NUCLEIC ACIDS AND THE PRODUCTION OF ANTIBODY BY PLASMA CELLS

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Plate 7

(Received for publication, March 19, 1949)

The formation of antibody in lymph nodes of mice and rabbits is an established phenomenon (1, 2). Following the injection of antigen into the footpads of rabbits antibody in the popliteal lymph node was found to reach a maximum by the 5th and 6th days, followed by a rapid decline (2, 3). On the 5th day the lymphoid cells of the efferent lymph of this node contained 5 to 7 times as much antibody as the supernatant lymph plasma; on the 7th day the ratio had dropped to 2 to 3 (4). Lymphoid cells from minced lymph nodes also contained high concentrations of antibody (5–7). It was concluded from these findings that the lymphoid cells[1] are concerned with antibody production (4, 8).

After the publication of the above observations important European contributions, made during the war, became known in this country. Bing and Plum (10), Undritz (11), and others pointed out that, patients with hyperglobulinemia have an increase of plasma cells in their tissues, and that the highest globulin levels are found in patients with plasma cell myeloma, whereas patients with lymphatic leukemia show no increase in globulins. Bjørneboe and Gormsen (12) observed that in rabbits hyperimmunization causes marked plasma cell proliferation in spleen and other organs. Recently Fagraeus (13) showed that antibody may be formed in cultures of spleens from rabbits previously injected with antigen. It was found that tissue which contained abundant plasma cells formed larger amounts of antibody than tissue which included Malpighian bodies. These observations suggest that the plasma cell, rather than the lymphocyte, is responsible for antibody production.

Recent studies (14, 15) have led to the thesis that the synthesis of protein is related to the nucleic acids. With special spectrographic and cytologic methods Caspersson and others have obtained evidence from which it would appear

[1] The lymphoid cells in lymph are generally regarded as lymphocytes (9) which led us to use the term *lymphocytes*. The lymphoid cells in lymph nodes are known to include plasma cells; therefore, Doughterty, Chase, and White, (5, 7) were correct in their use of the term *lymphoid cells*.

that the multiplication of chromosomes is associated with the formation of desoxyribose nucleic acid (DNA), whereas the production of cytoplasmic protein is linked with that of ribose nucleic acid (PNA). In studies of the metabolism of cytochrome *c* in regenerating rat liver Drabkin (16) has demonstrated that the production *in vivo* of certain specialized proteins, at least, is related to PNA.

As the relationship of nucleic acids to the synthesis of proteins appears to be pertinent in the problem of antibody production, we have undertaken to compare the formation of this protein with the changes in nucleic acids in lymph nodes.

EXPERIMENTAL

All experiments were performed on Chinchilla rabbits weighing about 1.8 kilos. Forty-one animals received 0.5 ml. of "febrile antigen typhoid 0" (Lederle) into each foot-pad. Nine others served as controls. The experimental animals were sacrificed 1, 2, 3, 4, 5, 6, and 9 days after the injection of the antigen.

DNA and PNA were determined by the method of Schneider (17) as adapted by Drabkin (16). Both popliteal lymph nodes were studied in 39 rabbits. Lymph drawn from the efferent lymph vessel of these nodes was examined in 10 animals; but though an average of 0.62 cc. of lymph was obtained from each leg, no measurable quantity of DNA or PNA was detected in either the cells or the supernatant plasma of this fluid.

Microscopic studies were carried out in 11 rabbits. It is well known that DNA is restricted mainly to nuclei, whereas PNA occurs largely in cytoplasm, but also in nucleoli. DNA can be demonstrated histologically with methyl green or malachite green, and PNA with pyronine or acridine red. One of the two nodes was fixed in 95 per cent alcohol and stained with methyl green and pyronine. The other was fixed in Zenker-formol and stained with malachite green and acridine red (18), and with Azur II and eosin.

RESULTS

The results of the experiments are given in Table I and Text-fig. 1. As four rabbits (Nos. 17, 23, 25, and 43) were found, at sacrifice, to have lost weight from diarrhea, they were omitted from the text-figure.

The weight of the two lymph nodes was increased 160 per cent after 2 days, 180 per cent after 4 days, and 188 per cent after 6 days. The DNA content of the nodes was increased at these dates 127 per cent, 157 per cent, an 185 per cent. The quantity of PNA had a different relationship, increasing 169 per cent after 2 days, 214 per cent after 4 days, and 306 per cent after 6 days. A study of the ratio of PNA to DNA revealed that the relative increase in PNA was greatest between the 4th and 6th days which is the period when antibody production is greatest.

