(y on x) relating results by the Henry-Chiamori (y) to the present method (x) was 202 (Fig. 4). The theoretical value for this slope, assuming that only maltose is measured in both methods, is 490 (approximate glucose equivalent for maltose in the Folin-Wu method taken as 0.4 and making a correction of + 13.5% for the difference in temperatures used in the 2 methods). Since it has been definitely established(1) that products other than maltose are measured in the Henry-Chiamori method the slope would be greater than 490 if only maltose was being measured in the present method. The finding of a slope of 202, therefore, indicates that (1) substance or substances other than maltose formed in the enzymatic hydrolysis are being measured by the ethylamine reaction, (2) the ratio of the absorptivity of color produced with ethylamine to the respective reducing capacity of this substance or substances is higher than the corresponding ratio for maltose.

Summary. A modification of Somogyi's saccharogenic method for serum amylase is presented in which maltose and other products formed in the enzymatic hydrolysis of starch are determined photometrically by reaction with ethylamine. Various aspects of this method were studied and results compared with the Henry-Chiamori modification of Somogyi's method.

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Rat Virus (RV) Infections of Pregnant, Fetal and Newborn Rats.* (29723)

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The present study concerns the pathogenesis of rat virus (RV) infections in rats. It is also a part of a continuing one on viral infections of pregnant, fetal and newborn animals, using rat(1) and H-1(2) viruses to explore circumstances under which congenital malformations and vertical transmission of viruses and possibly neoplastic transformations may take place. RV has attributes which suggest usefulness as a probe in these investigations. In addition to its unusually small size of 13 mμ(3), it has an ability to cross the placental barrier in hamsters(4) as well as to induce malformations in tissues and organs which continue their development after birth. Such tissues include the teeth, where RV may produce markedly different effects, depending on the stage of ontogenesis of the molars at time of inoculation(5); second, a generalized effect on skeletal development as shown in mongoloid dwarfism(6), and third, a cerebellar hypoplasia and ataxia resulting from destruction of the granular cell layer, following inoculation at birth(7).

The related H-1 virus is in the same size range as RV(3). It is a natural, latent virus of rats with many attributes similar to those of RV as well as a marked capacity for induction of congenital malformations in hamsters(8). It is planned to compare the pathogenesis of these two closely related parasites of rats in continuing studies.

Materials and methods. Virus. Of the 2 strains of rat virus employed, the 171 strain

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TABLE I. Results of Titrations of RV in Tissues of Non-Immune Pregnant and Non-Pregnant Rats Inoculated Intravenously.

Stage of gestation	Animal	Day of gestation Inoc	Har	Time inoc to har (days)	Uterus	Placenta	Fetus	Liver, spleen, kidney	Lung	Thymus	Gut	Feces
Early	A	9	12	3	2*	0	0	1	1	1	1	1
Mid	B	12	16	4	0	0	0	1.5	1.5	1	1.5	0
Late	C	14	18	4	2	0	0	2	2	2	—	1.5
	D	15	20	5	4	3	0	3	4	4	—	—
Non-preg	E	—	—	4	0	—	—	1	0	—	—	—
	F	—	—	4	—	—	—	0	—	—	0	—
	G	—	—	5	—	—	—	0	0	—	0	—
	H	—	—	6	0	—	—	0	—	—	—	—

* Infectivity titers (see text).

was isolated from preparations of the Moloney leukemia virus made from tissues of infected rats(9) and the RV 12 strain from a rat bearing *Cysticerus* sarcomas(1).

Animals. The Sprague-Dawley rats employed originated from Caesarean-derived stock and were random bred. They were obtained as pregnant females from Charles River Breeding Laboratories, North Wilmington, Mass., except for a few additional animals purchased from Carworth Inc., New York.

Titrations. Tissues harvested from infected rats were made up as 10% suspensions in medium 199, to which 2% agamma calf serum, penicillin and streptomycin had been added. Methods used for titrations and serologic procedures have been described(10). In making titrations of RV, suspensions of tissues from infected rats were diluted and the dilutions inoculated into rat embryo tissue cultures (RETC). The highest dilutions of such suspensions which were able to cause production of hemagglutinins at the end of 2 weeks were considered end points of the titrations. Titers presented in Table I are the inverse logs of such dilution end points.

Serology. All rats were bled on arrival at the laboratory and their sera tested both for presence or absence of inhibition of hemagglutination (HI) antibodies against rat virus.

