rabbit cells as homologous γ-globulin are therefore largely located on a segment comprising about 40 percent of the parent molecule. It will be necessary to obtain much smaller active fragments of FIII in order to establish the relationships of the chemical groupings responsible for these properties.

Summary. Most of the reactivity of rabbit γ-globulin with a pool of sera from rheumatoid arthritics was found to be associated with Fraction III of the papain-digested globulin molecule. Fraction II showed a low degree of reactivity while Fraction I appeared to be completely inactive.

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Rat Virus (RV) Infections in Hamsters. (26489)

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Rat virus (RV) was first recognized in rat embryo tissue cultures inoculated with materials from tumor-bearing rats. In such cultures the production of specific cytopathogenic effects (CPE) and of hemagglutinins indicated activity of the virus(1). Preliminary tests in animals failed to reveal pathogenic properties of RV. The present report describes (a) a fatal disease induced by RV in hamsters of one to 4 days of age, (b) occurrence of virus and viral hemagglutinins in the tissues and fluids of these animals and (c) spread of virus to uninoculated hamsters. Sucklings which happen to survive the acute disease may develop a stunted growth resembling mongolism. This dwarfism has been reported previously(2).

Materials and methods. Modifications and extensions of procedures not previously described(1) are given below.

Virus. The strain of virus used throughout current work was RV-13, which was carried through approximately 12 rat embryo tissue cultures and 4 passages in suckling hamsters prior to a final passage in rat embryo tissue culture. This tissue culture seed virus lot which was stored at –40°C, had an HA titer of 1:512 and an LD_{50} of 10^{-5} as measured by deaths induced in 1-day old suckling hamsters within a period of 10 days. As described below, a 10^{-6} dilution of the seed induced dwarfism but no acute fatal disease.

Inoculation and harvesting of hamsters. Syrian hamsters from the Animal Production Center of the NIH were inoculated as sucklings by the intracerebral route. Uninoculated control animals were retained in each litter tested; controls were identified by clipping a portion of their tails. Animals harvested at various stages of infection were anaesthetized, decapitated and exsanguinated into a dish moistened with heparin. Bladders were then clamped and incised in order

TABLE I. Effects of Rat Virus (RV) in Suckling Hamsters of Different Ages.

		Age of hamsters at time of inoculation*			
		<24 hr	4 days	5 days	6 days
Conditions induced by RV	Acute fatal disease	56/56	35/47	None	None
	Mongolism	None	12/47	"	"
	Latent infection	"	None	2/2	2/2
	Tissues†	Kidney	Kidney	Liv. & kid.	Liv. & kid.
Titrations	HA	81,920	40,960	1280	0
	Infectivity	7.3‡	7.3	7.0	4.8

* Hamsters inoculated intracer. with 10^4 LD_{50} of RV from 4th hamster passage.
† Tissue pools were from 2 to 3 hamsters, killed 5 days after inoculation.
‡ Titers expressed as inverse log of dilution killing 50% of hamsters <24 hr old.

to collect urine. A usual procedure was to freeze all specimens immediately after harvesting and to store them at −40°C until testing was undertaken. All specimens for determination of presence of virus were finally made into 1:10 suspensions with medium 199 containing 10% calf serum, plus penicillin and streptomycin, and clarified by repeated centrifugations at 2500 rpm.

Infectivity titrations. Titrations of tissue suspensions and other materials for infective virus were performed by intracerebral inoculation of hamsters less than 24 hours of age with serial, 10-fold dilutions. The animals wree then observed over a period of 10 days and 50% end-points, based on deaths and survivals, were calculated by the method of Reed and Muench(3).

Hemagglutination (HA). In these tests, the specimens of tissue and body fluids, clarified by centrifugation as described above, were carried through serial 2-fold dilutions in buffered saline (pH 7.4) in volumes of 0.4 ml. An equal volume of a 0.5% suspension of washed guinea pig erythroctyes was added and the cells were allowed to settle for one hour at room temperature. Titers were then read from patterns formed by the erythrocytes at the bottoms of the tubes. Results of these HA titrations were expressed in terms of actual dilutions of the virus suspensions prior to addition of red cells.

Serologic tests were of 2 types. In hemagglutination-inhibition (HI) tests, serial 2-fold dilutions of serum were made in buffered saline, in volumes of 0.2 ml and 8 to 16 HA units of RV were added in equal volumes. After this mixture had incubated for ½ hour at 36°C, 0.4 ml of 0.5% of guinea pig erythrocytes were pipetted into each tube. Results were read by the pattern method after one hour at room temperature. As in the HA tests, final results were read in terms of actual dilutions of the material tested prior to addition of other constituents, *i.e.* of virus and RBC. Neutralization tests (NT) were performed with equal volume mixtures of undiluted serum and of virus suspension containing 10 to 100 LD_{50}'s of RV; such mixtures, after ½ hour of incubation at 36°C, were inoculated intracerebrally into hamsters less than 24 hours of age. All sera used, in both types of serologic tests, HI and NT, were inactivated by heating at 56°C for ½ hour.

