whereas monochloramine will have only an extremely small amount of the monochlorammonium ion (NH_3Cl^+) present: one part in 10^7 of the chloro-compound present.

The presence of this considerable amount of monobromammonium ion would account for the strong bactericidal properties of the bromo-compound, and further it would facilitate the exchange reactions as exemplified in reaction 1, the nitrogenous substances originating from the bacterial tissue.

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Infectivity of Ribonucleic Acid from Mouse Brains infected with Semliki Forest Virus

Evidence has been recently presented to show that the ribonucleic acids isolated by phenol extraction from tobacco mosaic virus[1], from ascites tumour cells in mice infected with Mengo encephalitis virus[2] and from mouse brains with Eastern equine encephalitis virus[3] are infectious. In the present communication, the infectivity of the ribonucleic acid isolated from the brains of mice infected with Semliki Forest virus is reported. This virus can also multiply in mosquitoes[4] but causes no known human disease.

Three-day-old Swiss mice were inoculated intracerebrally with 0·02 ml. of a 10^{-4} dilution of a homogenate of mouse brain infected with Semliki Forest virus. One day after infection, the brains were harvested, frozen in 'dry-ice'–ethanol and homogenized with 0·14 *M* sodium chloride–0·01 *M* sodium phosphate, *p*H 7·0, to give a 10 per cent homogenate. The supernatant is here designated as 'virus preparation'.

The ribonucleic acid was prepared by phenol extraction and precipitated twice with 65 per cent ethanol and once with 1 *M* sodium chloride following the procedures of Wecker and Schäfer[3]. The final precipitate was resuspended in phosphate buffer to a volume equal to that of the original mouse brain before dilution.

Infectivity titres, expressed as *LD*50, were determined by the intracerebral injection of serial 10-fold dilutions in mice, 0·02 ml. for one-day-old mice and 0·03 ml. for 30- to 40-day-old mice. The 10 per cent brain homogenate and the ribonucleic acid preparation were regarded as 10^{-1} and 10^{0} dilutions respectively in calculating these titres.

The ribonucleic acid preparation exhibited about 0·1 per cent of the infectivity of the infected brain homogenate from which it was prepared (Table 1). The homogenate of the brains, harvested after infection with 10^{-3} dilution of the ribonucleic acid preparation, fixed complement with anti-Semliki Forest virus serum, an indication that the virus had multiplied in the recipient mice. Thus, the ribonucleic acid preparation contains all genetic information necessary for the production of complete Semliki Forest virus.

The ultra-violet absorption spectrum of the ribonucleic acid preparation was characteristic of nucleic acid, with a maximum at 257 mμ and a minimum at 230 mμ. The ratio between the optical densities was 2·1. Protein could not be detected by the biuret reaction. However, these results do not exclude the possibility that the infectivity might be due to small amounts of contaminating virus particles.

Table 1. Comparisons of Semliki Forest Virus and Ribonucleic Acid Preparations

		Log *LD*50	
		Virus preparation	Ribonucleic acid preparation
A	No treatment	6·9	3·8
B	Control	5·5	3·7
	After ribonuclease 50 μgm./ml. 25° C.; 10 min.	5·6	*
	After deoxyribonuclease 50 μgm./ml. 25° C.; 10 min.		3·3
C	Control	3·6	1·9
	After centrifugation (top supernatant)	1·6; 1·6	1·3
D	Control	6·0	3·5
	After sodium deoxycholate 0·1 per cent; 4° C.; 1 hr.	4·0	3·7
E	Control	4·1	2·3
	After sodium deoxycholate 0·1 per cent; 4° C.; 1 hr.	2·0	2·2

* No mice died at 10^0 dilution

In attributing the observed infectivity to the ribonucleic acid itself, rather than to contamination with intact virus, previous authors have relied on the differential effects of centrifugation and nuclease digestion on crude preparations of virus and ribonucleic acid[2,3] (cf. Table 1, *B* and *C*). In the present investigation, precautions were taken to make the two reaction systems reasonably comparable before repeating the differentiating procedures. The Semliki Forest virus was diluted ten-fold and centrifuged at 40,000*g* for 1 hr. at 3° C. to remove extraneous tissue material, and the supernatant was diluted with the ribonucleic acid preparation to give an infectivity only one to two hundred times that of the ribonucleic acid system. The concentration of free ribonucleic acid was equal in the two systems.

As shown in Table 1, *B* and *C*, even under these conditions ribonuclease treatment did not reduce the infectivity of the virus preparation, presumably because the ribonucleic acid of the intact virus is protected by its protein coat. Furthermore, centrifugation sufficient to remove 99 per cent of the original infectivity of the Semliki Forest virus preparation reduced the infectivity of the purified ribonucleic acid by only 50 per cent.

In an effort to provide further evidence, the two preparations were exposed to the action of sodium deoxycholate, which has been shown to inactivate many of the arthropod-borne viruses[5]. 99 per cent of Semliki Forest virus was inactivated, but the infectivity of the ribonucleic acid preparation was not affected (Table 1, *D* and *E*).

These results, although not decisive, add to the now impressive body of evidence that the infective entity in ribonucleic acid preparations is ribonucleic acid itself and not a contaminating virus particle.

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