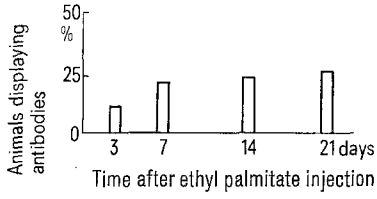
Material and methods. For the experiments, male Wistar rats (Velaz) of 220 ± 20 g of weight were used. The EP-emulsion in concentration of 0.5 g EP/ml was prepared as follows: The mixture of EP (Eastman, Rochester N.Y.) and 1% of Tween 20 in a 5% solution of glucose was first homogenized for 10 min in a glass homogenizer and then exposed to sonication for 5 min (ŠEBESTÍK et al.[6]). The particle size oscillated about 1 μm. This emulsion was injected to 4 groups of 10 animals each, into the tail vein, in amounts of 0.4 g EP per 100 g weight of the recipient. 2 days prior to injection of EP, 1.5 ml blood samples were collected from the incized tail vein of all animals for examination of antibody titers. After injection of EP, the second withdrawal for the group I, II, III and IV was performed on day 3, 7, 14 or 21 respectively. The antibodies against spleen were examined by the complement consumption test (CHUDOMEL et al.[7]). As antigen, the titrated rat spleen was used; thus less than 2% variances of biological results could be obtained.



Occurrence of auto-antibodies against spleen damaged by EP.

Results and discussion. Our experiments with rats demonstrate that auto-antibodies against spleen damaged by a single i.v. injection of EP are formed only in a limited number of animals, as shown in the graph. Where they do occur these autoantibodies are massive (semiquantitative evaluation).

The explanation why antibodies against spleens damaged by EP were formed in some animals only is difficult. We assume that a single injection of EP does not always induce antigenic changes massive enough to be normally detectable. Lastly, even the histological effects of EP have not always been of the same extent, and at a later stage after an injection of EP, signs of recovery in some spleens could be observed[3,4]. It may be useful to apply an immunization pattern using repeated smaller doses in order to establish suitable conditions for a permanent antigenic stimulus.

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Induction of Immunological Memory in Mice by RNA Extract

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Summary. RNA extract isolated from spleens of mice immunized with lipopolysaccharide from *E. coli* induced an immunological memory in normal mice. Application of small amounts of corresponding antigen provoked a specific secondary immune response in RNA primed mice.

RNA extracts have been shown to transfer immunological activity to non-sensitive lymphoid cells in certain in vivo and in vitro systems[1]. Recently MITSUHASHI and his collaborators have demonstrated that RNA extracted from mice or guinea-pigs, immunized with either diphtheria toxoid[2], sheep red blood cells (SRBC)[3], or *Salmonella flagella*[4], was capable of inducing immunological memory. RNA-primed animals are capable of responding to a small amount of corresponding antigen with a characteristic secondary immune response.

The following experiments were undertaken to ascertain whether it is possible to induce an immunological memory by RNA extract isolated from spleens of mice immunized with lipopolysaccharide (LPS) from *Escherichia coli*.

Materials and methods. Female mice of the AB strain, weighting 16 to 18 g, fed on a standard diet, were immunized with 10 μg LPS given in a single i.p. injection. LPS for immunization was extracted from *Escherichi coli* O 111:K 58 by the method of BOIVIN[5]. 5 days after antigen application, the mice were sacrificed and the spleens were pooled. RNA was isolated according to a modification of the method of ABRAMOFF[6] only with the exception of homogenizing the spleens in 0.05 *M* K-acetate buffer pH 5.8 containing 0.1% Na-dodecylsulphate and 0.001 *M* EDTA. Spleens of non-immunized mice were used for preparation of normal RNA (n-RNA). All procedures were carried out at 4 °C. RNA extracts were stored as pellets under ethanol at −20 °C until used in experiments. Spectrophotometric analysis of the RNA samples consistently showed an OD 260/280 of 1.98 to 2.10.

RNA extraction procedure provided a relatively pure preparation in which contaminations were limited to 1.5 to 2.5 μg protein and 0.5 to 1.5 μg DNA per mg RNA. Protein and DNA were measured by the method of LOWRY[7], using bovine serum albumine as standard, and the Indol test[8] respectively.

Gel chromatography of RNA samples on Sephadex G-100 (1.5 × 90 cm) in phosphate-buffered saline at pH 7.2 (PBS) yielded 3 peaks with the K_{av}-values 0.04, 0.38 and 1.00 respectively. Serum antibodies were determined by the indirect hemagglutination using the microtechni-

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Table I. Induction of secondary antibody response in mice primed with RNA extract

First stimulation	Second stimulation	Titer
500 µg n-RNA	0.01 µg LPS O 111	1:5
500 µg i-RNA	0.01 µg LPS O111	1:201
PBS	0.01 µg LPS O 111	1:5
500 µg i-RNA	500 µg i-RNA	1:5
0.01 µg LPS O 111	0.01 µg LPS O 111	1:153
0.01 µg LPS O 111	500 µg i-RNS	1:6

Titers are geometric mean of 6 mice 4 days after second stimulation. n-RNA, RNA isolated from spleens of normal mice; i-RNA, RNA isolated from spleens of immunized mice.