Results. Injection and disease. RV can produce acute fatal disease, a stunted form of growth resembling mongolism, or a latent infection, depending on dosage and age of the hamsters at time of intracerebral inoculation. These effects are outlined in Table I. The acute disease, induced in sucklings of from less than one to 4 days of age by 10^4LD_{50} of RV was manifested on the 4th to the 8th day by a slight anal exudate, a dark red color of the intestinal tract visible through the skin of the abdomen, and generalized weakness. Sucklings with these signs usually died within hours, with stomachs full of milk. Some of the hamsters in the group inoculated at 4 days of age had a severe anemia when moribund 7 to 8 days after inoculation. Thus only a small amount of thin, watery blood could be obtained when these animals were decapitated under anaesthesia.

A stunted form of growth associated with

TABLE II. Results of HA and Infectivity Tests for Presence of Virus in Tissues of Suckling Hamsters Inoculated with RV When 4 Days of Age.

Day after inoc.*	Titers of tissue suspensions†												
	HA						Infectivity for hamsters IC						
	Liv.	Kid.	Gut	Brain	Blood	Urine	Liv.	Kid.	Gut	Brain	Blood	Urine	Comment
2	None‡	None			None	None	<1 §				4.2	None	Well
4	20480	20480		80	"	"	6	6		3.6	5.7	3.6	"
5	40960	40960		640	"	"	7	7.3			5.8	3.8	Some mortality
6	20480	10240		640	"	80	7.6	7.6		4.8	5.7	5.6	Max mortality
7	2560	1280	2560				5.5	5.1	5.6				
8	None	None	None				3.6	5	5.5				Moribund

* Hamsters inoculated IC with 10^4 LD_{50} of RV.

† Tissues from 2 to 3 hamsters pooled for each determination.

‡ None: For HA test, means no hemagglutination at a starting dilution of 1:80. For infectivity test, means no virus isolated from a 1:10 suspension.

§ Titers expressed as inverse log of dilution killing 50% of hamsters <24 hr old.

defective incisor teeth was a second type of disease encountered among the sucklings inoculated as 4 day-olds. This form of disease has been described in newborn hamsters inoculated with sublethal amounts of RV(2).

Latent infection, a third condition induced by RV, was encountered in hamsters receiving RV at 5 or 6 days of age. Hemagglutinins were demonstrable in tissues from sucklings inoculated when 5 days old, but not in those inoculated at 6 days of age (Table I). Infective virus was recovered from hamsters of both age groups but the amount detectable in the older animals was less.

RV has been carried through 20 continuous passages in suckling hamsters. Tests similar to those done with 4th passage hamster virus (Table I) were undertaken with 10th passage hamster virus; the results were comparable. Most of these passages were made by means of 10% suspensions of pooled livers and kidneys, given by the intracerebral route. Intraperitoneal, subcutaneous and intranasal inoculations have also proved to be effective in transmission. In the later part of the work the inocula were held at 65°C for 15 minutes on an assumption that this heating might eliminate adventitious agents, if such were present. Such heating has had no apparent effect on the infectivity of RV.

Hemagglutinins and infective virus. The pathogenesis of RV-infections in suckling hamsters can be followed to some extent by testing various tissues and body fluids for amounts of RV present on successive days after inoculation. Table II gives results of 2 types of viral titrations on materials from sucklings inoculated as 4-day-olds and sacrificed 2 to 8 days later.

It is apparent from the tabular data that viremia was detected as early as the 2nd day by means of infectivity titration but circulating hemagglutinins were not demonstrated even on the 5th day when intensity of the viremia was at its peak and when hemagglutinins reached their maximum titer in the liver and kidney. Virus as determined by both procedures was present in largest amounts in liver and kidney but occurred also in brain, gut and urine. There was only a rough correlation between the results obtained by the 2 titration procedures. When hemagglutinins were demonstrable in a specimen the infective titer of that material was usually in excess of 10^{-5}. However, not all specimens with such amounts of infective virus gave positive results in the hemagglutin tests, *viz.*, blood. Neither hemagglutinins nor infective virus were demonstrated in tissues, urine or blood of 2 groups of normal unexposed hamsters sacrificed when 5 and 10 days of age respectively.

Spontaneous transmission of RV. Data presented in Table II suggested that RV was shed into the environment from both urinary and intestinal tracts and raised the question

TABLE III. Spontaneous Transmission of RV among Hamsters.

