# SPONTANEOUS HEPATITIS AND CEREBELLAR "HYPOPLASIA" IN SUCKLING RATS DUE TO CONGENITAL INFECTIONS WITH RAT VIRUS

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This paper describes how the chance discovery of spontaneous congenital infections caused by rat virus (RV) [1] in laboratory bred albino rats has provided an opportunity, 1) to isolate a strain which passes the placental barriers of its natural host, a potential not demonstrated by laboratory adapted strains of RV,[2] 2) to investigate a type of hepatitis which occurs in nature, and 3) to study further the process of spontaneous cerebellar hypoplasia of viral origin, an entity which we have already described in cats.[3]

## MATERIAL AND METHODS

*Rats.* Vertical transmission of RV is probably of sporadic occurrence in many rat colonies. The Sprague-Dawley rats observed in the present experiments were random bred and were received from a number of sources. Susceptible animals required for experiments were found by testing many pregnant rats for the presence of RV antibodies. Only a relatively few were free of antibody and hence utilizable for investigations. Gestation periods of the pregnant rats used were as long as 24 days in some cases, as estimated from the data sent to us with the animals from commercial breeding establishments.

*Virus.* SpRV, the strain of rat virus freshly isolated from spontaneous infections of congenital origin, was the only one used in the laboratory experiments described below, and then only as passed directly in rats or following a single transfer in rat embryo tissue culture.

*Tissue Culture.* Rat embryo tissue cultures (RETC) were prepared by removal, then trypsinization, of embryos from pregnant rats on the 16th day of gestation, followed by a re-trypsinization in 3 to 4 days, before final distribution in tubes. These tubes were inoculated when cell sheets had formed in another 3 days. For initial growth, the nutrient fluid was Eagle's Basic Medium containing 10 per cent calf serum from which the gamma globulin had been removed. Two per cent calf serum was used for maintenance. All fluids contained penicillin and streptomycin. Further description of RETC is given elsewhere.[1]

*Titrations.* Tissues harvested from infected rats were made up as 10 per cent suspensions in tissue culture fluid containing 2 per cent calf serum, whole embryos being rinsed in sterile saline prior to grinding. These materials were stored at −40° C. Tenfold dilutions of these preparations were subsequently inoculated into RETC, using

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TABLE I

ISOLATIONS OF SPRV FROM ALBINO RATS WITH SPONTANEOUS CONGENITAL INFECTIONS *

| Pregnant rat and litter | *Studies in embryos and neonates* | | | *Methods of Identification of rat virus* | | | | *Maternal studies* | |
|---|---|---|---|---|---|---|---|---|---|
| | Number in litter | Age at sacrifice | Condition | 1st Passages: Hamster | 1st Passages: RETC § | HA On tissue suspension ¶ | Inclusion body | Number of resorption sites | RV HI-AB |
| A | 11 Embryos † | 15th Day gestation | Normal | ++ | ++ | 1:512 (T.C. Fld) | | 3 | ND |
| B | 11 Pups | 2 @ 4 Days 1 @ 45 Days | 3 of 11 ill | ++ | ++ | 1:1024 | ++ | (not sacrificed) | 1:160 (at 3 weeks) |
| C | 2 Pups | 4 Days | Moribund, jaundiced | ++ | ++ | 1:1024 | ++ | 9 | 1:320 |
| D | 10 Embryos | 16th Day gestation | 1 of 10 ill | | ++ | 1:512 | | None | 1:20 |

* For further details see Table II.
† These embryos used for RETC.
§ RETC, rat embryo tissue culture.
¶ Hemagglutination titer.

3 tubes per dilution. The highest dilutions of the suspensions capable of causing production of hemagglutinins (HA) for guinea pig erythrocytes at the end of 2 weeks were considered end points; titers presented in Tables II to IV are in the inverse logs of these dilutions. Parallel titrations were performed on the same suspensions using a direct hemagglutination test and 2-fold dilutions. Further details of these procedures and of serologic methods have been given elsewhere.[1,2] Antibodies tested and referred to were those causing inhibition of hemagglutination.

*Inoculations.* The pregnant rats used were given 3 ml SpRV infected tissue culture fluid by intraperitoneal (I.P.) inoculation and neonatal rats were given inoculations by either the intracranial (I.C.) route (0.03 ml) or the I.P. route (0.1 ml) using an inoculum which had an HA titer of 1:256 and an RETC infectivity titer of $10^{-7}$.

*Fixation of Tissues.* Except for the central nervous system, which was perfused with *p*-toluenesulphonic acid,[4] tissues for histologic sections were fixed in Bouin's solution.

## Results

*Spontaneous Infections.* The group of congenital infections first observed developed in a colony of rats which had been free of RV and H-1[5] viruses and had then developed hemagglutination antibodies against both agents within a relatively few weeks, suggesting an epidemic spreading through a susceptible population. Circumstances which attended these spontaneous infections are outlined in Table I. Thus, the first observations were on rats A and B which arrived at the laboratory when 13 days pregnant. When rat A was sacrificed 2 days later for preparation of a standard lot of RETC, it was noted that in addition to 11 normal embryos, the placenta had 3 resorption sites. All tubes of this lot of RETC developed changes typical of RV infection within 5 days of being re-trypsinized. These cytopathic effects were observed in 36 tubes used for a variety of experiments, as well as in 28 uninoculated tubes, and were accompanied by the development of RV hemagglutinins in titers of 1:512 demonstrable in pooled supernatant fluids.