Table II. Specificity of immune response in mice primed with i-RNA

First stimulation	Second stimulation	Titer
250 µg n-RNA	0.01 µg LPS O 111	1:5
250 µg i-RNA	0.01 µg LPS O 111	1:108
250 µg i-RNA	0.01 µg LPS O 78	1:4

Titers are geometric mean of 6 mice 4 days after second stimulation. n-RNA, RNA isolated from spleens of normal mice; i-RNA, RNA isolated from spleens of immunized mice.

Table III. Comparison of inhibitory activity of various dilutions of *E. coli* O 111 antigen

Antigen concentration (LPS per ml)	Hemagglutination inhibition (titer)
2 µg	1:64
1 µg	1:32
0.5 µg	1:16
0.25 µg	1:8
0.12 µg	1:4

que of TAKATSY[9]. Blood samples were obtained from experimental and control mice by retro-orbital puncture at appropriate intervals. Inactivated sera were diluted two-fold in 0.05 ml PBS and 0.05 ml of a 0.5% suspension of SRBC were added. Erythrocytes were coated with LPS from *E. coli* 0 111:K 58, which was isolated by the method of WESTPHAL[10]. Hemagglutination titer are expressed as the last dilutions showing 2+ reaction.

By means of indirect hemagglutination inhibition, RNA isolated from immunized mice (i-RNA) was proved free of serological detectable antigen contaminations. RNA extracts were diluted two-fold in 0.05 ml PBS and 0.025 ml specific immune serum was added, containing 4 agglutining units. Following 1 h of incubation at 37 °C, 0.025 ml of a 1% LPS-coated SRBS suspension was added. Titers of hemagglutination inhibition are expressed as the last dilutions showing 2+ inhibition.

For inducing immunological memory, various doses of i-RNA were injected i.p. into normal mice. 3 week later, the mice were injected in the same way with 0.01 µg of the corresponding LPS as a second stimulus.

Results. Single application of 0.01 µg LPS from *E. coli* O 111:K 58 failed to elicit antibody formation in AB mice. But this amount of antigen was able to provoke specific antibody synthesis in mice primed with LPS or i-RNA extracted from the spleens of immunized mice. Priming with n-RNA isolated from the spleens of non-immunized mice was ineffective.

In most experiments normal AB mice were injected with 250 or 500 µg i-RNA, and 3 weeks later each animal received 0.01 µg of the corresponding antigen. As can be seen in Table I, already 4 days after the last injection specific antibodies were demonstrable in the sera. Table II shows that second stimulation with an RNA non-corresponding antigen as, for example, with LPS from *E. coli* O 78:K 80 did not provoke a second immune response. Single or repeated injection of i-RNA, however, failed to induce antibody synthesis. Also after injection of i-RNA in antigen-primed mice, no antibody activity was found.

By means of indirect hemagglutination inhibition test, the presence of antigen or antigenic fragments in i-RNA was demonstrable. By comparing hemagglutination inhibition caused by known amounts of i-RNA or of the corresponding antigen, the concentration of serologically demonstrable LPS in i-RNA was calculated as 0.05 to 0.20 µg LPS per mg i-RNA. But this catabolized LPS seems to have lost the immunogenicity, because the amount of LPS in the i-RNA did not elicit a secondary immune response in contrary to unaffected antigen.

Discussion. Opinions about the role of i-RNA ('Immune' RNA, 'Immunogenic' RNA) in the immune response are still contradictory. One view maintains that the i-RNA represents a messenger RNA able to induce antibody synthesis in lymphoid cells[11–13]. The opposing view suggests that the i-RNA is a carrier for the antigen or antigenic fragments. This RNA-antigen-complex is more immunogenic than the antigen alone and may act as a 'super antigen'[14–16]. These proposals may not be in conflict, since various investigators employed different techniques and antigens. Possibly more than one type of functional RNA exists.

It is generally agreed that radiolabelled antigen, after injection, is commonly found in the liver, spleen etc., but little is known about the nature of the retained antigen material. The results of our experiments indicate that the i-RNA induces an immunological memory in normal mice. This i-RNA is contaminated by catabolized antigen. The antigen moiety of the i-RNA was shown only to be able to bind to the specific antibodies but not to possess the capacity of inducing antibody formation. The antigen moiety differs from the original antigen because repeated injections of i-RNA do not provoke a booster effect. But it is not to be excluded that the antigen moiety may be able to prime lymphoid cells. Whether a combination of i-RNA and antigen is responsible for the induction of antibody formation or the i-RNA itself, remains to be clarified.

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