Exposure to RV infected litter	Uninoculated hamsters: Age group	No. animals in group	Duration of exposure	Disease	HI-antibodies: No. sera tested	No. positive	Range of titers
Direct (in same jar)	Newborn	9	6-9 days	Fatal 6-9 days	None	—	—
	4 days old	12	3 wk	None	6	6	320-1280
	Mothers (whose y'ng were inoc.)	8	3 "	"	8	8	160-1280
Long, indirect (same room); transient, direct (ate inoc. young)	Mothers†	5	1-2 days	"	5	None*	
	" †	4	3 wk	"	4	"	

* None: No hemagglutination at starting dilution of 1:80.
† These mothers lost their young, through cannabalism, within a few days of birth.

as to the extent to which spontaneous transmission might take place. Table III shows that such transmission occurs. In one experiment involving litters of newborn hamsters, 9 of the 9 uninoculated controls developed the same type of fatal illness as their inoculated littermates. However, the former died within 6 to 10 days while the latter succumbed in 4 to 5 days. Pooled livers and kidney tissues from several of the spontaneously infected hamsters had HA titers of 1:10,240. In contrast, uninoculated animals among the 6 litters which were injected when 4 days of age, remained well even though they became infected as evidenced by the development of HI antibodies (Table III). Table III also shows that RV spread, spontaneously, to the mothers of infected litters. Although these adults displayed no signs of infection, they had circulating HI antibodies when tested 3 weeks after inoculation of their young. Postpartum hamsters, nevertheless, do not readily acquire infection from brief, direct exposure to animals having RV infections. Thus 4 mothers which ate their babies within a day after inoculations with RV and subsequently lived in the same room with infected families for 3 weeks, failed to develop RV antibodies (Table III). The 5 mothers mentioned in Table III which had no RV antibodies at the time their young died several days after inoculation merely add to the experience, throughout these studies, that adult hamsters do not ordinarily possess RV antibodies.

Some attributes of hemagglutinins. The hemagglutinins recovered from infected hamster tissues appeared to be specific for rat virus. Thus they had the same general characteristics of hemagglutinins produced by RV in rat embryo tissue culture *i.e.*, they agglutinated guinea pig erythrocytes at room temperature without elution. Secondly, guinea pig antisera prepared against RV inhibited the hemagglutinins from infected hamster tissue in high titer. An illustrative experiment is summarized in Table IV. Sera from guinea pigs prior to immunization inhibited HA in low titer. This apparently non-specific inhibition of RV hemagglutination at low titers has been demonstrated in normal sera from a number of species. Its exact nature is not clear.

Discussion. RV is a virus which has caused no apparent disease on inoculation into rats, its natural host, or into mice which are the host of polyoma virus, a seemingly related agent(1,2). Hamsters are susceptible to both viruses, however, and the diseases induced offer points of contrast. One difference is the failure of RV to induce neoplasms. It is conceivable that RV is too lethal in its effects on hamster tissue to achieve that "delicate balance—between virulence of virus and resistance of host," as phrased by Stewart(4) which is possibly essential if a virus is to induce cancer. Another contrast between RV and polyoma is found in their patterns of multiplication in suckling hamsters. Rowe and associates(5)

TABLE IV. Specificity of Hemagglutinins Found in Tissue of RV-infected Hamster.

Guinea pig serum	HA units of RV-infected liver susp.	Dilutions of guinea pig serum 20	40	80	160	320	640	1280	2560	5120
Pre-inoc.	16	0	+	+	+	+	+	ND	ND	ND
	8	0	0	+	+	+	+	"	"	"
	4	0	0	0	+	+	+	"	"	"
3 wk post-inoc.*	16	0	0	0	0	0	0	+	+	+
	8	0	0	0	0	0	0	0	+	+
	4	0	0	0	0	0	0	0	0	+

* Guinea pig immunized with RV-infected rat embryo tissue culture fluid.

have shown that polyoma virus reaches peak titers in 4 to 6 days after inoculation, but titers of infective virus are low, hemagglutinins fail to appear and there is no immediate disease or subsequent dwarfism. Neoplasms are the characteristic signs of polyoma infection in infected hamsters. The multiplication of polyoma virus in suckling mice (4) follows patterns which are closer to those described above for rat virus in suckling hamsters. Thus Rowe *et al.*, encountered infective virus in titers of 10^{-6} and 10^{-7} in kidneys and livers of infected animals and these were associated with presence of hemagglutinins. Polyoma virus was also demonstrable in both blood and urine. The fact that this agent can induce dwarfism associated with defective incisor teeth in mice is an additional point of similarity with RV (2).

Summary. 1) Rat virus (RV) can induce 3 different conditions in suckling hamsters according to dose of virus and age of the animal at time of inoculation. These conditions are (a) a fatal disease in sucklings 1 to 4 days of age; (b) a stunted growth in those surviving a minimal dose, and (c) a latent infection of older sucklings. 2) Spontaneous transmission of RV can take place from inoculated to uninoculated hamsters. 3) Infected sucklings have infective virus in high titer in livers, kidneys and other organs as well as in lesser amounts in blood and urine. Specimens with the highest infectivity titers have also had considerable amounts of hemagglutination activity. 4) Rat virus has been carried through 20 passages in suckling hamsters. Intracerebral, intranasal, subcutaneous and intraperitoneal routes of inoculation have all been effective in inducing acute disease.

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Studies of Placental Morphogenesis I. Radioautographic Studies of Human Placenta Utilizing Tritiated Thymidine.* (26490)

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The manner in which the syncytiotrophoblast takes origin and is maintained has occupied the attention of many investigators interested in the placenta. This paper presents preliminary data from investigations of this problem.

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Method. Fresh sterile human placental tissue was obtained from 4 therapeutic abortions ranging in gestational age from 14 to