Observation of these spontaneous infections led us to examine the litter of rat B (Tables I and II) which had arrived in the same shipment as rat A. At the time of study, rat B had delivered a litter of 11 sucklings 4 days previously. Eight of these were normal. Three others, however, were somewhat smaller and were sacrificed for further examination, 2 being used for purposes of virus isolation and 1 for histologic sections. The tissues for the single animal examined histologically in the early stages of the infection were found to have intranuclear inclusion bodies and destructive cytopathic effects typical of RV infections (Figs. 1, 2 and 5). The presence of RV in the other two afflicted sucklings was confirmed by 3 methods: 1) by demonstration of specific RV hemagglutinins in suspensions of various tissues, of which a pool of gut had a highest titer of 1:1024, 2) by isolations of virus in RETC, and 3) by I.C. inoculations into newborn hamsters. The tissue culture isolations were

## TABLE II

INFECTIVITY TITRATIONS FOR RV PERFORMED IN RETC *
(TISSUE SUSPENSIONS OF RATS WITH SPONTANEOUS CONGENITAL INFECTIONS) †

| Litter | *At sacrifice* | | *Tissues tested* | | | | | | | | Comment |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Age | Condition | Brain | Thymus | Lung | Liver | Spleen | Kidney | Gut | Feces | |
| B | Pups<br>1 @ 4 Days<br>1 @ 45 Days | Moribund | 6‡ | | | | 5‡ | | 6‡ | | 8 of 11 well<br>One adult survivor ataxic |
| C | Pups<br>4 Days | Moribund, jaundiced | 6¶ | | | 7‡ | | 5‡ | 6‡ | | Only 2 pups born |
| | Mother | Well | | 3 | 2 | 0 | | 3 | 2 | 2 | Placenta had 9 resorption sites |
| D | Fetus<br>16 Days | Mottled | | Whole fetus .....6 *<br>Placenta .....5 | | | | | | | Other 9 embryos normal |

* Rat embryo tissue culture.
† For further details see Table I.
‡ These tissue suspensions also positive by direct HA (see text).
¶ Infectivity titers expressed as inverse log of RETC end points.

actually made on 3 separate occasions from 3 different lots of RETC, all of which developed a characteristic cytopathic effect and hemagglutination following inoculation. Uninoculated control cultures from each lot remained normal. One of the apparently normal pups from this litter survived in general good health, but developed clinical ataxia; atypical cerebellar hypoplasia was found upon sacrifice at 45 days (Fig. 6).

The litter of rat C, an animal received a week later than rat B, differed in that it consisted of only 2 pups, which were puny at birth and were moribund with deep jaundice when sacrificed at 4 days of age (Tables I and II). Mother rat C had 9 resorption sites in her uterus when sacrificed at the same time. It was thus manifest that there had been a normal complement of fetuses early in pregnancy, but only 2 had survived until birth. The presence of RV infection in the tissues of these two sucklings was revealed 1) by the existence of intranuclear inclusions and destructive cytopathic effects typical of infections with this agent (Figs. 7 to 12), 2) by the demonstration of specific hemagglutinations in titers of up to 1:1024 on direct examination of pooled tissue suspensions, and 3) by the isolation of virus, by both *in vivo* and *in vitro* methods. The *in vivo* isolations were performed by I.C. inoculations in newborn hamsters. As described elsewhere,[6,7] such animals usually show no signs of acute illness on original passages of RV, but remain in otherwise general good health, even after the onset of a chronic, intractable cerebellar ataxia which becomes manifest within 2 to 3 weeks of inoculation. SpRV induced this characteristic syndrome on first hamster passage and histologic sections confirmed that the typical viral action on the cerebellum had resulted in the destruction of the external germinal layer. These cerebellar lesions were identical to those encountered in rats (Figs. 3 and 4).

A fourth instance of spontaneous congenital infection was found by chance examination of a 16-day pregnant rat (rat D, Table I), which in addition to 9 normal fetuses, and a uterus free of resorption sites, had a single fetus with a somewhat whitish head and mottled placenta. Ten per cent suspensions of this fetus and placenta demonstrated RETC infectivity titers of $10^{-6}$ and $10^{-5}$, respectively, while the fetus had, in addition, an hemagglutination titer of 1:512 (litter D, Tables I and II). The normal appearing fetuses in this litter were not saved for testing.

Table II summarizes infectivity titers obtained from original tissue suspensions. The spontaneously infected sucklings of rats B and C, as well as the single proven affected fetus of rat D, had relatively high titers ranging from $10^{-5}$ to $10^{-7}$, as contrasted with mother rat C which had titers of $10^{-2}$ and $10^{-3}$. Mother C, however, had a hemagglutination antibody titer of 1:320 (Table I) at the time of sacrifice. This indicated that

## TABLE III

INTRAUTERINE INFECTIONS WITH RV INDUCED BY INTRAPERITONEAL INOCULATIONS IN PREGNANT RATS

| Litter | Day of gestation mother inoculated | *At sacrifice* | | | *Tissues tested* | | | | | | | Maternal HI antibody |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Days post-inoculation | Condition | Number of resorption sites (RS) Number of young born (YG) | Brain | Liver | Spleen | Gut | Lung | Other | Milk | a) Pre-inoculation b) At sacrifice |
| E Mother | 16 | 8 At birth | Well | 9 RS | 4* | 4 | 6 | | | Feces 7 | | a) Negative b) 1:160 |
| E Young | | 8 At birth | Well? | 2 YG | 7 | 7 | 7 | 7 | 7 | | 5 | |
| F Mother | 20 | 3 At birth | Well | 10 RS | | | | | | | | a) Negative b) 1:160 |
| F Mother | | 3 At birth | Well | 2 YG | 4 | 6 | | 4 | | | 0 | |
| G Young | 20 | 3 At birth | Well | 7 YG | | 0 | | 0 | | | 0 | a) 1:160 |
| H Mother | 5 | 16 (Just before birth) | Well | 9 RS 3 full-term fetuses | 2 | 2 | 4 | 3 | 0 | Feces 4 Resorption sites 4 | | a) Negative b) 1:320 |
| I Adult | Non-pregnant | 16 Non-pregnant | Well | | | | 5 | | | Thymus 3 | | |

* Infectivity titers expressed as inverse log of RETC end points.

the peak of her infection had been passed and could also explain the fact that the suspensions of her tissues were all negative at $10^{-1}$, with higher dilutions becoming positive, presumably as some inhibitor was removed by dilution.

