INCREASING RESISTANCE TO INFECTIONS WITH NUCLEIC ACID PREPARATIONS

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Experiments on mice showed that prophylactic administration of certain RNA preparations considerably increases the nonspecific resistance of the animals to infection with Salmonella equi. This increased resistance is formed 4 h after administration of the RNA. By combining prophylactic (before infection) and therapeutic (after infection) administration of sodium nulceate the percentage of surviving animals was increased. The antitoxic immunity was potentiated by administration of a combination of crude tetanus toxoid with RNA.

The writer has shown previously [2] that prophylactic administration of yeast RNA to mice increased their nonspecific resistance to infection with Salmonella equi.

This paper describes the further study of this phenomenon.

EXPERIMENTAL METHOD

Albino mice weighing 20-25 g were used. The animals received one or more intraperitoneal injection of various preparations of yeast RNA: American, British (Gee Lawson), Soviet TU 10P 197-68 (I), and high-molecular-weight TU 10P 224-68 (II), produced at the Olainskii Factory; total yeast transfer RNA [3], and also sodium nucleate in doses of 8-16 mg per mouse.

The animals were infected with various doses of a 22-h agar culture of S. equi. In some experiments the mice were immunized with 3 or 10 standard units native tetanus toxoid (batches 924 and 935) and with yeast RNA, which was incubated for 1 h at 22°C and then injected subcutaneously into the right flank of the mice. Two weeks later the animals received an injection of glycerinerated tetanus toxin (batch 655) diluted 1.7500 with physiological saline. Death of the mice receiving the toxin was recorded as a rule at intervals of 1-2 h for the first 24-30 h, and then every day for 10-11 days. In the analysis of the results the time of death of the mice was defined as the logarithm ($\log_2$) of the time of their death in hours, and a conventional survival time of 9.91 h was assigned to the surviving mice [4]. The value of LD_{50} also was determined by the method of Ashmarin and Vorob'ev [1], with the aid of the χ^2 criterion.

EXPERIMENTAL RESULTS

When all the RNA preparations were tested they were found to be effective and they considerably prolonged the life of the infected animals (Table 1). Both transfer and high-molecular-weight RNA (6.9 S) and sodium nucleate were found to be effective.

It was important to determine the minimal time during which increased resistance to infection develops. The results in Table 2 show that nonspecific resistance begins to rise slightly 2 h after injection of RNA. However, in this period the difference from the control is not yet significant. After 4 h nonspecific resistance was considerably increased; this was taken as the minimal effective time. Subsequently the resistance increased still further. Integrity of the RNA molecule is not essential for its pro-

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TABLE 1. Increase in Resistance of Mice to Salmonella Infection Produced by Various RNA Preparations (M± m)

Expt.	Preparation	Infecting dose of S. equi (number of bacterial cells)	Number of mice	Log_2 of mean life span (in h)	P
1	Transfer RNA, 7.5 mg 18 h before	640 million	9	7.62± 1.43	< 0.01
	RNA (USA), 7.5 mg, 18 h before	640 "	10	7.17± 0.7	< 0.001
	RNA-I (USSR), 7.5 mg, 18 h before	640 "	10	7.75± 1.13	< 0.001
2*	Not injected	640 "	10	4.88±1.04	–
	RNA (USA), 16 mg, 21 h before	1.28 billion	10	5.64± 0.9	< 0.05
	RNA-II (USSR), 16 mg, 21 h before	1.28 "	10	6.02± 0.68	< 0.001
	Sodium nucleate, 16 mg, 21 h before	1.28 "	9	6.59± 1.63	< 0.02
	Not injected	1.28 "	9	4.52± 0.3	–

*Death of the animals was recorded every 24 h.