The variations in the amounts of the two nucleic acids observed in our experiments correspond to those of the antibody titers reported previously (2–4). They appear conditioned by variations in the state of nutrition and health of the animals as revealed by data from four diseased rabbits, which showed

TABLE I

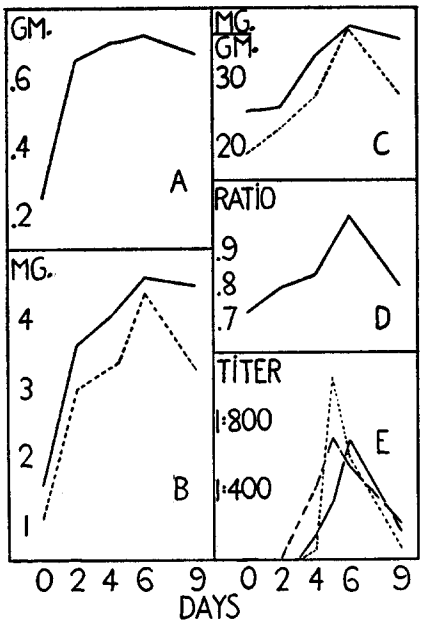
DNA and PNA Concentrations and Contents in the Two Popliteal Lymph Nodes Following the Injection of Typhoid Vaccine into Each Foot-Pad

	Rabbit		Lymph node weight	DNA			PNA			Ratio $\frac{\text{PNA}}{\text{DNA}}$
	No.	Weight		Concentration		Total	Concentration		Total	
				Wet	Dry		Wet	Dry		
		kg.	*gm.*	*mg./gm.*	*mg./gm.*	*mg.*	*mg./gm.*	*mg./gm.*	*mg.*	
Control	11	1.7	0.19	6.73	25.4	1.33	4.35	16.4	0.825	0.62
	12	1.7	0.19	6.73	25.4	1.33	4.35	16.4	0.825	0.62
	13	1.7	0.31	5.7	25.4	1.77	5.3	23.6	1.64	0.93
	14	1.65	0.36	5.8	9.5	2.09	4.6	7.5	1.66	0.79
	15	1.65	0.21	5.0	—	1.05	3.5	—	0.735	0.70
	36	1.8	0.19	—	—	—	3.6	20.6	0.68	—
	37	1.85	0.37	6.34	33.4	2.35	4.1	21.6	1.52	0.65
	38	1.85	0.18	6.24	34.9	1.12	4.2	23.5	0.76	0.68
2 days	5	2.0	0.67	3.55	20.5	2.31	—	—	—	—
	6	2.0	0.98	4.1	20.9	4.25	—	—	—	—
	16	1.85	0.63	7.4	25.8	4.66	5.6	19.5	3.53	0.76
	17*	1.65	0.20	6.1	17.8	1.34	3.7	10.8	0.81	0.60
	18	1.9	0.80	5.1	25.5	4.08	3.2	16.0	2.56	0.63
	33	1.95	0.72	6.67	22.5	4.8	7.0	30.6	5.04	1.05
	34	1.95	0.39	6.55	35.2	2.55	5.3	28.5	2.07	0.81
	35	1.6	0.39	6.45	29.2	2.52	4.4	19.9	1.72	0.68
4 days	1	1.85	0.72	5.0	23.0	3.6	5.15	21.7	3.505	1.03
	2	2.05	0.72	5.0	23.0	3.6	4.3	21.7	3.505	0.86
	19	1.75	0.63	5.4	34.4	3.4	3.7	23.6	2.33	0.69
	20	1.6	0.615	6.8	39.8	4.18	6.0	35.1	3.69	0.88
	21	2.05	0.6	5.2	24.8	3.12	4.1	19.5	2.46	0.79
	39	1.85	0.76	5.92	38.3	4.52	4.8	30.9	3.65	0.81
	40	1.75	0.93	6.55	40.3	6.09	5.25	32.3	4.88	0.80
	41	1.75	0.6	6.61	40.3	3.97	5.1	31.1	3.06	0.77
6 days	3	2.0	0.97	6.17	37.1	5.98	4.6	27.6	4.46	0.75
	4	1.7	0.63	6.6	36.8	4.16	4.3	24.0	2.71	0.65
	22	1.7	0.71	5.4	31.1	3.83	6.3	36.3	4.47	1.17
	23*	1.45	0.32	6.6	38.7	2.11	6.8	39.9	2.18	1.03
	24	1.65	0.64	6.7	40.5	4.29	6.9	41.7	4.42	1.03
	42	1.85	0.61	6.55	41.1	4.0	8.3	52.5	5.06	1.285
	43*	1.6	0.31	5.78	27.8	1.79	7.75	37.3	2.4	1.34
	44	1.9	0.74	6.5	40.3	4.81	7.0	43.4	5.18	1.08
9 days	7	2.15	1.04	6.84	39.5	7.11	2.78	16.	2.89	0.41
	8	1.85	0.47	7.45	35.6	3.5	3.22	15.4	1.51	0.43
	25*	1.75	0.36	6.3	33.3	2.27	5.9	31.2	2.12	0.94
	26	1.75	0.63	7.2	29.7	4.54	7.2	29.7	4.54	1.00
	27	1.4	0.65	6.0	30.7	3.9	5.4	27.6	3.51	0.90
	46	1.8	0.54	6.28	40.8	3.39	7.5	48.7	4.05	1.19