*Experimental Infections.* These experiments were designed to reproduce the disease states observed in the spontaneous outbreaks and thus to gain an understanding of processes involved. Results obtained with pregnant rats varied with the timing of inoculations and the immune status of the animal. Mother E (Table III), for example, exhibited no RV antibodies when given I.P. inoculation on the 16th day of gestation, but had developed a hemagglutination titer of 1:160 when sacrificed 8 days later, along with her newborn young. The presence of 9 resorption sites in her uterus and the survival of only 2 fetuses until birth indicated that her disease had resulted in severe fetal infection with multiple deaths. These two surviving fetuses had uniformly high titers of RV in various organs as well as a titer of $10^{-5}$ in milk removed from their stomachs. It thus appeared that the mother was transmitting virus through her mammary glands at the end of a 24-day period of gestation. She also had relatively large amounts of RV in spleen and in rectal feces.

The finding of virus titers exceeding those of the mother in these newborn animals, studied immediately after birth indicated that SpRV had crossed the placental barriers and proliferated in the fetuses. In that only 2 fetuses of a large litter survived until birth, the results of this experiment closely resembled the example of spontaneous congenital infection observed in the litter of rat C (Table I).

Results obtained with a second pregnant rat, mother F, were confusing since she may have been harboring a spontaneous infection at the time of inoculation. Circumstances which suggested this possibility are shown in Table III. Thus, although inoculated I.P. on the 20th day of gestation, when she exhibited no RV antibodies, she resembled mother E in having many resorption sites, indicative of fetal deaths, and in giving birth to only 2 young, at which time her hemagglutination antibodies had reached a titer of 1:160. All of these events occurred within 3 days of inoculation. Since the comparable results obtained with mother E (Table III) had taken 8 days, it seemed likely that mother F had been infected for an equal if not longer period. Lower virus titers in the organs of the young, as well as an absence of virus in the milk, suggested that the peak of the RV infection had been passed by the time of sacrifice. Although these findings are open to other interpretations, it is especially difficult to understand how 10 nearly full-term fetuses could have become infected via the placenta and have been nearly completely resorbed, all within 3 days. Regardless of whether the end results were due to a spontaneous or to an

TABLE IV

INFECTIVITY TITERS AND HISTOLOGIC FINDINGS IN RATS INOCULATED AT BIRTH

| Litter | *At sacrifice* | | | *Tissues tested* | | | Maternal antibody (HI) before delivery | Histology |
|---|---|---|---|---|---|---|---|---|
| | Routes of inoculation | Day post-inoculation | Condition | Brain | Liver | Gut | | |
| J | I.C. & I.P. | 8 | Moribund, jaundice (3 controls) (Well) | 4 * | 6 | 5 | Negative | Inclusion bodies, liver and cerebellum Control infected from inoculated siblings |
| K | I.C. | 5 | Small | 3 | 5 | 4 | Negative | Cerebellar hypoplasia at 18 and 37 days |
| | I.C. | 14 | Small | | 4 | 5 | | |
| L | I.C. | 5 | Well | 0 | 2 | 0 | 1:160 | Normal |

* Titers expressed as inverse log of RETC end points.

experimental infection, they do provide an apparent example of *in utero* transmission.

The young of an RV-immune rat (litter G, Table III) served as controls to those of mother F. These pups exhibited no demonstrable virus at birth, which took place 3 days after inoculation of the mother, and revealed no evidence of infection upon histologic examination. The mother's uterus contained no resorption sites.

Results obtained with 2 other rats, one pregnant and one non-pregnant, and with longer-term infections are also shown in Table III. The uterus in mother H, inoculated on the fifth day of gestation, contained 9 resorption sites and 3 full-term fetuses when sacrificed just prior to birth. No virus was recovered from the 3 fetuses. This suggested that they had escaped the infection, to which their 9 litter mates had succumbed, as evidenced by an RV-titer of $10^{-4}$ in a single resorption site. This titer was higher than obtained for all of the maternal tissues except for the spleen. Viral titers of $10^{-5}$ in the spleen and of $10^{-3}$ in the thymus in non-pregnant rat I (Table III), sacrificed at the same time as mother rat H, 16 days after inoculation, indicated that pregnancy was not essential to a substantial proliferation of virus in adult female rats.

None of the above inoculations led to striking evidences of disease since all of the adult rats remained well regardless of whether they had spontaneous or experimental infections. Their young were sacrificed before they could expectedly have developed other manifestations, as suggested by events shown in Table IV. We were consequently led to another series of experiments to see what types of disease might be induced by inoculations of SpRV. In the first of these studies, litter J received SpRV by I.C. and I.P. routes of inoculation with a result that all 10 sucklings had developed jaundice and were either dead or moribund within 8 days. Three litter mate controls, which received normal tissue culture fluid by the same routes, remained well. We had thus been able to reproduce the picture of hepatitis observed in the spontaneous infections, but our experiments had taken 8 days to produce a comparable stage of the process. Since the sucklings with spontaneous hepatitis were only 4 days of age, the indication is that their infections antedated delivery and began late in gestation.

An unexpected finding was that a control suckling (see litter J, Table IV), which appeared well when its litter mates were dying at 8 days of age was found to have typical RV inclusion bodies in the external germinal layer of its cerebellum, indicative of early infection. Its inoculated litter mates sacrificed at the same time exhibited advanced, destructive cerebellar lesions. Our interpretation was that the control suckling had acquired SpRV spontaneously from its infected siblings, a situation of

special interest not only in showing the capacity of this strain to pass from rat to rat through natural barriers to infection but also to penetrate the blood-brain barrier and reach the cerebellum as a target organ.

