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# A NEW VIRUS ISOLATED FROM HEMORRHAGIC DISEASE IN RABBITS

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In the spring of 1984, an acute infectious disease occurred in Angora rabbits imported from Federal Germany. The disease could not be cured or controlled by various antibiotics and sulfonamides, consequently it was suggested that the causative agent might be a virus, named rabbit hemorrhagic disease virus (RHDV) tentatively. The suggestion was confirmed by laboratory examinations.

## Description of the Disease

The disease affects rabbits older than two months, never occurs in those below that age and sucklings. There is no difference in sex and breeds. The mortality may be highter than 80% in some colonies, but some may be quite resistant(Liu, S.J.et al, 1984). Experimental infection shows that the incubation period is very short, predominantly 1-2 days, occasionally 3 days. The virus invades the host via skin scratches and respiratory and alimentary tracts. Oral, intramuscular or intraperitoneal inoculation with infected tissue suspension can produce typical disease. In natural peracute cases, any clinical sign may not be observed. Many rabbits that are normal in appetite and appearance die abruptly after several hours. Some of them squeal with pain before death. In experimental infections, it is found that the body temperature rises by 1-1.5 C 12-24 hours postinoculation and drops rapidly.

## Pathological Lesions

The skin round the nostrils is stained with incompletely clotted blood. Petechial hemorrhages of various size are observed on the lung, ranging from pin head to mung bean. Trachea, bronchi and bronchioles are full of foamy fluid and on their mucous membranes there are many petechial and diffusive hemorrhages. The liver is swollen extremely, brownish red in color and fragile. The gall bladder is distended with bile and part of its mucous membrane is detached. The spleen and kidney swell by 2-3 times and are fragile with significant congestion and hemorrhage. Many lymph nodes are also swollen and distributed with petechial hemorrhage. On the gastroenteric mucous membrane there are many petechial and diffusive hemorrhages and adheres much mucus(Xu, F.N. et al, 1985).

## Hemagglutination

Various tissues from infected rabbits can agglutinate human red blood cells despite the blood groups. The titers reach $10X2^{10-18}$ in liver, serum and spleen and even higher. Erythrocytes of chicken, goose and sheep can be agglutinated at low titer ( $10X2^{2-4}$) while those of other animals, including cow, goat, pig, rabbit, rat, guinea pig, duck, and quail cannot. Hemagglutination is inhibited by specific antiserum(Du,N.X. et al 1986 and Yan,H.C. et al 1986).

The hemagglutination is not affected significantly at various temperature(4-37 C). The requirement of pH value is not strict, optimal at pH 6-7.2. The virus releases from agglutinated RBC at pH 9.2. The hemagglutinating activity can be destroyed by 0.5% trypsin, 2% sodium borohydride or chloramine T but not by receptor destroying enzyme(RDE) or formaldehyde. 0.1 mol/L potassium periodide, chloroform and ether can lower the hemagglutination titer of the crude virus samples but not of the purified preparation.

## Properties of the Virus

Negatively stained and thin section specimens are examined under the electron microscope. The virion is icosahedral, 32-34 nm in diameter and nonenveloped. The capsid is composed of 32 capsomers 5-6 nm in diameter. It possesses a core with diameter of 19-20 nm. In CsCl solution, the virions are banded at a buoyant density of 1.36-1.38g/ml. The sedimentation coefficient of the virion is 162 S, as determined by sucrose density gradient centrifugation combined with ultraviolet scanning.

## The Viral Nucleic Acid

Applying SDS-pronase K-phenol method, nucleic acid is extracted and purified from infected tissues. It shows a typical spectral curve as determined by ultraviolet spetrometry. The absorbance ratio of 260nm/280nm is 2:1 and the peak of absorption is at 261nm(Deng,R,T. et al. 1986).

The results of polyacrylamide gel and agarose gel electrophoresis indicate that the virus nucleic acid is non-segmented. It gives blue color in diphenylamine test and dark blue in orcinol test. It is completely degraded by DNase I and not by RNase A. It is completely degraded by DNase $S_1$ and is resistant to Alu I. It does not denature on heating as determined by ultraviolet spectrometry. It gives red in acridine orange staining and the electron micrograph of the nucleic acid is like a " collapsed bush".