Inoculation of pregnant rats. The day following breeding was regarded as the first day of gestation in these animals, with implantation occurring on the 6th(11) and parturition on the 21st or 22nd day. Pregnant rats were anesthetized for inoculation with 10% chloral hydrate, using 10 mg/30 g of body weight. In intravenous inoculations, the animals were given 1.5 ml of T.C. virus into the lingual vein(12) and in intraembryonic inoculations, 0.1 ml of virus suspension, directly into the fetus through the uterine wall. This was done following a small, midline abdominal incision which exposed the fetuses *in utero*. The maternal abdomen was then closed with silk sutures.

Harvesting of tissues. On the day of sacrifice rats were anesthetized with ether and cardiac blood withdrawn into a heparinized syringe. Representative samples of maternal tissues were then collected under sterile conditions, the fetuses being removed from the uterus and washed in 2 rinses of sterile saline to remove any traces of maternal blood or amniotic fluid. The uterus was next separated from the combined chorioallantoic and yolk sac placentas. All specimens, when finally ground and made up as 10% suspensions in regular T.C. fluid, were stored at —40°C.

Results. I. Intravenous inoculations of pregnant rats. Table I shows recoveries of rat virus which were made following intravenous inoculations of female rats which had no demonstrable RV-antibodies. Pregnancy appeared to increase susceptibility to this agent. Thus RV was recovered from all of the gravid animals inoculated, with highest titers in those inoculated late in pregnancy (Rat D, Table I). On the other hand, rat virus was recovered from only 1 of 4 non-pregnant rats inoculated in a similar manner and then only from a single tissue, the spleen. An important finding was that RV failed to reach embryos, or at least to proliferate in them, since

there was no detectable virus in fetuses from any of the pregnant rats. This was in spite of the fact that in Rat D there were substantial amounts of RV in specimens of both uterus and placentas. None of the above rats developed illness as a result of the intravenous inoculations.

II. *Intraembryonic inoculations.* When fetuses were inoculated directly *in utero* 3 to 5 days prior to birth, the health of the mothers was unaffected and about 50% of the young were born in good condition. Table II shows that titers of RV were relatively high in fetuses taken at time of birth and that those in the accompanying placentas and uteri were higher than in visceral organs of the mothers. No virus was recoverable from the tissues of a mother having HI-antibodies in a titer of 1:80 at time of intraembryonic inoculation.

Several aspects of results obtained when suckling rats were sacrificed 4 to 11 days after birth, were unexpected (Table II). One was that sucklings inoculated intraembryonically 5 to 6 days prior to birth developed rat virus infections of equal degree, regardless of whether their mother was free of RV-antibodies (Rat 2D, Table II) or had antibody titers of 1:40 to 1:160 at time of intraembryonic inoculations (Rats 2E and 2F). A second finding was that RV was recoverable from lungs of the sucklings in relatively high titers, for as long as 11 days after birth. Lungs behaved differently than other tissues in tissue culture, apparently due to the presence of an inhibitor. In some specimens, for example, where no virus was recoverable in the lowest dilutions of 10^{-1} and 10^{-2} it was recoverable at dilutions of 10^{-3} or above.

III. *Inoculations of newborn rats.* Suggestion of ways in which RV might be transmitted in nature was given by the findings in lungs, gut, and urine shown in Table III. Thus RV was recoverable from lungs in titers of 10^{-5} and 10^{-6}, 8 to 19 days after inoculations made at birth and results were comparable whether inoculations were by parenteral or intranasal routes. Virus was recovered in the even higher titers of 10^{-8} from G.I. tracts from some of the rats and a titer of 10^{-5} was found in the urine of a single individual. The agent was apparently able to persist in such situations, even when circulating antibodies were present. The last rat in Family D, Table III, for example, had relatively high titers of virus in intestines, lungs and urine in spite of a serum HI-antibody titer of 1:320 when sacrificed 19 days after inoculation.

A finding of possible significance, in relation to leukemia, (see final discussion) was the relatively high titer of RV obtained from the thymus glands of those rats in which this organ was tested (B and C, Table III). Among other results presented is the fact that RV, when recoverable, was present in only minimal concentrations of 10^{-1} in samples of heparinized blood, and that rat virus apparently failed to persist in sucklings born of an RV-immune mother.