* These four rabbits were found to have diarrhea when sacrificed.

little or no increase in weight or nucleic acid content of their lymph nodes. However, the ratio of PNA to DNA was qualitatively the same as in the healthy animals, indicating that diseased animals reacted similarly to healthy ones.

A study of the slides of the rabbits showed that during the first 6 days when PNA and antibody formation were greatest, the cellular response was chiefly that of plasma cells. The lymphocytes began to proliferate significantly on the 3rd or 4th day, and germinal centers began to appear on the 4th or 5th day.



TEXT-FIG. 1. Weights (A), and the DNA (——) and PNA (- - -) contents (B), dry weight concentrations (C) and ratios (D), of the two popliteal lymph nodes following the injection of typhoid vaccine into each foot-pad. E shows antibody titers in these nodes following the injection of typhoid vaccine (——), dysentery vaccine (- - -), and sheep erythrocytes (.....).

These cells, however, were fully active only on the 9th day when PNA and antibody formation had passed their peaks.

Controls (No. 28).—Cortex and medullary cords were poorly developed and contained mostly lymphoid cells resembling small lymphocytes. In the medullary cords and the cortical tissue adjacent to the sinuses one saw a few plasma cells. The lymphocytes revealed little granular PNA in their scanty cytoplasm, whereas the plasma cells showed abundant cytoplasm diffusely filled with pyronine-positive material. There were a few immature lymphoid cells in both cortex and medullary cords. They differed from the mature forms by their larger nucleus and by the presence of abundant PNA not only in their cytoplasm, but also in their nucleolus. The reticulo-endothelial cells contained some coarse inclusions, some of which were pyronine-positive.

Experimental Findings.—

After 24 hours (No. 49).—The lymph nodes were now inflamed. The afferent lymph vessels and the sinuses were filled with granulocytes, macrophages, and fibrin, and there were many granulocytes in the connective tissue surrounding the nodes. The macrophages showed all transitions from monocytes to histiocytes and contained faintly pyronine-positive granules in their cytoplasm. The granulocytes contained no PNA. In addition to these exudative changes, one saw signs of proliferation in the medullary cords and the cortical tissue adjacent to the sinuses. There were a good many immature lymphoid cells with large PNA-containing nucleoli and a heavily pyronine-stained cytoplasm. Many of these cells showed mitotic figures.

2 days (Nos. 29, 48).—The exudative reaction which had been so prominent on the first day was considerably diminished. The macrophages of the sinuses were markedly swollen and contained many vacuoles; they showed singularly little pyronine-positive material. The medullary cords and the cortical tissue adjacent to the sinuses were now greatly enlarged and contained many immature lymphoid cells with large PNA-positive nucleoli and heavily pyronine-stained cytoplasm (Fig. 1). There were many mitotic figures. More mature plasma cells were also seen. These contained little or no nucleolar PNA, but their cytoplasm was heavily stained with pyronine and often showed a light vacuole near the nucleus. The bulk of the cortex, on the other hand, was still at rest (Fig. 2). It did not differ significantly from that seen in untreated animals.

