# NUCLEIC ACID CONTENT OF LYMPHOID TISSUE IN RABBITS IMMUNIZED WITH HEATED TYPHOID VACCINE

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It has been demonstrated that there is a close relationship between antibody formation and the nucleic acid metabolism of organisms [6, 10, 11, 15]. Information is available about the accumulation of nucleic acids in cells producing antibodies [1, 2, 3, 16] and the participation of the nucleic matrix in the process of antibody output from the producer cells [7, 9]. The participation of RNA in this process is of particular significance. It has been established that RNA may convey information concerning antigens from macrophages to lymphoid tissue [8], that it may also bring about a transformation of intact lymphoid elements to those which are immunologically competent [12] and take a direct part in antibody synthesis [6, 11] during single or repeated immunizations. Considering all this, it seems only reasonable that in order to develop rational schemes and methods for carrying out immunization, the state of nucleic acid metabolism in an organism subjected to the effect of an antigen should be taken into account.

The present work is a study of the quantitative changes which occur in the nucleic acid content of lymphoid tissue during the response reaction of the organism to immunization.

## EXPERIMENTAL METHOD

In the experiment we used 72 rabbits of weight 2.2-2.5 kg. Of these animals 54 were given a single intravenous immunization with heated typhoid vaccine prepared from S. typhi, strain No. 44-46. The antigen dose consisted of 3 billion microbial bodies. Eighteen rabbits were used as control animals. At various lengths of time after immunization the rabbits were killed by exsanguination and after perfusion of the blood system with physiological saline to remove all traces of blood, their axillary, subscapular and popliteal lymph nodes and spleens were dissected out. The spleens were given supplementary washings with physiological saline and afterwards dry weight determinations were carried out on all the organs mentioned.

The total nucleic acid content and free nucleotide content of the isolated lymph nodes and the spleen was determined by Schmidt and Thannhauser's method using the modification suggested by P. G. Tsanev and G. G. Markov [5]. At the same time as we killed and carried out these determinations on the immunized rabbits, total nucleic acid content and individual RNA content determinations were carried out on the control animals.

The antibody titer was determined by the agglutination reaction using a double dilution method involving blood serum and homogenates of the lymph nodes and spleen.

The results of the nucleic acid content determinations for lymphoid tissue were expressed in terms of 1 mg dry weight for the tissue and analyzed statistically using students' t test.

## EXPERIMENTAL RESULTS

I. Total Nucleic Acid Content and Free Nucleotide Content of Lymph Nodes and Spleen in Immunized Rabbits. Immunized rabbits were killed at 1, 2, 3, 4, 5, 7 and 9 day intervals after injection of antigen, using 5-9 animals at each interval of time. We took 18 intact rabbits at each time interval and used them as control animals.

TABLE 1. Changes in the Total Nucleic Acid Content and Free Nucleotide Content of Lymph Nodes and Spleens from Rabbits Subjected to Intravenous Immunization with Heated Typhoid Vaccine (As μg/mg Dry Weight)

| Organ | Statistical index | "The norm" | Nucleic acid content after immunization | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | After 1 day | After 2 days | After 3 days | After 4 days | After 5 days | After 7 days | After 9 days |
| Spleen | $M \pm m$ / $P$ | 20,04±0,370 | 19,14±0,710 >0,25 | 20,51±0,490 >0,25 | 21,94±0,332 <0,001 | 21,20±0,650 >0,1 | 21,25±0,410 <0,05 | 20,96±0,430 >0,1 | 20,29±0,646 >0,5 |
| Axillary lymph node | $M \pm m$ / $P$ | 22,70±0,550 | 21,85±1,140 >0,25 | 24,00±1,459 >0,25 | 26,24±1,259 <0,02 | 27,60±0,730 <0,001 | 27,21±1,280 <0,01 | 25,11±0,710 <0,02 | 24,12±0,604 >0,05 |
| Subscapular lymph node | $M \pm m$ / $P$ | 23,16±0,601 | 22,40±0,709 >0,25 | 23,92±1,591 >0,5 | 24,87±1,008 >0,1 | 27,71±0,790 <0,001 | 27,90±1,590 <0,02 | 23,97±0,770 >0,25 | 23,13±0,710 >0,5 |
| Popliteal lymph node | $M \pm m$ / $P$ | 20,47±0,680 | 22,47±0,443 <0,05 | 22,77±0,600 <0,02 | 24,51±1,202 <0,01 | 24,14±1,320 <0,05 | 22,44±0,960 >0,1 | 23,35±0,230 <0,001 | 21,68±0,631 >0,25 |