Highly purified virus nucleic acid, obtained by electrophoresis-electrodialysis of virus nucleic acid preparation, is degraded by formic acid and analysed for base composition by high performance liquid chromatography(Xu,S. et al. 1987). Based on retention time, cytosine(C), guanine(G), thymine(T), and adenine(A) 4 peaks appear successively in case of RHDV nucleic acid, similar to the DNA of calf thymus, but unsimilar to the yeast RNA, in which uridine(U) appears next to C and thymine does not appear. The mole percentages of the 4 bases of RHDV nucleic acid are C=28.7, G=20.5, T=31.9, A=24, the mole percentage of G+C is 44.2. The value of C is significantly different from G, and so T from A. The result suggests the RHDV nucleic acid is a single stranded DNA.

The data of agarose gel electrophoresis and urea-denatured PAGE indicates that the molecular weight of the nucleic acid is approximately $2.4X10^6$ daltons(Deng, R.T. et al. 1986).

## The Viral Polypeptides

Purified virions along with reference polypeptides whose molecular weights are known are submitted to SDS-PAGE, and the bands formed are stained with silver nitrate(Gou,X.F. et al. 1987). Seven viral polypeptides are detected, they are VP1, VP2,VVP3, VP4, VP5, VP6 and VP7, whose molecular weights are 75 Kd, 54.3Kd, 51.9Kd, 51.3Kd, 49Kd, 18.7Kd and 17Kd respectively. The cumulative molecular weight is 317.6Kd.

## Isolation and Cultivation

10% suspensions of liver, spleen and lung collection from infected rabbits are inoculated onto various cell cultures, including primary rabbit cells(kidney, liver, lung and testis), cell lines (PK-15, MA-104, IBRS-2, HeLa and Vero ) and a diploid cell strain (rabbit kidney). Blind passages are made and every passage is detected for hemagglutinating activity and cytopathic effect. The results are negative, and healthy adult rabbits inoculated with cell culture fluid of the third passage remain uninfected(Du,N.X. et al 1986).

Infected tissue suspensions are inoculated into the embryonated eggs via various routes, but no evidence of virus growth is observed.

## Discussion

Based on the manifestations of epidemiology, clinical signs and pathological changes, it is proved that RHD is a new viral disease, never reported in China or other countries. Though it is firstly discovered in Angora rabbits imported from Federal Germany, the source of the causative agent is so far unclear.

Suspensions of liver and spleen from infected rabbits can agglutinate hyman red blood cells, with agglutination titer much higher than other agglutinating viruses. the agglutinating

activity associated with virions can be destroyed by 50 C or pH 3.0, but the pathogenicity of the virus does not diminish accordingly. These peculiar properties need to be elucidated.

The viral nucleic acid is sensitive to DNase but resistant to RNase, positive in diphenylamine test but negative in orcinol test. These indicate that it is DNA. It is completely degraded by restriction enzyme Alu I, does not denature on heating and is similar to phage λ DNA in electron micrograph. These facts suggest that the nucleic acid of RHDV is single stranded. Though the viral DNA close to that of Parvovirus(Siegl, G. et al 1985 and Fenner, F. et al 1982), the size and sedimentation coefficient of RHDV are larger. Many properties of RHDV have to be studied thoroughly before its situation in virus classification is affirmed.

An effective immunity is produced at 5 days postinoculation, and lasts no less than 6 months. This is rarely seen in other inactivated vaccines, but the immunological mechanism is not clear yet.

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# A new virus isolated from hemorrhagic disease in rabbits

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Abstract

A new infectious disease of rabbits occurred successively in China since 1984. Small virus particles detected from extracts of visceral tissues of infected rabbits, could reproduce typical disease when inoculated experimently into susceptible rabbits, from which same virus was recovered. Consequently the causative agent was confirmed to be a virus, which was tentntively named the rabbit hemorrhagic disease virus(RHDV), after the name of the disease——the rabbit viral hemorrhagic disease (RVHD). The virion was 32-34 nm in diameter, icosahedral symmetry, and without envelop. Its buoyant density in CsCl was 1.36-1.38g/cm$^3$, and its sedimentation coefficient was 162S. The nucleic acid was single stranded DNA, with molecular weight of $2.4 \times 10^6$d. The virus could agglutinate human erythrocytes, resist the treatment of ether and chloroform, and was stable to pH3.0 and 50C for 60 minutes. Nuclear inclusion bodies were found in hepatic, renal and encephalic cells. According to the results described above, it was suggested that the virus was a new species, whose properties were basically in accord with those of Parvovirus. The cryptogram could be written as follows: D/1, 2.4/*, S/S, V/O,R,I.