3 days (No. 50).—The medullary cords and the cortical tissue adjacent to the sinuses showed a picture similar to that on the 2nd day. The follicles and other portions of the cortex now contained a few immature lymphoid cells resembling the immature cells in the medullary cords except that they contained less cytoplasm and stained fainter with pyronine. A section through the efferent lymph vessel of the node revealed mostly small lymphocytes. A few immature cells which were present showed PNA-positive nucleoli, while plasma cells were not observed.

4 days (Nos. 31, 51).— In the medullary cords and the cortical tissue adjacent to the sinuses mature plasma cells predominated now in many places. Some of these cells showed what may be interpreted as shedding of cytoplasm. In other places, however, actively dividing immature lymphoid cells still prevailed. The cortex was considerably enlarged and showed a good many immature lymphoid cells not unlike those in the medullary cords except that they contained less pyronine-positive material. In a few places early germinal centers were detected. It appeared that the lymphoblasts rapidly differentiated into small lymphocytes, and this would account for the enlargement of the cortex.

5 days (No. 52).—The medullary cords and the cortical tissue adjacent to the sinuses were now crowded with mature plasma cells (Fig. 3). Dividing plasmoblasts were still present but greatly diminished in number. The cortex was greatly enlarged and contained many immature lymphoid cells with large PNA-positive nucleoli and abundant pyronine-positive cytoplasm. There were many mitotic figures. But again the PNA concentration was lighter than in the immature cells in the medullary cords. There were many early germinal centers with numerous lymphoblasts and medium sized lymphocytes. Their cytoplasm was less abundant and more faintly stained with pyronine than that of the plasmoblasts. The PNA occurred in fine granules rather than in the diffuse and compact distribution observed in the plasma cell series (Fig. 4). The cells contained in the efferent lymph vessel of this node were mostly small lymphocytes. However, some immature lymphoid cells were also present, and there were a few plasma cells with a small round nucleus and abundant heavily pyronine-stained cytoplasm showing a light vacuole near the nucleus.

6 days (Nos. 30, 45).—One of the rabbits (30) showed essentially the same picture as that sacrificed on the 5th day (Fig. 3) except that there was less proliferation of lymphocytes in the cortex. The other animal revealed a reduction in the number of plasma cells in the medullary

cords and elsewhere. The remaining plasma cells showed what may be interpreted as shedding of cytoplasm. Some showed pyknosis indicating disintegration. The germinal centers resembled those of rabbit 52 (Fig. 4). Nuclear phagocytosis was not yet conspicuous.

9 days (No. 32).—The medullary cords and the cortical tissue adjacent to the sinuses were now markedly depleted of plasma cells. The cortex, on the other hand, contained very large typical germinal centers with many dividing lymphoblasts and medium sized lymphocytes, and there were many phagocytes containing nuclear debris. The immature lymphocytes of the germinal centers contained distinctly less pyronine-positive material than the immature plasma cells described earlier. The PNA occurred in fine granules, and not in the diffuse or compact distribution of the plasma cell series. The cells contained in the efferent lymph vessel of the node appeared to be small lymphocytes. Cells resembling plasma cells or immature lymphoid cells were not seen.

DISCUSSION

It has long been known that lymphoid tissue is rich in both DNA and PNA. The thymus of the rabbit, which contains no plasma cells, but consists largely of lymphocytes, has been found to have from 1.8 to 2.5 mg. of DNA phosphorus per gm. of wet tissue. This is a very high value for the concentration of DNA in mature tissue. The PNA content of the thymus is also considerable, about 0.9 to 1.0 mg. of phosphorus per gm. of wet tissue. However, the ratio of PNA to DNA in thymus (sheep and calf) is among the smallest observed, only 0.2 to 0.3, whereas that of liver is 2.5 to 4.5 (sheep, rabbits, and rats) (14–16).