Inoculations of the above rats resulted in several types of disease, dependent somewhat on age. Thus sucklings which became moribund at 8 to 12 days of age were runts in size and had marked distention of the stomach and intestinal tracts. On the other hand, 2 (Family D, Table III) which did not become ill until over 40 days of age were dwarfs, being only half of the expected size for their age. One of them, ill at 43 days, had obviously defective teeth which were found by Dr. Paul Baer of Nat. Inst. of Dental Research (personal communication) to have resorptions of the roots of the molars, while the other, moribund at 46 days after inoculation, had a marked degree of hydrocephalus, such that only a thin layer of brain tissue remained. A number of inoculated rats, on the other hand, survived in apparent good health.

Discussion. An aim of present studies was to determine whether RV might be able to cross the placental barrier and thus have the potentiality of being transmitted "vertically" *in utero,* as was suggested by a finding of antibodies in germ-free rats(1). Another objective was to determine whether RV might induce congenital malformations which it has done, to a minor extent in hamsters(8). RV crosses the placenta readily in these latter animals(4). Present experiments, however, give no evidence that rat virus is able to cross the placental barrier in rats. That RV

TABLE II. Results of Titrations of RV in Tissues of Suckling Rats Which Had Been Inoculated *in utero* in Late Pregnancy and of Mother Rats Whose Fetuses Had Been Similarly Inoculated.

Category of rat	Animal No.	RV-antibody of mother (HI)	When harvested	Day of gestation: Inoc	Born	Tissues titrated: Uterus	Placenta	Fetus	Liver, spleen, kidney	Lung	Gut	Other	Condition young at birth
Mothers	2A	Neg	At delivery	19	22	4*	5	7	1.5	—	—	—	1 born dead 1 resorbed
	2B	"	" "	18	22	3	3	6	1.5	—	—	2†	5 born well 5 " dead
	2C	1:80	" "	18	22	0	0	0	0	0	—		2 born well 5 " dead
Young	2D	Neg	6 days of age	18	22	—	—	—	1.5	6	1.5	2‡	7 born well
	2E	1:160	4 " " "	17	22	—	—	—	1.5	6	3		4 " "
	2F	1:40	11 " " "	16	21	—	—	—	3	5	—	—	4 " "

* Infectivity titers (see text). † Feces. ‡ Thymus.

TABLE III. Results of Infectivity Titrations of RV Isolated from Rats Inoculated at Birth.

Family and strain of rat	Age at sacrifice (days)	Inoculations: Route	Strain of RV	HI antibody of young	Tissues titrated: Blood	Lung	Thymus	Liver	Spleen	Kidney	Gut	Excreta†	Condition at sacrifice
Family A	3	I.N. Sub.	171	(Mother neg)	—	4*	4	4	5	6	—	—	W
Strain SD	7	I.P.			1	4	4	4	3	4	6	—	W
	10				1	4	4	3	4	3	5	—	Ill
	17			1:80	0	1	—	1			—	—	W
	40			1:160	—	0	0	0			—	F–1	W
Family B	1	I.N. S.C.	171	(Mother neg)	—	4	—	4			5	—	W
Strain SD	12	I.P.			—	5	6	5			6	—	W ill
	19			1:320	0	5	—	4			5	U–5	Ill
Family C	8	I.P. S.C.	RV	(Mother neg)	—	6	8	3			8	—	Moribund
Strain WR-SD	43				—	—	—	0			—	F–0	Ill
	46			1:320	—	0	0	0			—	—	Moribund
Family D	1	I.N. S.C.	171	(Mother 1:80)	—	—	—	3			—	—	W
	12	I.P.			0	—	—	0			—	—	W
	47				—	—	—	0			—	—	W

* For infectivity titers—see text. † F, feces; U, urine.

can proliferate in fetal tissue, however, is suggested by its propagation in rat embryo tissue cultures as well as by its apparent ability to reach high titers in fetuses when inoculated directly. It might be objected that virus had simply been retained in the fetus under these circumstances without actual multiplication. While this possibility is difficult to rule out, it was noteworthy that fetuses which survived birth had relatively high titers of RV in some of their tissues as late as 16 days after inoculation, in spite of having developed or acquired circulating antibodies within this period. In a further set of experiments it was found that presence of circulating antibodies in the mother did not protect those young inoculated before birth from developing RV-infections, and even typical illnesses later on. One presumes that neonatal rats require an appreciable time to acquire protective antibody levels from ingestion of milk.

Neither intranuclear inclusions nor significant pathologic changes in any of the experimental animals examined were noted. Continuing studies of the histology of RV-infections, however, are in progress. It should be pointed out that when young wild rats were inoculated at birth with a combination of the Moloney leukemia virus and RV, one of them had numerous intranuclear inclusions when examined, in a moribund condition, 16 days later(9).