A second litter of newborn rats received SpRV by I.C. inoculation alone. These animals (litter K, Table IV) survived in general good health, although 2 of them exhibited moderately high titers of RV in pooled visceral organs when sacrificed at 5 days of age. Similar results were obtained with 3 more of the sucklings sacrificed at 14 days. The tissues for an additional 2 rats in the same litter were examined histologically at later periods, 18 and 37 days post-inoculation. Both pups were ataxic and demonstrated severe cerebellar hypoplasia, characteristic of RV infection (Fig. 4). Litter L, Table IV, had received inoculation I.C. in the same manner as litter K. Mother L was immune to RV at the time of the inoculations and her young sacrificed when 5 days of age exhibited no histologic abnormalities.

*Histologic Changes.* Descriptions given below are of pathologic findings in the hepatitis and cerebellar disease first encountered in the spontaneous infections and then reproduced by inoculations into suckling rats already described.

*Cerebellar Disease.* The changes observed were essentially the same as those described elsewhere in hamsters[7,8,9] and cats.[3,10] They represented a selective attack upon the external germinal layer of the cerebellum, which took place in successive phases. Initially a latent period of a few days in the case of the inoculated rats was followed by an inclusion body phase in which characteristic RV intranuclear inclusions appeared (Figs. 1 and 2). This was followed in turn by a cytopathic phase, in which fulminant lytic effects were produced in the infected cell population. A depletion phase (Figs. 3 and 5), characterized by premature disappearance of the external germinal layer, led to a hypo- and dysplastic state (Fig. 4) in which the main feature was a varied degree of failure of the definitive granular layer to develop. This was occasionally combined with persistence of irregular islands of heterotopic granule cells (Fig. 6). A disordered disposition of Purkinje cells in the molecular layer was a constant feature (Figs. 3 to 6). Less frequently, meningocytes and endothelial cells were involved, exhibiting inclusion bodies (Fig. 11) in the early phase of the infection. The end effect of these changes was a granuloprival cerebellar hypoplasia.

*Hepatitis.* Rats which became jaundiced as sucklings fell into 2 groups, namely a single animal selected from a pair of moribund pups at 4 days of age following a spontaneous congenital infection (litter C, Table I) and 2 sucklings which had received I.C. and I.P. inoculations of SpRV at birth and were moribund at 8 days of age (litter J, Table IV).

Since all of these animals had a similar acute hepatitis when examined, they can be described together. The outstanding change was the presence of intranuclear inclusions. These were found in hepatic parenchymal cells (Figs. 7 and 8) in a few Kupffer cells (Fig. 9) and more sporadically in bile duct epithelium, connective tissue and endothelial cells (Fig. 12). Cytopathic effects were also evident (Fig. 10). These included parenchymal cell nuclear pyknosis as well as cytoplasmic eosinophilia, rounding and ballooning, with detachment from adjacent cell structures. Among the findings were a retention of bile in the liver cell parenchyma and pigmented casts in renal tubules.

The capacity of SpRV to pass placental and blood-brain barriers may reflect its ability to proliferate in endothelial cells lining small blood vessels (Figs. 11 and 12).

## Discussion

The spontaneous infections of rats fell into 3 categories, of which the more severe were (1) death and resorption of all fetuses and (2) the same findings except for survival of a few pups which manifested hepatitis with jaundice within 4 days after birth. A third category represented lesser degrees of infection. Here only a few of the neonates were obviously diseased while the majority appeared normal. Yet one of these apparently normal pups presented characteristic clinical features of cerebellar ataxia, with pathologic findings compatible with RV disease when necropsied as a young adult. Variations observed in the 3 groups probably resulted from the fact that initial infections came at different stages of gestation. Infections occurring early in pregnancy might, expectedly, be more apt to produce fetal death and infections of later onset lead to manifestations of disease, delayed until after birth.

A pregnant rat infected late in pregnancy may give birth without having had time to develop antibodies and, hence, would transmit RV in her milk for a short time. Milk transmission would appear unlikely with earlier onset of infection, since antibodies, when such are present, are well transmitted by lactating rats.[11]

The findings described herein should have interest to breeders of laboratory rats from several points of view. One conclusion is that the manifestations of disease in the uterus of the pregnant rat or in its newborn offspring may be the only indications of RV infection within a colony, since this virus usually persists only as a latent infection.[1] Another observation is that RV could, conceivably, be present in rats delivered by Cesarian section. If only one such rat were used in starting a new colony, the agent might as well persist by transmission *in utero* as spread laterally by infected urine, feces and possibly other means.

The spontaneous infections included, seemingly by chance, three disorders which we had been studying under laboratory conditions, without knowing whether they might exist in nature. The conditions being studied were viral hepatitis of rats, cerebellar hypoplasia, and vertical transmission of RV, all of which appeared to have bearings on various diseases of man. RV hepatitis, for example, offers some points of similarity to the human disease. As we plan to show in detail in a subsequent publication, this similarity lies not only in parenchymal necrosis and a later nodular hyperplasia, but also in bile duct proliferation, and scarring with distortion of architecture. Some of the properties of RV and H-1 such as small size, resistance to physical agents, and adaptation to either *in utero* or fecal transmission suggest, in a general way, those described for the etiologic agents of human viral hepatitis.

A second subject of our previous investigations, cerebellar hypoplasia, was first discovered in an attempt to adapt freshly isolated strains of RV to newborn hamsters.[7] This likewise appeared to be a purely laboratory phenomenon. Although spontaneous cerebellar ataxia is a common condition in cats, and presents a histologic pattern resembling the viral cerebellar lesion,[12] it had hitherto been considered to be of a genetic or toxic origin. Subsequently, however, we were able to induce a cerebellar disease in newborn kittens with RV,[10] as well as with a newly discovered agent, the feline ataxia virus (FAV), isolated directly from cats.[3] A further extension of information came on finding that rats as well as cats can have spontaneous ataxia. In both animals it appears to stem from an infection initiated at, or not long prior to birth, which leads to a destruction of the external germinal layer of the cerebellum in the initial phase followed by a slower attack on Purkinje cells.