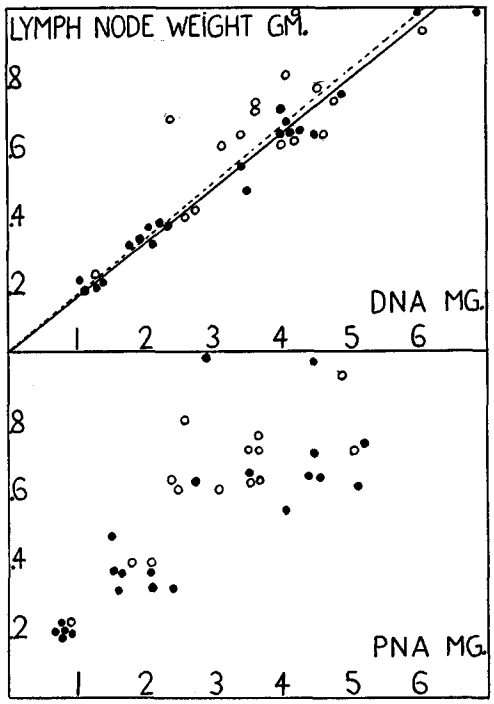
The nucleoproteins of individual lymphocytes have been studied by Thorell (19), who stated: "... large lymphocytes have an endocellular nucleic acid metabolism indicating a high intensity of growth. The small lymphocytes contrary to this seem to have a very low intensity of growth." The various observations are in accord with the fact that lymphocytes consist largely of nuclear material. Unlike other cells, they develop little cytoplasm nor do they form a complex Golgi apparatus (20), or any other conspicuous cytoplasmic organelle.

The nucleic acids of lymph nodes, which contain plasma cells as well as lymphocytes, have received little attention. Andreasen (21, 22) who studied the total nucleic acid content of thymus, lymph nodes, and spleen of rats from 12 hours to 2 years of age, found that in lymph nodes the concentration of nucleic acid phosphorus rose from 1.71 mg. per gm. of wet tissue at the age of 15 days to 2.19 mg. per gm. at 180 days and then fell again.

The nucleoproteins of individual plasma cells have been studied by Zylberszac (23), van den Berghe (24), and Bing, Fagraeus, and Thorell (25). It was shown that the basophilia of the cytoplasm of these cells was due to PNA (23, 24), and that in this respect plasma cells were greatly different from histiocytes (macrophages) (24). Plasma cells produced experimentally in rabbits were derived from plasmoblasts resembling early erythroblasts and myeloblasts. During maturation, their nucleoli like those of other hemocytoblasts diminished gradually, while the PNA concentration in the cytoplasm unlike that of other blood cells was fully retained (25).

A study of the DNA turnover in normal lymphoid tissues of rats with radioactive phosphorus (P^{32}) as measured by the percentage ratio of nucleic acid P^{32} over free plasma P^{32} (22) revealed that in the thymus of adult rats 5 to 6 per cent was renewed in 3 hours, whereas in lymph nodes and spleen only 1 to 2 per cent was turned over.

In 1 month old animals the renewal was 6.5 to 8.5 per cent in the thymus and 1.5 to 5.2 per cent in lymph nodes and spleen. These figures are in accord with the known fact that lymphocytes are short lived cells and therefore are constantly regenerated, whereas the plasma cells and other elements contained in lymph nodes and spleen have a longer life and are reproduced at a slower speed.



TEXT-FIG. 2. DNA and PNA contents of the two popliteal lymph nodes compared with the weights of these nodes following the injection of typhoid vaccine into each foot-pad. The broken line represents the average wet weight concentration of the nucleic acids in all animals (• and ◦), the continuous line those with the exception of the rabbits sacrificed after 2 and 4 days (◦).

The results reported in the present communication are consonant with the above reports in the literature. It was observed that the changes in total content of DNA in the popliteal lymph nodes paralleled the changes in weight of these nodes. The mass of the nodes plotted against the content of DNA yields a straight line (Text-fig. 2). Quantities of DNA which were significantly lower than the average of 6.1 mg. per gm. of wet tissue (of all animals) or 6.3 mg. per gm. (if the animals sacrificed after 2 and 4 days are excluded) were found only in the nodes of some rabbits sacrificed on the 2nd and 4th days of the experiment when inflammatory exudate was still present. Thus, our findings are in

accord with the view that the production of DNA is connected with the multiplication of chromatin. They suggest that the increase in weight of the lymph nodes following the injection of antigen after the initial exudative phase is due largely to multiplication of cells.

The PNA concentration of the lymph nodes was considerably higher than that of the thymus observed by other investigators. The ratio of PNA over DNA varied from 0.41 to 1.34. It resembled that of the spleen of sheep and rats (14, 15). Moreover, there was no direct relation to the weight of the nodes (Text-fig. 2). While the total quantity of PNA (Text-fig. 1 B) increased with the weight of the node (A), both the dry weight concentration (C) and the ratio of PNA over DNA (D) show clearly that the greatest rise in PNA occurred between the 4th and 6th days, that is appreciably later than the rise in DNA or the increase in mass.

