were observed for 2 weeks. Methods employed for the 2 types of titration, hemagglutinin and infectivity, have been described (6). Titrations for infectivity in rat embryo tissue cultures gave results comparable to these in hamsters, when the cultures were maintained for 2 weeks and then tested for presence of hemagglutinins.

Results. Rise and decline of RV-infection in pregnant hamsters. Fig. 1 gives the main events in pregnancy in hamsters in relation to times chosen for intravenous inoculations of RV. Preliminary experiments had revealed that placentas and embryos of the hamsters did not attain sufficient size for removal and titration of virus before the 9th to 10th days of gestation, hence these times were used as a rough baseline for the first studies presented in Table I. The results of titrations of tissues harvested 1 to 72 hours after inoculation are included in Group I. It is apparent that, in spite of recovery of RV in blood and placenta an hour after inoculation, the kidney was the only organ tested in which the virus persisted in recoverable amounts over the next 3 days. Hamsters of Group II were inoculated earlier and their tissues harvested 4 to 5 days later. The highest titers obtained in these animals were in tissues associated with the gravid uterus: placentas, fetuses and uterus itself. These titers could not be explained by the blood the tissues contained, for viremia was either absent or of low degree in the hamsters involved (see Table I). Groups III and IV give results for tissues harvested at longer intervals and at later stages of the gestation period. RV content fell progressively or disappeared entirely in blood, livers, and kidneys used as control tissues, but remained at comparatively high levels in placentas. There

TABLE I. Results of Titrations of RV in Tissues of Pregnant Hamsters Taken at Progressively Longer Time Intervals Following Intravenous Inoculations.

		Conditions of experiment			Tissues titrated					
					From gravid uterus			Others used as controls		
Group	Animal No.	Day of gest. Inoc.	Har.	Time, inoc. to har.	Uterus	CA-placenta-YS	Fetus	Mat'l blood	Liver	Kid.
I †	H 376	10	10	1 hr	0	1.0	0	1.0	ND	2.0
	H 378	9	10	24 "	0	0	0	0	ND	2.0
	H 374	8	10	48 "	2.0	0	0	0	ND	2.0
	H 377	8	11	72 "	0	0	0	0	ND	2.0
II	H 209	4	9	5 days	5.5	5.5	5.7	0	4.8	1.0
	H 212	5	10	5 "	7.0*	7.3*	4.2	2.1	5.5	4.5
	H 194	6	10	4 "	ND	6.3*	5.5	2.2	5.0	4.7
	H 195	6	10	4 "	ND	7.6*	4.2	1.0	3.8	4.5
III	H 210	4	11	7 "	5.5	4.6	1.0	0	3.8	1.8
	H 246	4	13	9 "	ND	3.4–4.5	ND	0	3.0	0
	H 241	5	12	7 "	3.5	4.3–3.7	0	0	2.8	0
IV	H 237	4	15	11 "	<1	1.6–1.3	0	0	0	0
	H 235	5	14	9 "	2.8	4.0–3.5	1	0	0	1.0
	H 242	6	14	8 "	1.5	4.5–2.7	0	0	1.3	0

* HA titers (see text).

† Titrations in Group I were performed in tissue culture.

TABLE II. Results of RV-Titrations in Non-Pregnant Female Hamsters Used as Controls.

Animal No.	Time, inoc. - har.	Tissues titrated			
		Uterus	Blood	Liver	Kidney
H 266	4	0	0	4.5	0
H 267	6	0	0	3.7	0
H 268	8	0	0	1.5	0
H 269	10	0	0	4.5	0

were no sharp differences in titers between the chorio-allantoic and yolk sac portions of placentas.

Titrations of RV in non-pregnant hamsters. Table II gives results of titrations in non-pregnant female hamsters 4 to 10 days after intravenous inoculations. RV in these animals was limited to their livers where it persisted during the entire period.

Hemagglutinins in infected tissues. In a previous study of suckling hamsters(6) it has been shown that specific hemagglutinins were demonstrable in all tissues which had RV infectivity titers of 10^{-5} or above. Hemagglutination (HA) tests were of less value in the present study due to the smaller amounts of virus encountered in most of the specimens. However, the 4 tissues with infectivity titers of 10^{-6} or greater had HA titers ranging from 1:64 to 1:1024 (Table I).

Development of antibodies. Pregnant hamsters were inoculated with RV on the 4th day of gestation and were bled on the 8th, 11th and 15th days in order to follow the development of antibodies in pregnancy. The HI antibodies had reached maximal titers of 1:320 to 1:640 within 7 days of the inoculation (Table III). Low titers of 1:20 in the normal sera were regarded as non-specific.