Our experience with a spontaneous outbreak of congenital infections has suggested to us that RV as it occurs under natural conditions may have properties different from those of strains of the same agent propagated by laboratory methods. One laboratory strain of RV, for example, failed to pass through the placental barrier of the natural host under experimental conditions and was thus incapable of inducing fetal infection.[2] Lack of this capacity in the laboratory strain may be explained by the fact that in the course of passage by syringe it had not been acted upon by the usual selection pressures which operate in transmission from one host to another by natural means. Artificial transmission may favor mutants and variants which might otherwise be weeded out by natural selection. In this regard, it may be well to consider studies of the natural history in the pathogenesis of viral infections in the light of evolutionary processes. As Huxley [13] has aptly remarked, "Evolution is the overriding fact for biology, the comprehensive framework to which separate bio-

logical facts and functions are related. The evolutionary approach will prompt us to ask the right questions of nature, and, when we have asked them, will help us to find the right answers."

## Summary

The fact that a strain of rat virus, SpRV, can pass the placental barrier and induce congenital infections of varying severity was established by observations made on three pregnant rats. These arrived in the laboratory from a commercial breeding colony with spontaneous infections; we have as well transmitted the disorder by parenteral inoculation of pregnant rats. These findings were unexpected, since previous attempts to induce such infections with laboratory-passaged strains of RV had been unsuccessful. Their implications in terms of vertical transmission of viruses in general and in colonies of germfree and conventional rats in particular, are to be the subjects for later discussion.

Of two litters of rats having spontaneous infections acquired *in utero*, one exhibited marked hepatitis with jaundice. In the other, in which many of the young were seemingly normal and unaffected, a destruction of the external germinal layer of the cerebellum appeared in a few and cerebellar ataxia developed subsequently in one.

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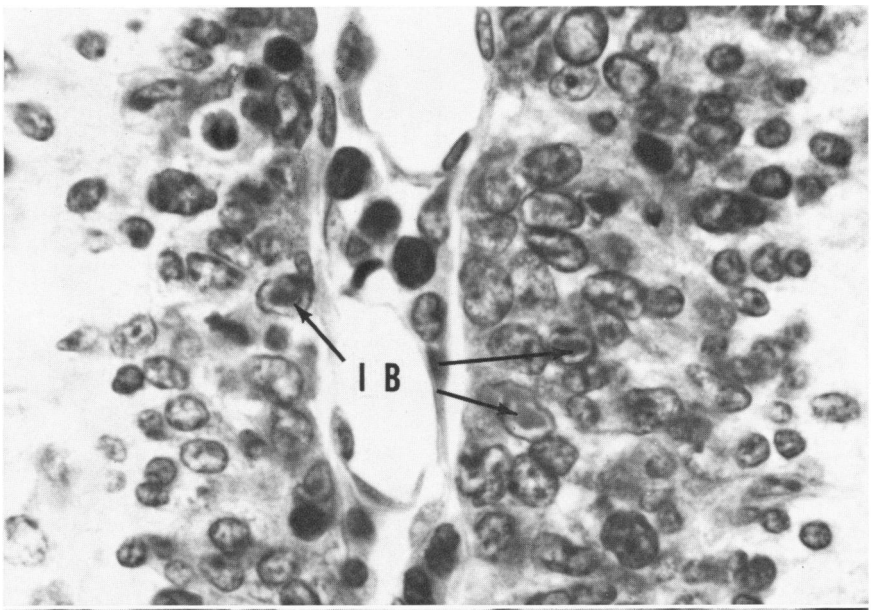
## LEGENDS FOR FIGURES

Photomicrographs were prepared from sections stained with hematoxylin and eosin.

FIG. 1. Cerebellum, 4-day-old spontaneously infected rat. The inclusion body phase of RV infection is shown. The central fissure is bordered on each side by the external germinal layer, several cells of which contain intranuclear inclusion bodies (IB). These are best seen in the 3 cells indicated by arrows. × 900.

FIG. 2. Detail of Figure 1. Two cells containing intranuclear inclusion bodies (arrows) are especially well seen in the plane of focus. Two cells with pyknotic, fragmenting nuclei to the left of the central fissure exhibit the typical destructive cytopathic effects of RV. Although mononuclear cells are found in the leptomeninges the parenchymal changes have invoked no inflammatory exudate. × 1,700.

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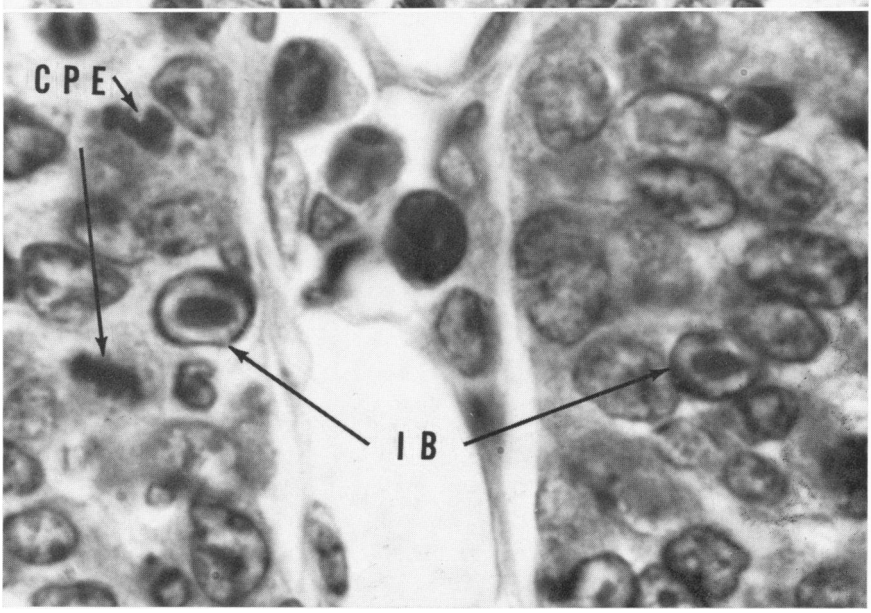