If the chemical results are compared with the histologic findings, it is evident that during the first 4 days when the increase in DNA was greatest the cellular reaction was one of multiplication especially of plasmoblasts. Mature plasma cells began to appear in large numbers only on the 4th day; they were the predominating cells on the 5th and 6th days; thereafter they diminished rapidly. Thus, the greatest rise in PNA concentration occurred when the plasma cells reached maturity. The highest figures of PNA were observed when the cells were fully mature.

The lymphocytes began to proliferate in significant numbers on the 3rd and 4th days, and germinal centers began to appear on the 4th and 5th days; the latter were fully active only on the 9th day. It is worth noting that the rise in lymphocytes did not prevent the PNA from dropping. This accords well with the repeatedly demonstrated comparatively low PNA content of lymphocytes.

It has been mentioned earlier that it has been demonstrated that following a single injection of vaccine antibody formation in the regional lymph nodes occurs chiefly between the 4th and 6th days (Text-fig. 1 E). The highest concentrations were found on the 5th and 6th days. This is precisely the time when the concentration of PNA was highest and mature plasma cells were found to be present in largest numbers. On the other hand, the lymphocytes at this time were in an early stage of proliferation; they reached their greatest development only several days after the peak of antibody formation. These observations would appear to leave little doubt that in these lymph nodes the antibody was formed by plasma cells.

Our previous finding of antibody in the lymphoid cells of the efferent lymph (4) has been interpreted to signify that the lymphocytes may likewise form antibody. Against this interpretation stands the fact that lymphatic leukemias in contrast to plasma cell myeloma are not associated with hyperglobulinemia. Also, Harris, Rhoads, and Stokes (26) were unable to extract significant quantities of antibody from the thymus of immunized animals. The present study

of methyl green- and pyronine-stained sections through efferent lymph vessels suggests that some of the lymphoid cells that leave the lymph nodes during the period of antibody formation are plasmoblasts and plasma cells. It is possible also that large shed fragments of cytoplasm of plasma cells were contained in the efferent lymph and spun down with the lymphoid cells when they were separated from the lymph plasma.

Fagraeus (13) and others advocated the view that the plasma cells are derivates of the reticulo-endothelium. Her results with tissue cultures led her to believe that the formation of antibody "takes place side by side with, and during, the development of the reticulum cells into plasma cells. With the appearance of mature plasma cells the titre *in vitro* will again decline." She concluded that antibody is "formed within reticuloendothelial cells. In case of intense antibody formation a differentiation of these cells into plasma cells takes place. Thus the mature plasma cell is to be regarded as the final link in a chain of developments, a cell which has already passed the stage of its greatest functional intensity."

It should be pointed out that Fagraeus, like many others, does not distinguish between immature reticulum cells (undifferentiated mesenchymal cells) and mature reticulo-endothelial cells (macrophages). There is no evidence to show that the highly differentiated macrophages have prospective potentialities. In fact, this assumption is contrary to all knowledge of ontogeny. There is little doubt, however, that the undifferentiated mesenchymal cells if properly stimulated give rise to immature plasma cells (transitional cells of Fagraeus).

In our experiments, plasma cell production was well under way 24 hours after injection of vaccine. There were many plasmoblasts, many of which showed mitotic figures, indicating that multiplication of these cells was accomplished, like that of other blood cells, chiefly by mitotic division at the hemocytoblastic level. Maturation of plasmoblasts to plasma cells was associated, as in other blood cells, with reduction in the size of the nucleus and disappearance of the PNA in the nucleolus. It was recognizable on the 2nd and 3rd days, it was conspicuous on the 4th day, and it was completed on the 5th day of the experiment. The maturation of the plasma cells thus paralleled the rise in PNA and antibody concentration in the lymph nodes. The concentration of both was greatest when the plasma cells had reached full maturity.

Fagraeus' observation that tissue cultures of splenic tissue containing mature plasma cells at the time of explantation produced less antibody than cultures with immature elements may well be explained by the greater vulnerability of mature lymphoid cells which has been noted repeatedly in experiments with salicylates and other alarm-reacting stimulants which will be reported elsewhere. It is conceivable that the manipulations to which the explanted tissue was exposed damaged the mature cells more than the immature elements. However, it is likely that antibody synthesis in the cytoplasm of plasma cells,

like hemoglobin synthesis in erythrocytes, is accomplished during maturation of these cells, and that synthesis ceases when maturity has been reached. However this may be, our experiments seem to show that maximum concentration of antibody in the plasma cells is attained only when these cells are fully mature.