Effect of virus on maternal and fetal tissues. Intravenous injection of RV failed to produce any effect, which was apparent by gross or microscopic examination, on the pregnant or non-pregnant control hamsters. The fetuses appeared to be normal in size and numbers for each day of gestation injected. Examination of H & E stained paraffin sections of placental and fetal tissues failed to reveal pathological changes. No abnormalities of skeletal development were noted in the Alizarin-stained fetuses. Those fetuses injected directly *in utero* on the 12th or 13th day of gestation continued to grow until the time of parturition (day 16). Then they were either stillborn or died within a few hours after birth. RV was recovered from random fetuses of this group at birth.

TABLE III. Result of HI-Tests on Sera of Pregnant Hamsters, Showing Rise of Antibody in Course of Gestation Following an Intravenous Inoculation of RV.

	Day bled		Individual HI-titers		
	Of gestat.	Post-inoc.	H #1	H #2	H #3
Pregnant	8	4	1:40	1:40	1:40
"	11	7	1:640	1:320	1:320
"	15	11	1:640	1:320	1:320
3 non-pregnant uninoculated control hamsters			<1:20	<1:20	<1:20

Discussion. Present investigations are part of a continuing effort, first to find viral agents which have a special affinity for tissues associated with the gravid uterus of laboratory animals, and second, to demonstrate whether such viruses have any particular effects on the embryo or mother. A systematic approach to this problem has appeared to be justified in several respects. Thus, information on the course of certain viral infections in pregnancy may give insight into such problems as the vertical transmission of virus from mother to fetus, viral induction of genetic change, and induction of congenital malformations.

This latter problem presents well-known difficulties. A teratogenic virus must produce only a minimal type of injury if the embryo of early pregnancy is to survive to the stage of birth and beyond. It must also be able to penetrate the placenta without being over virulent for tissues related to conception. Rat virus has some attributes favorable for such investigation. It will proliferate, as shown above, in uteri and placentas, and it will infect embryos in early stages of gestation, the titer of virus in infected tissues declining at the same time as a rise of circulating antibodies takes place. Thus factors other than simple propagation within the embryo must be considered as being responsible for the effects of teratogenic viruses.

Pregnant hamsters are more susceptible than non-pregnant to infection with RV, and this virus appears to grow preferentially in

the gravid uterus. This suggests that pregnancy in hamsters is a host factor which increases their susceptibility to infection with rat virus. The rapidly differentiating tissues of the placenta and the embryo as well as the striking metamorphic changes in the endometrial stroma during gestation might provide the proper milieu for viral propagation. RV produces no recognizable histological disease in either pregnant or fetal hamsters. A similar absence of histological change has been described in lung infections induced in newborn mice with polyoma virus(7). The main pathogenic effects of RV appear to be in young suckling hamsters in which it proliferates to high titers(6), inducing inclusion bodies for only a limited period at peak of the infection, a situation similar to that described in the Mastomys rat(8). Inclusion bodies were not observed in the infected adult hamsters.

Whether RV can induce tumors or has potentialities for inducing mongolism *in utero* remains unknown. It is becoming apparent from our continuing studies that RV actually represents a spectrum of strains, all interrelated but having differing properties, as has been recently discussed by Moore(9). The strain used in the present studies was highly hamster-adapted. It is conceivable that effects other than those obtained might have resulted from use of the strain in a still more hamster adapted form, or, at the opposite extreme, as the form originally isolated from rats. Both of these possibilities are being investigated.

Conclusions. 1. RV injected into pregnant hamsters during early stages of gestation has a predilection for tissues associated with the gravid uterus and embryo, reaching significantly higher titers in 4 days as compared to control tissues, then decreasing as maternal antibody levels begin to rise. 2. Direct intraembryonic injection of RV into fetal hamsters on the 12th or 13th day of gestation produces fetal death at, or soon after, parturition. 3. Following intravenous injection, RV causes no apparent ill-effect on either the maternal or embryonic tissues.

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ACTH Content of Dog Kidneys.* (28122)

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Richards and Sayers(1) have reported that in rats relatively large amounts of injected ACTH accumulate in the kidneys, and others have confirmed this observation(2,3). Since little if any of the ACTH appears in the urine, it has been suggested that ACTH might be returned to the blood stream from this extrapituitary storage site. In Richards and Sayers' study, there were small but detectable amounts of ACTH in the kidneys of stressed normal rats that had not been injected with exogenous ACTH(1). The present studies were undertaken to quantitate the amount of endogenous ACTH in the kidneys of surgically stressed normal dogs, and to determine whether ACTH was released from the kidney into renal venous blood following laparotomy and hemorrhage.

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