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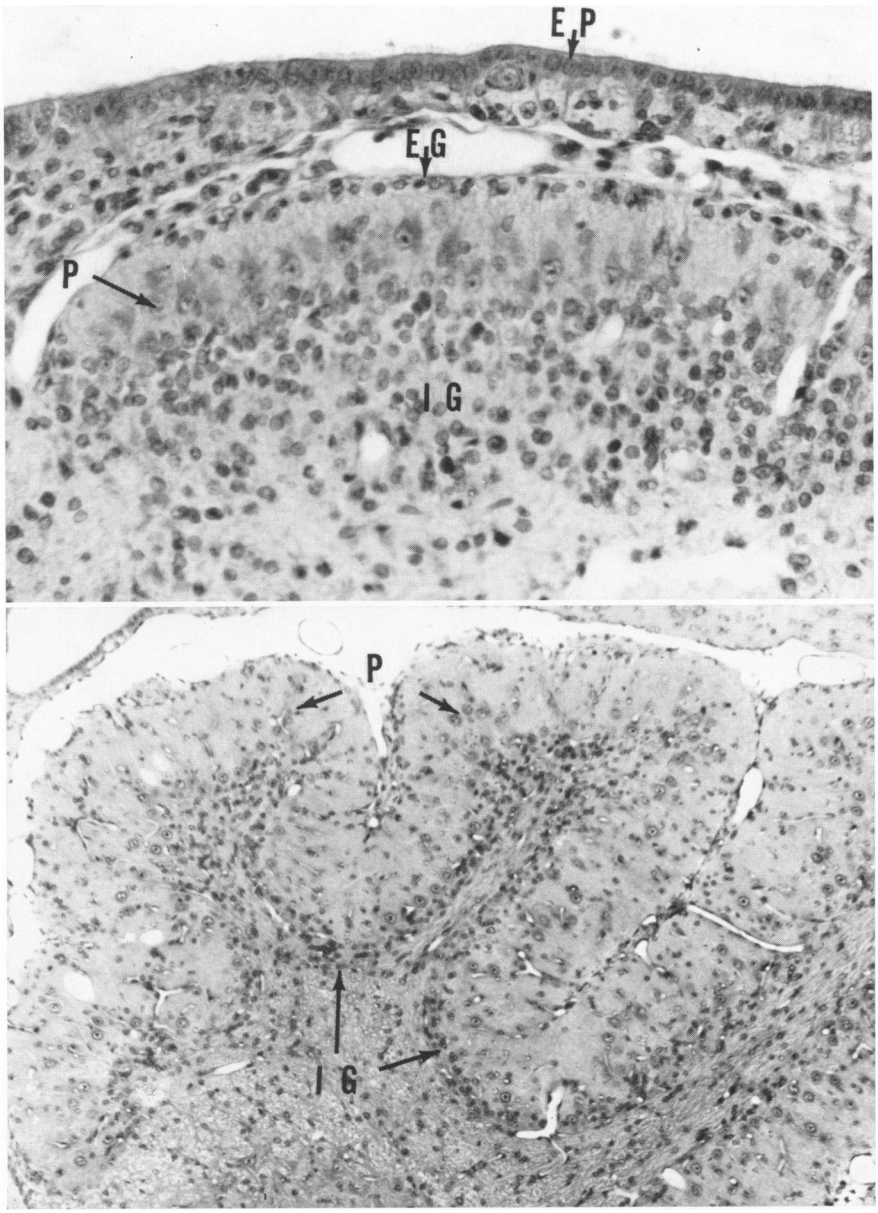


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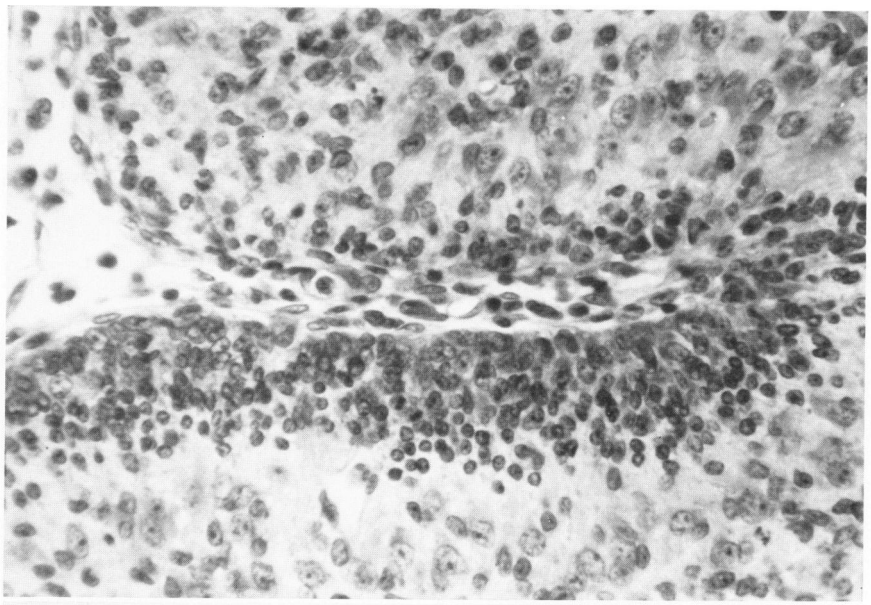
FIG. 3. Cerebellum, 8-day-old rat. The depletion phase of the lesion produced by intracerebral injections of SpRV at birth is demonstrated. Only a single, incomplete layer of cells persists in the external germinal layer (EG) which at this stage is normally a rapidly proliferating zone several layers deep. The Purkinje cell layer (P) is irregularly disposed. The internal granular zone (IG) exhibits hypocellularity. Both it and the external germinal zone contain numerous pyknotic nuclei. A few inflammatory cells are observed in the leptomeninges. The ependyma (EP) of the fourth ventricle is free of involvement. × 200.