SUMMARY

A study has been made of the relationship of antibody formation and the changes in amount of the nucleic acids in rabbit lymph nodes draining areas injected with typhoid vaccine.

The increase in DNA was found to parallel the increase in weight of the nodes, as might be expected from the active multiplication of cells.

The peak of PNA increase occurred between the 4th and 6th days after vaccine injection when antibody formation was at its maximum.

A histologic study of methyl green- and pyronine-stained sections of the nodes revealed that during the first 6 days of the experiment the cellular reaction was chiefly one of plasma cells. During the first 3 days plasmoblasts predominated; on the 5th and 6th days mature plasma cells were the prevailing cells. Most of the PNA was contained in the plasma cells.

The lymphocytes began to proliferate in significant numbers on the 3rd and 4th days, and germinal centers began to appear on the 4th and 5th days. They showed their greatest activity only on the 9th day when PNA and antibody formation had passed their peaks.

These results are interpreted as indicating that the plasma cell and not the lymphocyte is responsible for antibody formation.

BIBLIOGRAPHY

1. McMaster, P. D., and Hudack, S. S., *J. Exp. Med.*, 1935, **61,** 783.
2. Ehrich, W. E., and Harris, T. N., *J. Exp. Med.*, 1942, **76,** 335.
3. Ehrich, W. E., Harris, T. N., and Mertens, E., *J. Exp. Med.*, 1946, **83,** 373.
4. Harris, T. N., Grimm, E., Mertens, E., and Ehrich, W. E., *J. Exp. Med.*, 1945, **81,** 73.
5. Dougherty, T. F., Chase, J. H., and White, A., *Proc. Soc. Exp. Biol. and Med.*, 1944, **57,** 295.
6. Kass, E. H., *Science*, 1945, **101,** 337.
7. White, A., and Dougherty, T. F., *Endocrinology*, 1945, **36,** 207.
8. Ehrich, W. E., *Ann. New York Acad. Sc.*, 1946, **46,** 823.
9. Drinker, S. K., and Yoffey, J. M., Lymphatics, Lymph, and Lymphoid Tissue, Cambridge, Harvard University Press, 1941.
10. Bing, J., and Plum, P., *Acta med. scand.*, 1937, **92,** 415.
11. Undritz, E., *Helvet. med. Acta*, 1938, **5,** 548.
12. Bjørneboe, M., and Gormsen, H., *Acta path. et microbiol. scand.*, 1943, **20,** 649.
13. Fagraeus, A., Antibody Production in Relation to the Development of Plasma cells, Stockholm, Esselte aktiebolag, 1948.

14. Symposia of the Society for Experimental Biology (Great Britain), Cambridge University Press, 1947, **1.**
15. Nucleic acids and nucleoproteins, in Cold Spring Harbor Symposia on Quantitative Biology, Cold Spring Harbor, Long Island Biological Association, 1948, **12.**
16. Drabkin, D. L., *J. Biol. Chem.*, 1947, **171,** 395.
17. Schneider, W. C., *J. Biol. Chem.*, 1945, **161,** 293; 1946, **164,** 747.
18. Hitchcock, C. H., and Ehrich, W. E., *Arch., Path.*, 1930, **9,** 625.
19. Thorell, B., *Nord. Med.*, 1945, **28,** 2115.
20. Ehrich, W. E., *Ergebn. allg. Path. u. path. Anat.*, 1934, **29,** 1.
21. Andreasen, E., *Acta path. et microbiol. scand.*, suppl. 49, 1943.
22. Andreasen, E., and Ottesen, J., *Acta physiol. scand.*, 1945, **10,** 258.
23. Zylberszac, S., *Acta biol. belg.*, 1941, **1,** 429.
24. Van den Berghe, L., *Acta biol. belg.*, 1942, **2,** 390.
25. Bing, J., Fagraeus, A., and Thorell, B., *Acta physiol. scand.*, 1945, **10,** 282.
26. Harris, T. N., Rhoads, J., and Stokes, J., *J. Immunol.*, 1948, **58,** 27.