TABLE 2. Changes in RNA Content of Lymph Nodes and Spleens from Rabbits after Intravenous Immunization with Heated Typhoid Vaccine (μg/mg Dry Tissue)

| Organ | Statistical index | "The norm" | RNA content after immunization | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | After 1 day | After 2 days | After 3 days | After 4 days | After 5 days | After 7 days | After 9 days | After 14 days |
| Spleen | $M \pm m$ / $P$ | 5,30±0,150 | 5,10±0,210 >0,5 | 4,81±0,519 >0,25 | 6,47±0,172 <0,001 | 5,84±0,560 >0,25 | 6,11±0,230 <0,02 | 6,79±0,470 <0,01 | 5,28±0,210 — | 4,47±0,190 <0,01 |
| Axillary lymph node | $M \pm m$ / $P$ | 5,03±0,299 | 5,25±0,238 >0,5 | 5,03±0,577 — | 6,72±0,550 <0,02 | 7,66±0,610 <0,01 | 6,45±0,346 <0,01 | 7,64±0,580 <0,01 | 5,88±0,579 >0,25 | 5,06±0,590 >0,5 |
| Subscapular lymph node | $M \pm m$ / $P$ | 5,22±0,296 | 5,23±0,273 — | 4,84±0,860 >0,5 | 6,59±0,550 <0,05 | 6,80±0,967 >0,1 | 6,88±0,340 <0,01 | 7,31±0,340 <0,001 | 6,28±0,453 <0,05 | 4,30±0,390 >0,05 |
| Popliteal lymph node | $M \pm m$ / $P$ | 5,13±0,400 | 5,13±0,304 — | 4,56±0,688 >0,25 | 6,25±0,270 <0,05 | 6,05±0,510 >0,1 | 5,90±0,210 >0,1 | 6,77±0,750 >0,05 | 5,16±0,322 — | 5,06±0,370 >0,5 |

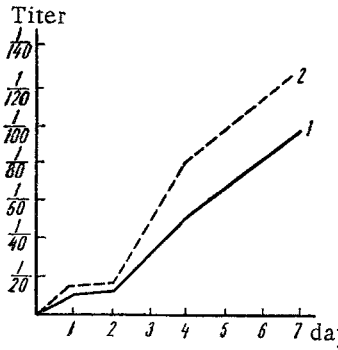


Fig. 1. Agglutinin titers of lymph node homogenates (1) and spleen homogenates (2) from immunized rabbits.

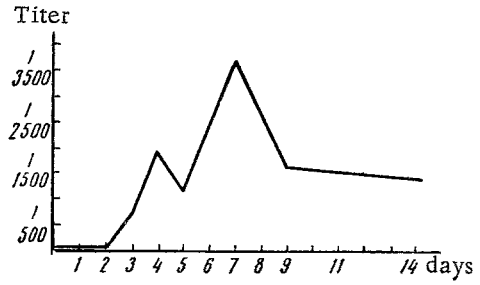


Fig. 2. Agglutinin titers of immunized rabbit serum.

The results of the experiment are set out in Table 1. As the total nucleic acid content of the organs under investigation showed an insignificant amount of variation in normal rabbits, we regarded it as practical to combine these values together in the table under the expression "the norm".

The observable difference between the nucleic acid content of the popliteal lymph node on the one hand and the axiallary and subscapular lymph nodes on the other is statistically (in the first case $P < 0.02$ and in the second $P < 0.01$).