Some features of the pathogenesis of RV in rats parallel the behavior of polyoma virus in mice(13). This suggests that both agents may be transmitted in nature in a similar manner, through contamination of the local environment by heavy excretions of virus. Suckling animals may be particularly adapted to such transmission. RV, for example, was recovered in substantial amounts from gut, urine, and lungs of suckling rats inoculated at birth and, in the case of one rat, was shown to be persisting in these situations in relatively high titers 18 days later, in spite of the presence of circulating antibodies.

Further comparisons with RV are suggested by the studies of Cordray *et al*(14) on the behavior of polyoma in infections of mouse lung. Three points pertinent in this regard were the persistence of polyoma virus in this situation in titers well above those in kidney and spleen; second, absence of pathologic changes in the polyoma infected lungs; and third, the presence of a viral inhibitor demonstrable on grinding lung tissues. Rat lungs infected by RV have an inhibitor which appears to act in a similar manner.

The roles which viral proliferation in thymus glands may play in association with leukemia(15,9) suggest other possible parallels between polyoma and RV. Kodama and Moore (16) have speculated for polyoma virus proliferation in thymus may explain the special sensitivity of AKR mice to leukemia.

Many problems remain concerning the pathogenesis of RV infections in rats. One question is why this agent which proliferates readily in fetuses and sucklings was unable to do so in adult, non-pregnant females and why it has not been isolated from normal individuals. It is possible that RV may have peculiar properties arising from its small size of 13 mμ(3). Kassanis and Nixon(17), for example, found that Tobacco Necrosis Virus was actually a mixture of 2 agents of which the smallest, because it could not initiate an infection alone, was termed a satellite virus. It is not inconceivable that RV may require additional factors for proliferation in adult rats. Among these might be pregnancy, the presence of a neoplasm, or the activity of some larger virus, such as that of leukemia, which would render the local cellular environment favorable for a satellite virus.

An alternative concept suggested by the work of Payne *et al*(18) is that rat virus may appear under conditions which favor the development of neoplasms, as they discovered in their efforts to produce mammary tumors in rats with irradiation and methylcholanthrene.

Whatever mechanisms may be involved, spontaneous neoplasms do not occur in isolated systems and RV should be worthy of continued study as an agent which may be directly or indirectly concerned with carcinogenesis in rats.

Summary. RV was found to proliferate in pregnant but not in non-pregnant rats fol-

lowing intravenous inoculation early in pregnancy. There was no indication that this virus is able to cross the placenta. It was, however, able to proliferate there and also in fetuses, if these were inoculated directly, late in gestation. Directly inoculated fetuses surviving birth developed illness characteristic of RV-infections in the suckling period. Rats inoculated at birth might develop an acute disease characterized by a distended intestinal tract and die within 8 to 12 days or survive 40 or more days, dwarfs in size and with deformed teeth. One individual developed an extensive hydrocephalus. Additional findings were the recovery of RV in relatively high titers from lung, intestine, and urine and the fact that sucklings inoculated directly *in utero* might develop RV-infection or disease, even though the mother was RV-immune.

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Threshold for Pressor and Renal Ischemic Effects of Angiotensin.* (29724)

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The threshold dose for slowly infused angiotensin in man is in the neighborhood of 2-5 mμg/kg/minute(1,2,3). The renal ischemic effect has a threshold perhaps somewhat lower than this: in the study of Bock, Dengler, Krecke and Reichel(1), an infusion rate of 2 mμg/kg/min, the lowest administered, still depressed the PAH clearance to 70% of normal. In their study, an indirect method of measuring renal blood flow was employed, that of PAH clearance. Because with reduced renal blood flows, the indirect methods sometimes give erroneous results, it was thought desirable to ascertain the threshold dose for the renal ischemic effect when measuring renal blood flows directly. At the same time the threshold dose for the pressor effect was ascertained, under our conditions, for comparison with the renal ischemic effect.

Methods. Dogs anesthetized with sodium pentobarbital, 30 mg/kg, were suspended in the prone position and both kidneys dissected free except for the hilar blood vessels and the ureter. The left ureter was cannulated so that urine flow could be observed. Then the right kidney was removed, leaving a good exposure of the right side of the vena cava. A shunt from the left renal vein to the jugular vein was next established: a special trocar

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