EXPLANATION OF PLATE 7

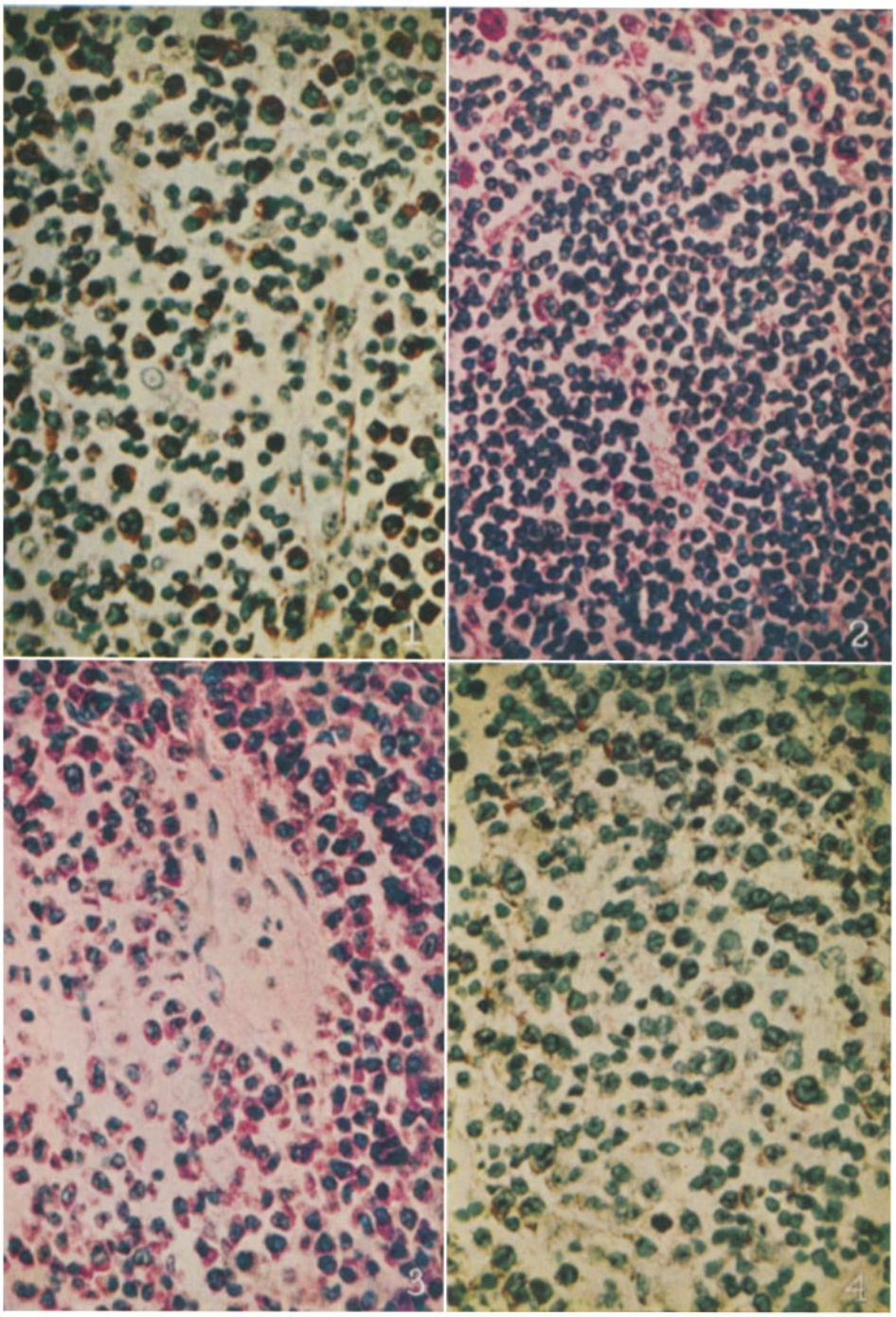
The tissue was fixed in alcohol and stained with methyl green and pyronine. The magnification of the sections is about × 400.

FIG. 1. Rabbit 29. 2 days after injection of vaccine. Medullary cord showing many lymphoid cells (plasmoblasts) with heavily pyronine-stained cytoplasm.

FIG. 2. Same. Cortical tissue showing small lymphocytes with little pyronine-stained material.

FIG. 3. Rabbit 30. 6 days after injection of vaccine. Medullary cord crowded with mature plasma cells.

FIG. 4. Same. Cortical tissue showing proliferating lymphoblasts and lymphocytes.



(Ehrich *et al.*: Nucleic acids and production of antibody)