The nucleic acid content of the lymph nodes and the spleen increases after intravenous injection of heated typhoid antigen. A statistically significant increase in the nucleic acid content of the popliteal lymph node can be detected as early as 1 or 2 days after immunization (the increase is 9.7 and 11.2%). By the third day a significant increase is detectable in both the popliteal lymph node (19.7%) and the axillary (15.5%), whereas on the fourthday it is shown by all 3 groups of lymph nodes (11, 21.5 and 17.9% respectively). On the fifth day the level of nucleic acid in the axillary and subscapular lymph nodes shows a further rise but that of the popliteal node has begun to fall. By the seventh day the amount of nucleic acid in the axillary and popliteal lymph nodes exceeds its initial level by a statistically significant amount. In all the investigated groups of lymph nodes the nucleic acid content has returned to approximately normal value by the ninth day.

The level of nucleic acid in the spleen shows a statistically significant increase by the third day after immunization and this is repeated on the fifth day. However, it has returned to its original value by the ninth day. The data obtained suggest that as a result of immunization, the nucleic acid level of the lymphoid tissue is increased such that it reaches a maximum value on the third, fourth or fifth day of the experiment; this level is maintained in some of the organs under investigation until the seventh day.

II. RNA Content of Lymph Nodes and Spleen of Immunized Rabbits. We used 12 rabbits as control animals and 54 rabbits as experimental material in these investigations. The latter were killed at 1, 2, 3, 4, 5, 7, 9, and 14 day intervals after injection with a single dose of antigen, 4-9 animals being taken at each time interval. The RNA content of the specified lymph nodes and spleen was determined. The results of the experiment are set out in Table 2.

During the first 2 days after intravenous injection of corpuscular vaccine the RNA content of lymphoid tissue showed no statistically significant deviation from the norm. However, a significant increase in the amount of RNA in the organs under investigation had taken place after 72 h as follows:—axillary lymph node (by 33.6%), subscapular lymph node (by 26.6%), popliteal lymph node (by 21.8%). By the fourth day of the experiment, a significant increase in the RNA level was recorded only in the case of the axillary node (52.2%) but on the fifth and seventh days significant increases were found for the axillary (28.2 and 51.8% respectively) and the subscapular (31.8 and 40%) lymph nodes.

No statistically significant change in the RNA content of the popliteal lymph node took place over this period, but the nature of the RNA content curve for this node corresponded to that for the nucleic acid content values in the other nodes. By the ninth and fourteenth days after injection of antigen the RNA content of all lymph nodes had returned to within the limits of the initial value.

The amount of RNA in the spleen after intravenous immunization increased by a statistically significant amount during the period of the third, fifth, and seventh days of the experiment; the increases were 22, 15.2 and 28.1% respectively. By the ninth day the RNA content had returned to its normal value, but on the fourteenth day it had dropped to a significantly lower value ($P < 0.01$).

III. Antibody Content of Serum and Lymph Node and Spleen Homogenates from Immunized Rabbits. The tissues of the named organs were homogenized after refrigerating in physiological saline at a dilution of 1:10. After centrifugation (30 min at 4000 revs/min), the agglutination reaction of the supernatant liquid was tested.

Homogenates of lymph glands and spleens from rabbits 24 and 48 h after immunization gave low antibody titers (0-1:15). By the fourth day after immunization the titers had markedly increased (Fig. 1). This tendency continued until the seventh day. After that the agglutination titer of the homogenates decreased.

The antibody titer for serum began to rise on the third day after immunization, it attained a high value on the fourth day, decrease somewhat on the fifth day and then rose again to a maximum on the seventh day (Fig. 2). Hence, there is a definite parallelism between the accumulation of antibody in lymphoid tissue and the level of antibody in the serum.

The facts recorded above suggest that the amount of nucleic acid in the lymphoid tissues increases as a result of immunization. On comparing the changes which occur in total nucleic acid and free nucleotide levels with the curve for changes in RNA level, it is seen that there is a general similarity between the two. In both cases a statistically significant increase in value is reached for all the organs under investigation on the third day of the experiment and this higher value is preserved in the majority of cases throughout the fourth, fifth, and seventh day period, disappearing on the ninth day of investigation.