FIG. 4. Cerebellum, 37-day-old rat. Granuloprival hypoplasia has been produced by intracerebral inoculation of SpRV. The virtually complete absence of the definitive granular layer and the haphazard disposition of the Purkinje cells constitute the outstanding features of this lesion. × 75.



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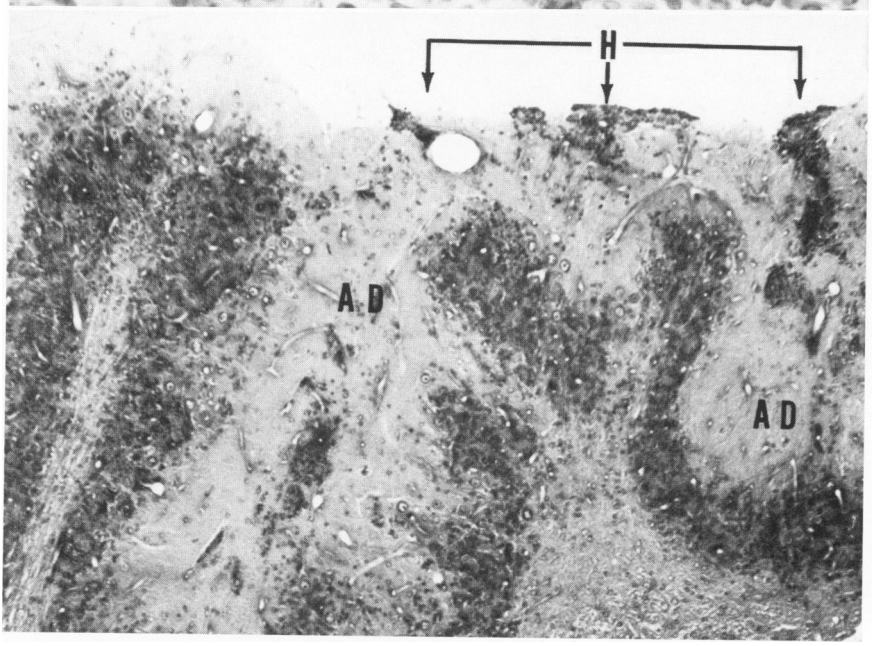
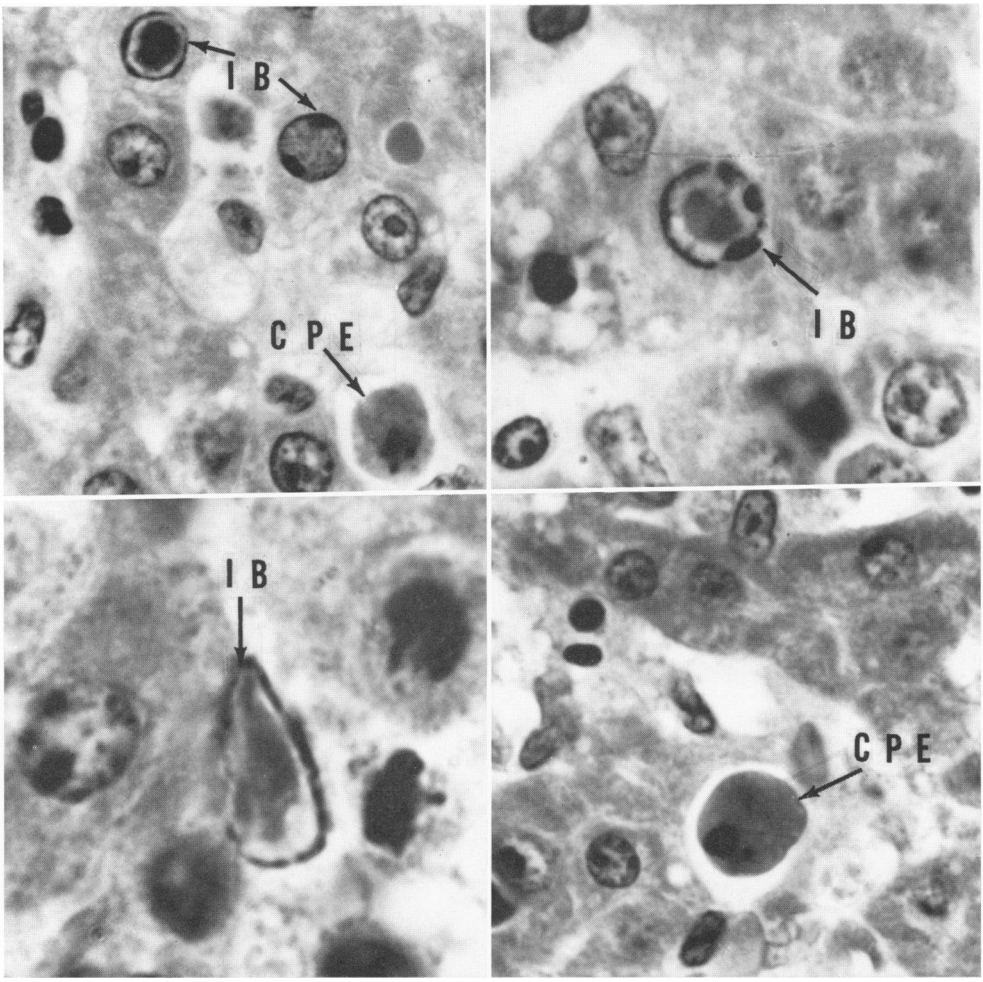



6

FIG. 5. Cerebellum, spontaneously infected 4-day-old rat. The external germinal layer exhibits irregular destruction by RV. In the adjacent folia there is sparing of this zone below and depletion above. The leptomeninges show a moderate infiltration with mononuclear cells and increased vascularity. × 370.

