

# CHANGES IN NUCLEIC ACID SYNTHESIS IN THE SPLEEN DURING IMMUNOGENESIS

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After injection of typhoid vaccine into rabbits, nucleic acid synthesis in their spleen is modified. Synthesis of D-RNA is stimulated after 2-4 h, but on the 4th day (the peak of antibody formation) this stimulation is less marked. Synthesis of R-RNA is also increased after 2-4 h, and remains at the same level on the 4th day. Synthesis of DNA is increased after 6-8 h and continues to increase to a lesser degree until the 4th day.

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It can now be taken as established that an antigen stimulates DNA synthesis in an immunologically competent tissue [12, 13, 17]. Only two papers have so far been published on the synthesis of individual RNA fractions, and their results were dissimilar [14, 15].

The object of the present investigation was to study synthesis of different RNA fractions and of DNA in the early stages of immunogenesis and at the peak of antibody formation.

## EXPERIMENTAL METHOD

The index chosen to reflect the rate of nucleic acid synthesis was the intensity of incorporation of  $P^{32}$ . Since the level of incorporation of the label may depend on the specific activity of the labeled precursor, a series of preliminary experiments was carried out to determine the ratio between the specific activity of the nucleic acid and the specific activity of inorganic phosphate in the spleen.

Rabbits were immunized with a single intraperitoneal injection of heated typhoid vaccine in a dose of  $3 \cdot 10^9$  bacterial cells. At various times after immunization,  $Na_2HP^{32}O_4$  was injected intravenously in a dose of  $2 \cdot 10^5$  pulses/min/g body weight. In parallel experiments the isotope was injected into rabbits of the control group. The animals were sacrificed 2 h later. The spleens of the experimental and control rabbits were pooled respectively, the RNA fractions, DNA, and inorganic phosphate were isolated from them, and their specific activity was determined. For each experiment 4 or 5 immunized rabbits and the same number of controls were used. In experiments to study DNA synthesis only, the DNA was isolated from each spleen separately.

RNA was isolated by Georgiev's method of thermal fractionation [2]. This method yields RNA preparations with a DNA-like nucleotide composition (D-RNA), and preparations with the same nucleotide composition as ribosomal RNA (R-RNA). According to Georgiev's findings [3], D-RNA consists mainly of messenger RNA, but also contains certain other DNA-like fractions whose biological role has not yet been fully explained. R-RNA contains newly formed ribosomal RNA and its precursors.

To obtain the characteristics of the RNA preparations obtained from the spleen, their nucleotide composition was determined.  $P^{32}$ -labeled RNA preparations were hydrolyzed in 0.5 N KOH for 18 h at 37°. The nucleotides were separated by chromatography on paper [8]. The nucleotide composition of the RNA was calculated from the radioactivity of the individual nucleotides (newly formed), and also from their content (total) [9]. For comparison, the nucleotide composition of high-polymer cytoplasmic RNA, which consists almost completely of rRNA, was determined. The preparation was isolated from the spleen by Georgiev's method [1].

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TABLE 1. Ratio between Nucleotides in Isolated RNA Preparations

Preparation	$\frac{G + C}{A + U}$	$\frac{G + U}{A + C}$
D-RNA		
newly formed	0.86	1.03
total	0.91	1.07
R-RNA		
newly formed	1.73	1.01
High-polymer cytoplasmic RNA (rRNA)	1.88	1.04

Legend: G – guanylic acid, U – uridylic acid, C – cytidylic acid, A – adenylic acid.

DNA was isolated by the method of Orlov and Orlova [7] with slight modifications.

Inorganic phosphate was precipitated with a magnesium mixture [6]. The precipitate was dissolved in 0.1 N HCl. One part of the solution was used for measurement of the radioactivity, the other for determination of the phosphate concentration [16].

#### EXPERIMENTAL RESULTS

The mean data for the relative proportions of nucleotides in the isolated RNA preparations are given in Table 1. The coefficient of specificity (0.86, 0.91) for D-RNA preparations is close to the coefficient of specificity of DNA. The ratio between the nucleotides was the same in the newly formed and the total RNA of this fraction. The coefficient of specificity of the newly formed R-RNA was 1.73, and of the high-polymer cytoplasmic RNA (i.e., of rRNA, was 1.88).

The data for nucleic acid synthesis given in Tables 2 and 3 show that D-RNA synthesis was considerably stimulated in the spleen 2–4 h after injection of antigen, while synthesis of R-RNA was activated to a lesser degree.

During the first day after injection of typhoid vaccine no changes were observed in the RNA concentration in the spleen [4, 5], and judging from the DNA synthesis, the increase in cell proliferation in the first 2–4 h was still very slight. Consequently, in this period, the RNA content in the spleen did not increase and the observed increase in synthesis probably took place on account of relatively unstable molecules or as a result of the fact that the amount of newly formed RNA was small by comparison with its total content in the tissue.

This increase in RNA synthesis was only partially associated with cell division, for on the 4th day, when cell proliferation was far more intensive, the activation of D-RNA synthesis was less marked. Evidently, it was reflected in the intensification of protein synthesis observed by I. Ya. Uchitel' and co-workers [11].

On the 4th day, at the peak of antibody-formation, the level of activation of R-RNA synthesis was the same as 2–4 h after injection of antigen, while activation of D-RNA synthesis was less marked. This is in agreement with previous findings [14]. Probably the increase in RNA formation on the 4th day after injection of antigen was at least partly associated with the intensive cell proliferation taking place at this time.

The results of the study of DNA synthesis show that within 6–8 h after injection of antigen cell proliferation is intensified and continues to increase at least until the 4th day.

TABLE 2. Changes in RNA Synthesis in Spleen after Injection of Typhoid Vaccine

Time after injection of vaccine	Index	D-RNA		% activation	P	R-RNA		% activation	P
		expt.	control			expt.	control		
2-4 h 4th day	Specific activity	12 030±1 070	6 772±440	78	<0.01	6 160±310	4 295±670	44	<0.05
	Relative specific activity	8 255±1 440 2.83±0.42	5 912±320 2.16±0.14	40 31	>0.05 >0.05	7 000±480 2.41±0.29	4 852±320 1.75±0.04	44 38	<0.02 <0.05

Note. Here and in Table 3, specific activity is expressed in pulses/min/mg.

$$\text{Relative specific activity} = \frac{\text{Specific activity of RNA}}{\text{Specific activity of inorganic phosphate in spleen}} \cdot 100.$$

TABLE 3. Changes in DNA Synthesis in Spleen after Injection of Typhoid Vaccine

Time after injection of vaccine	Index	DNA		% activation	P
		experiment	control		
2-4 h 6-8 h	Specific activity	1 215±120	990±140	22	>0.05
	Relative specific activity	1 164±100	824±80	41	<0.05
18-20 h 4th day	Specific activity	0.365±0.021	0.266±0.035	37	<0.05
	Relative specific activity	1 654±150	1 065±170	54	<0.05
	Specific activity	0.638±0.036	0.438±0.057	46	<0.02
	Relative specific activity	1 748±160	943±70	86	<0.01
		0.688±0.103	0.344±0.026	100	<0.02

Note. Relative specific activity =  $\frac{\text{Specific activity of DNA}}{\text{Specific activity of inorganic phosphate in spleen}}$

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