A certain amount of variance in the results is noticeable on the fourth day following immunization, for on that day the total amount of nucleic acid in the lymphoid tissue attains a maximum, but the RNA level in 2 lymph nodes and in the spleen shows no significant difference from the original. This variance is evidently related to the process of mitosis accompanying the immunological reorganization of lymphoid tissue [17]. As is well known, mitosis is preceded by activation of cellular metabolism, the appearance of the mitotic apparatus and an increase in the RNA content of the cell [13]. During mitosis, the metabolic activity of the chromosomes decreases, which is partly attributable to a suppression of RNA synthesis [14]. A comparison between the changes in RNA content of lymphoid tissue and the concentration of antibody in the serum of immunized animals provides us with a basis for asserting that the following parallelism exists between RNA content and serum antibody titer: increase in the serum antibody titer on the third day is accompanied by an increase in the RNA level of lymph organs; a certain amount of fall in the amount of RNA content on the fourth day is accompanied by a drop in the antibody titer on the fifth day and finally, an increase in the RNA content on the fifth and seventh days is accompanied by a rise in the antibody titer on the seventh. The presence of such a parallelism suggests that during the period of active mitotic division synthesis of antibody by the cells does indeed suffer some diminution.

The results of our experiments lead us to the conclusion that increase in the RNA content of lymphoid tissue following intravenous immunization with corpuscular vaccine is related to an activation of protein synthesis—the synthesis of specific antibodies and also, possibly to an increase in the mitotic activity of cells which produce the antibodies. This latter hypothesis, however, requires further confirmation.

The data which have been obtained confirm the view that all lymphoid tissues are implicated in the immunological reaction resulting from intravenous immunization. Our particular method of investigating the total nucleic acid content in lymphoid tissues makes possible a more valid appraisal of the processes which occur during immunological reorganization of the organism and this may be useful in evolving rational schemes and methods of immunization.

## LITERATURE CITED


1. A. P. Gindin and Kh. V. Forshter. In book: "Histochemical Methods in Normal and Pathological Morphology," 205 (1958).
2. S. I. Ginzburg-Kalinina. Zh. Mikrobiol, 9, 52 (1961).
3. V. N. Kartasheva, E. F. Vakarina, and V. P. Vodis. In the book "Problems of Immunity", 22 (1960).
4. A. S. Spirin, Biokhimiya, 5, 656 (1958).
5. P. G. Tsanev and G. G. Markov, Biokhimiya, 1, 151 (1960).
6. R. W. Dutton and J. D. Pearce, Nature, 194, 93 (1962).
7. M. Feldman, D. Elson, and A. Globerson, Ibid., 185, 317 (1960).
8. M. Fishman, J. exp. Med., 114, 837 (1961).
9. B. D. Jankovic and H. F. Dvorak, Proc. Soc. exp. Biol. (N. Y.), 108, 564 (1961).

10. M. F. LaVia, F. W. Fitch, and C. H. Gumolerson, In book: J. H. Heller (ed.) Reticuloendothelial Structure and Function. New York, 45 (1960).
11. O. Mäkelä and G. J. V. Nossal, J. exp. Med., 115, 231 (1962).
12. J. A. Mannick and R. H. Egdahl, Science, 137, 976 (1962).
13. D. Mazin and D. Prescott. In the Book "Problems of Cytophysiology", Moscow, 38 (1957).
14. D. Mazia. In the Book: The Cell. Biochemistry, Physiology, Morphology. New York, 3, 77 (1961).
15. J. Mitchell and G. J. V. Nossal, Nature, 197, 1121 (1963).
16. M. Simic, D. Circovic and D. Marincovic. Bull. Sci. Conseil. Acad. RPF Yougoslavie, 6, 103 (1961).
17. R. Wissler, F. Fitch, M. LaVia et al., J. cell. comp. Physiol., 50, 1, 265 (1957).

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**All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.**

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