FIG. 6. Cerebellum, 45-day-old spontaneously infected rat, litter mate of the animal shown in Figure 1. A striking maldevelopment of the cortex has occurred, consisting of the following features: a) irregular hypoplasia of the definitive granular layer; b) persistent heterotopic islands of granule cells in the leptomeninges (H); c) obliteration of fissures and subarachnoid space and production of adhesions (AD) between the folia by proliferated heterotopic glia. × 75.



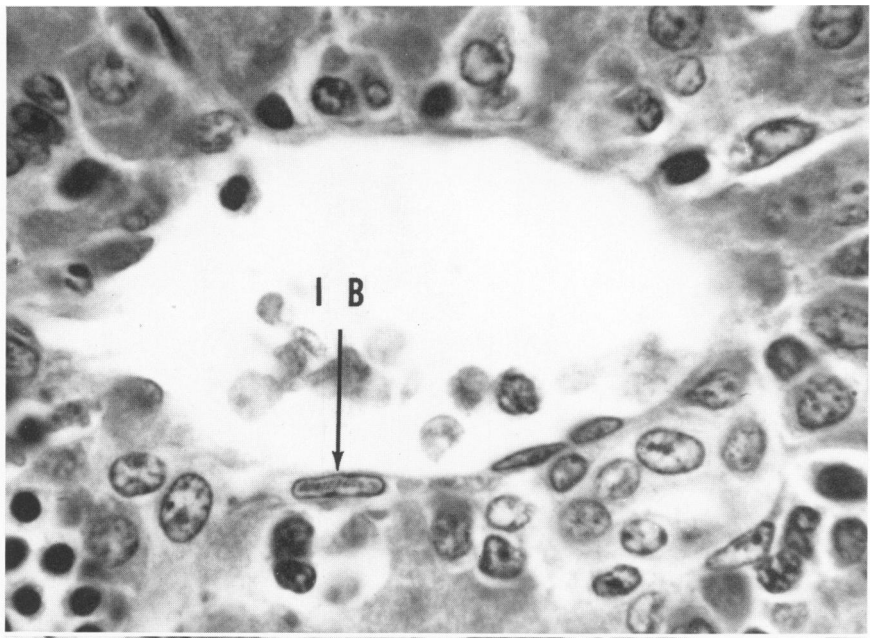

Figures 7 to 11 are from the liver of a 4-day-old rat with spontaneous RV infection.

FIG. 7. Two phases of inclusion body formation (arrows) are demonstrated in hepatic parenchymal cells. A rounded eosinophilic necrotic hepatic cell (arrow) is also shown. × 750.

FIG. 8. The striking displacement of nuclear chromatin towards the membrane and typical halo formation are demonstrated in an inclusion-containing hepatic cell. × 1,750.

FIG. 9. An RV inclusion body appears in the nucleus of a Kupffer cell. To the right a mitosis in an hepatic cell has just been completed. × 1,500.

FIG. 10. A deeply eosinophilic rounded, detached hepatocyte is illustrated. The dense, pyknotic nucleus is almost obscured in this monochromatic photograph. × 1,750.




11

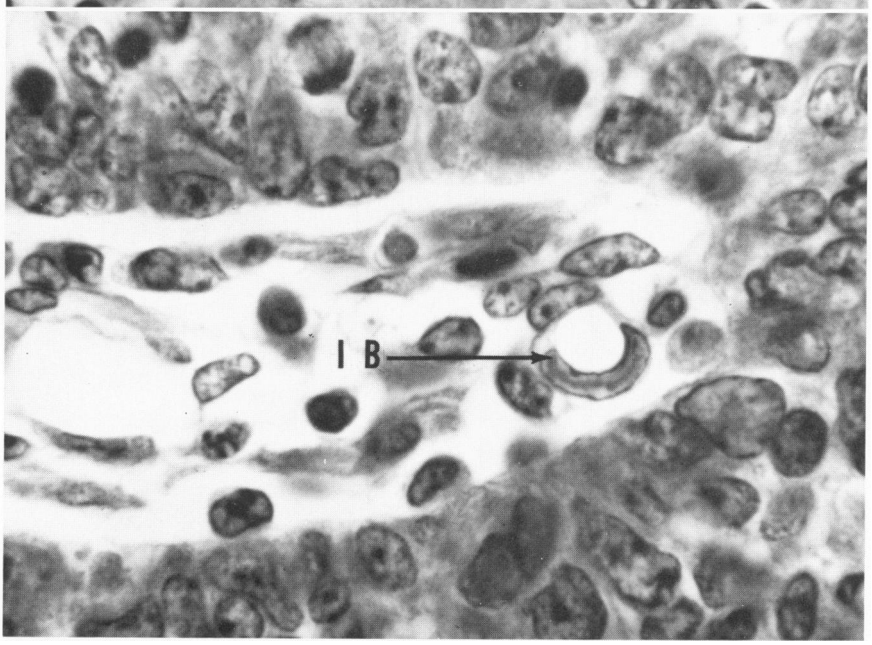



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FIG. 11. An endothelial cell in an hepatic vein exhibits an elongated centrally placed intranuclear RV inclusion (arrow). × 1,750.

FIG. 12. An endothelial cell in a vessel in the subarachnoid space of a cerebellar fissure. A linearly orientated intranuclear inclusion body is evident (arrow). This section is from the same animal as shown in Figure 11. × 1,750.