TABLE 2. Dynamics of Increase in Resistance of Mice to Salmonella Infection After Injection of RNA* (M± m)

Time of injection of RNA (8 mg per mouse) before infection (in h)	Number of mice	Log_2 of mean life span (in h)	P
2	10	4,11±0,88	>0,05
4	10	4,19±0,77	<0,05
6	10	4,56±0,9	<0,02
8	10	4,94±0,9	<0,01
10	10	5,1±1,13	<0,01
12	10	4,85±0,93	<0,01
12†	11	6,59±0,91	<0,001
14	10	4,91±1,04	<0,01
16	10	5,43±1,56	<0,02
Not injected	10	3,35±0,36	—

*Mice infected with 1.28 billion S. equi cells.
†RNA heated to 100°C for 30 min.

tective effect. This effect was not abolished even after boiling the RNA for 30 min (Table 2). Indeed, it may even have been strengthened.

Tests of the protective action of sodium nucleate against Salmonella infection showed that its action is similar to that of RNA preparations [2]. In experiments on 80 mice it was more effective (injection of 8 mg per mouse 20 h before infection) if the animals were infected with large doses.

The protective action of sodium nucleate against infection was strengthened if the preparation was injected twice, once before infection and once 40 min after infection, and then given daily for 10 days thereafter. LD_{50} in this experiment was significantly ($P < 0.01$) higher than LD_{50} for the control (Table 3), although in this case also the sodium nucleate was most effective when the mice were infected with large doses of the culture.

Finally, in the last series of experiments the effect of RNA was studied on the formation of immunity to tetanus by injection of tetanus toxoid. By combining the toxoid with RNA it was possible to increase the level of immunity produced, although the effect depended on the dose of stimulator or of toxoid (Table 4).

TABLE 3. Increase in Resistance of Mice to Salmonella Infection by Means of Sodium Nucleate

Injection of sodium nucleate	Infecting doses of S. equi (in billions of bacterial cells)	Number of mice	Life span: Log_2 of mean life span (in h)	Life span: P	LD_{50}	P
Doses of 8 mg 48 and 20 h before infection	1,28	10	6,58±0,9	<0,001	$3,96 \times 10^8$	<0,01
Dose of 4 mg 40 min after infection	0,64	10	7,69±1,13	<0,01		
21 h after infection	0,32	10	8,47±1,11	>0,05		
Daily for 10 days	0,16	10	9,61±0,75	>0,05		
Not injected	1,28	10	3,89±0,77	—	$2,12 \times 10^8$	—
	0,64	10	5,2±1,54	—		
	0,32	10	7,22±1,54	—		
	0,16	10	8,52±1,09	—		

TABLE 4. Stimulation of Immunity Against Tetanus by Means of RNA*

Expt.	Injection of preparations	Number of mice	No. dying	No. surviving	χ^2	P
6*	3 standard units toxoid+16 mg RNA (British)	20	8	12	0,39	>0,05
	The same + 80 mg RNA (British)	23	3	20	6,76	<0,01
	The same + 80 mg RNA (Soviet)	22	9	13	0,34	>0,05
	3 EC units toxoid	20	10	10	—	—
7†	10 standard units toxoid + 16 mg RNA (British)	41	4	37	9,7	<0,002
	10 standard units toxoid	40	16	24	—	—
8‡	10 standard units toxoid + 16 mg RNA (British)	58	38	20	13,01	<0,001
	10 standard units toxoid	57	53	4	—	—
	Unimmunized animals	20	20	0	—	—

*Immunity with tested by injection of toxin (1:7500) 2 weeks after immunization.

†Toxin of batch 924.

‡Toxin of batch 935.

The level of immunity could be raised by increasing the dose of toxoid and giving the same dose of stimulator (experiments Nos. 6 and 7) or by increasing the dose of stimulator and giving the same dose of toxoid (experiment No. 6).

The mechanism of stimulation of antitoxic immunity is not yet clear. It is logical to suppose that the molecules of toxin and RNA may form a complex [5], thereby conferring increased immunogenicity on the antigen. However, this has not yet been demonstrated experimentally.

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