INFECTIVITY OF TOTAL RNA DERIVED FROM NEWBORN MICE INFECTED WITH COXSACKIE VIRUS A - 10

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A study of the function of virus nucleic acids combines investigation of genetical mechanisms with research into the elucidation of the part played by nucleic acids and proteins in determining inherited characteristics and the property of infectivity. In addition, we note that the question of the infectivity of virus nucleic acids is one which is widely discussed but has received insufficient experimental investigation.

Several research workers [1, 5, 10] have shown that RNA isolated from cells infected with poliomyelitis virus does itself possess infectivity. However, the question of the infectivity of RNA from other members of the enteric groups of viruses, particularly the Coxsackie viruses, appears to be completely ignored in the literature [6, 9, 12].

The aim of this particular work was to investigate the possibility of obtaining infective RNA from the bodies of newborn mice infected with Coxsackie virus A - 10.

EXPERIMENTAL METHODS

Experiments were carried out using newborn white mice of no definite line (351), aged 1-3 days and weighing 1.3-1.7 g. The RNA was isolated from the bodies of the newborn mice infected with A - 10 Coxsackie virus using a modification of the phenol technique [2]. The titer of virus prepared from the tissues of such animals reached a level of $10^{6.5}$ - $10^{7.5}$ per 0.05 ml in 10% suspension.

In several cases, we used Crimean bentonite, converted into the Na-form and treated by the Brownhill technique [7], to isolate the RNA.

The level of RNA in the preparations was determined spectrophotometrically [7] and was found to vary from 0.5 - 1.0%. The protein content, as determined by the Lowry method [11] did not exceed 0.5% of the amount of RNA. It was not possible to demonstrate the presence of the DNA reaction using diphenylamine [8].

The results of the experiments were finally evaluated 7 days after the infection of the animals.

EXPERIMENTAL RESULTS

RNA solutions in the abovementioned concentration were injected under the skin on the back of newborn mice or intra-abdominally in amounts of 0.05 ml. After a period of 48-72 h the symptoms of the disease, as characteristic of the particular species, developed in the affected animals. These symptoms included an initial excitatory phase followed by depression, debility, ataxia, cyanosis, rapid but difficult breathing of the abdominal type, rotatory movements about a single axis, tremor, emaciation, paresis, together with paralysis of the muscles of the pelvis, back and both anterior and posterior extremities.

Identification of the strain of virus derived from mice infected by RNA injection was carried out by means of a neutralization reaction with serum, type specific for A-10 Coxsackie virus. All the diseased animals subjected to this virological test proved to contain A - 10 Coxsackie virus in high concentration: 0.05 ml 10% virus suspension contained $10^{5.5}$ - $10^{7.5}$ LD_{50} for newborn mice.

TABLE 1. The Effect of Mode of Injection of the RNA Solution on its Infectivity

Preparation injected	Total mice used	Those which died from the infection	
		absolute	%
RNA from bodies of infected mice injected subcutaneously	80	76	95
Same, intra-abdominal infection	45	28	62
RNA from bodies of healthy mice, subcutaneous injection	40	—	0

TABLE 2. The Effect of Nuclease on the Infectivity of RNA

Preparation injected and its treatment	Number of mice	Those which died from the infection	
		absolute	%
RNA + RNA-ase* , subcutaneous injection	57	1	0.18
RNA + DNA-ase† " "	23	19	83
Virus + RNA-ase‡ " "	41	1	100

* Wenger's preparation of RNA-ase, supplied by the firm of Reanal. RNA-ase was added at a rate of 100 micrograms per ml of RNA solution. The effect of the enzyme was prolonged for 20 min at room temperature.

† A highly purified preparation of DNA-ase was kindly supplied by the Genetical Laboratory of the Zoological Institute, Ukrainian SSR Academy of Sciences. The DNA-ase, activated with magnesium ions, was added to the RNA preparation at a rate of 10 micrograms per ml solution and incubated at room temperature for 20 min.

‡ A suspension of A - 10 Coxsackie virus consisting of $10^{1.2}$ LD_{50} per 0.05 ml, was treated with RNA-ase by the method described above.

TABLE 3. Stabilization of the Infectivity of RNA with the Aid of Bentonite

Preparation injected	Total mice	Those which died from the infection	
		absolute	%
RNA from bodies of infected mice, isolated using bentonite	38	23	60
RNA from bodies of infected mice, isolated without bentonite	27	4	15

Note. The preparations were preserved for 24 h at 4°.

The infectivity of RNA by subcutaneous injection is considerably higher than by intra-abdominal (Table 1). The diminution in infectivity of RNA as a result of using the latter method of injection may be due to the effect of RNA-ase in the coelomic fluid 12.

Preparations of RNA obtained from the bodies of healthy newborn mice possessed no infectivity.

In order to demonstrate that the active substance in the preparations obtained from infected animals was actually RNA and not some contaminant, we treated the RNA solutions with nucleases. The results of these experiments, which are set out in Table 2, suggest it is the RNA derived from the bodies of mice infected with A - 10 Coxsackie virus and no other substance which possesses the property of infectivity. The possibility of the infectivity of RNA being due to intact virus particles in the preparations is disproved by the complete resistance of the latter to the effect of RNA-ase.

Solutions of RNA, extracted without the use of bentonite, rapidly lose their infectivity when preserved in a refrigerator at 4° (Table 3). However, when bentonite was used in the extraction, the infectivity of the preparations were considerably stabilized, a finding which may be explained by the inactivation of RNA-ase in the presence of bentonite [13, 3].

The data obtained from this research suggests that the total RNA, obtained by the described method from the bodies of newborn mice infected with A - 10 Coxsackie virus, possesses a definite infectivity. However, it is not clear whether protein plays any part in the infectivity of the RNA, or whether the infectivity resides in the total RNA by itself. These problems require further investigation.

SUMMARY

Infective RNA was recovered from the bodies of suckling mice infected with Coxsackie A - 10 virus. Infectious RNA preparations lost their infectivity upon treatment with RNA-ase. Treatment of similar preparations with DNA-ase had no influence on infectivity. The infectivity of such preparations in subcutaneous injections to newly-born mice was considerably higher than in intra-abdominal injection. This phenomenon is attributed by the author to the inhibiting effect of RNA-ase of the cavity fluid. The infectivity of RNA preparations is almost completely lost upon their storage at 4°C during 24 h. However, bentonite used in RNA recovery markedly stabilizes it during storage, which depends upon the inactivation of RNA-ase in the presence of bentonite.

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