NUCLEIC ACID SYNTHESIS AFTER EXPERIMENTAL BCG VACCINATION AS SHOWN BY HISTOAUTORADIOGRAPHY

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After vaccination of guinea pigs with BCG, nucleic acid synthesis is activated in the lymph glands and spleen. Synthesis begins to increase 3-7 days after injection of the vaccine and reaches a maximum from 3 weeks to 1.5 months later in the case of DNA and 1.5-3 months later in the case of RNA, thereafter decreasing between the 3rd and 8th months.

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In connection with the concepts of tissue and cellular immunity in tuberculosis, the study of proliferative and synthetic phenomena during its formation is extremely important. It has long been known that after BCG vaccination, resulting in the development of immunity, proliferative reactions take place in the lymph glands, spleen, and lungs, and the synthesis of RNA and proteins is stimulated [1-3, 7, 9]. However, information concerning the times of development of these reactions, the morphological basis of vaccination, and the cells taking part in them is extremely contradictory.

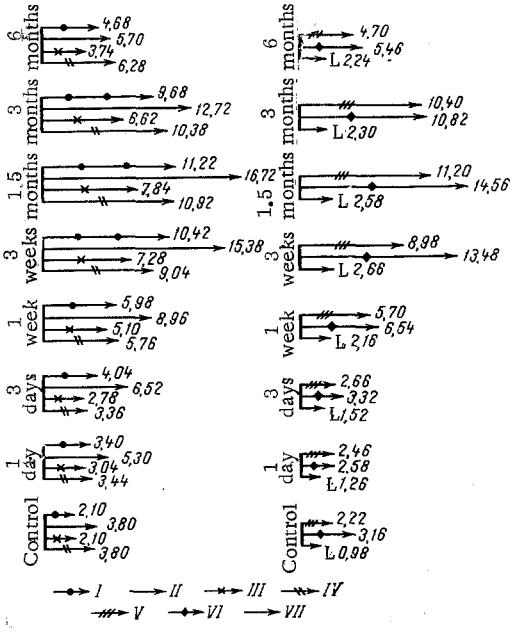

Fig. 1. Dynamics of incorporation of thymidine-H^3 into cells of immunocompetent organs. I and II) regional lymph glands, central and peripheral zones respectively; III and IV) distant lymph glands, medullary cords and follicles respectively; V and VI) red and white pulp of spleen respectively; VII) lung.

In the present investigation the dynamics of synthesis of nucleic acids, whose role in immunologic processes is difficult to overestimate [4-6, 8-10], was studied after BCG vaccination.

EXPERIMENTAL METHOD

Experiments were carried out on 80 guinea pigs, 70 of which were vaccinated with a 2-week BCG culture (0.1 mg per animal) and sacrificed from 1 day to 8 months thereafter. All the animals were injected 2 h before sacrifice with DNA precursor (thymidine-H^3) or RNA precursor (uridine-H^3) in a dose of 0.5 μCi/g body weight. Labeled cells (per thousand cells) were than counted in zones of sections from different organs.

EXPERIMENTAL RESULTS

Analysis of the quantitative results obtained in the study of DNA synthesis (Fig. 1) shows that soon after vaccination (1-3 days) a marked increase in incorporation of radioactive label was observed only in the regional lymph glands losing their follicular structures (mainly in the cortical zone). Starting from one week after vaccination, a marked increase in DNA synthesis (compared with the control) took place in the remote lymph glands and spleen. Later, an increase in label-incorporating activity was observed in all the lymph glands and spleen between 3 weeks and 1.5 months after vaccination (Fig. 2). After 3 months

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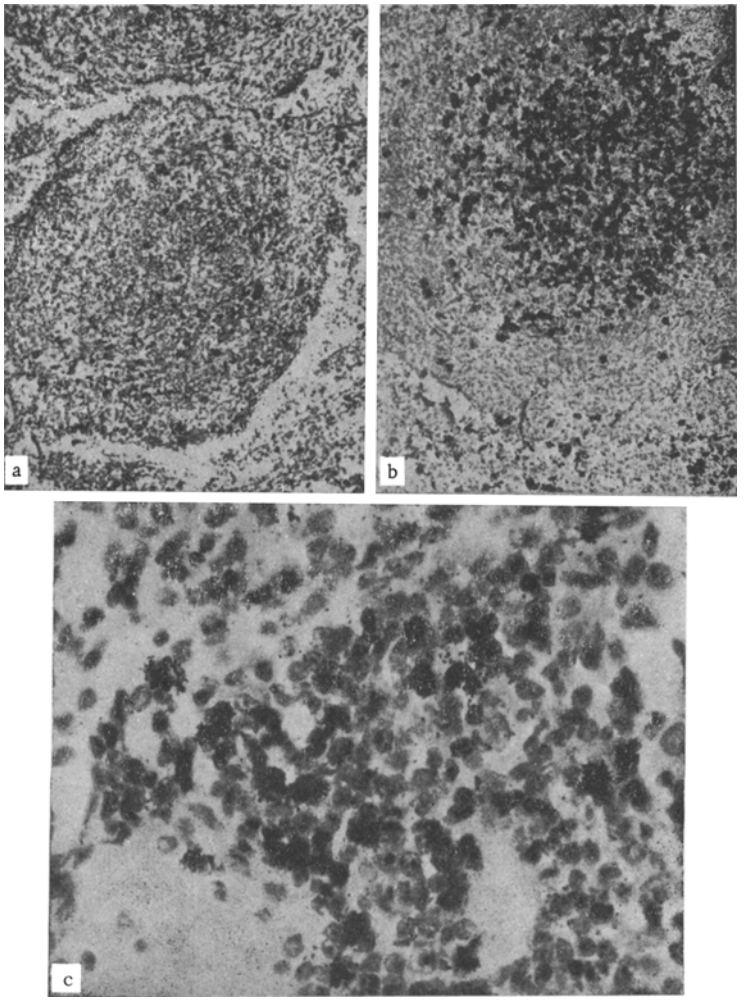

Fig. 2. Incorporation of thymidine-H^3 into spleen follicle cells (a) of unvaccinated animal and (b) 1.5 months after vaccination, 180×; c) incorporation of uridine-H^3 into red pulp cells of spleen 3 months after vaccination, 400×.

DNA synthesis began to decrease slightly, subsequently falling sharply until 6 months after vaccination, although still remaining at a higher level than in the control animals.

A statistically significant increase in DNA synthesis in the regional lymph glands ($P < 0.005$) was observed from 1-3 days after vaccination, continuing until 3 weeks, while the intensity of synthesis fell significantly between 3 and 6 months. A statistically significant increase in synthesis took place in the distant lymph glands and spleen from 1 to 3 weeks after vaccination, with a decrease also between 3 and 6 months. None of the changes in DNA synthesis in the lungs were statistically significant.

Analysis of RNA synthesis in the organs showed (Fig. 3) that cells of the regional lymph gland also participated in synthesis most rapidly and actively, and in the early periods (until 1 week) they were labeled much more intensively than cells of the spleen and distant lymph glands. From 3 weeks to 1.5 months a further increase in labeling activity took place, the quantitative differences between RNA synthesis by the different immunocompetent organs at these periods largely disappearing. After 3 months the intensity of RNA synthesis by all organs fell somewhat, except in the spleen, where synthesis reached a maximum during this period (Fig. 2), while 8 months after vaccination labeling activity had fallen considerably although it still remained at a higher level than in nonvaccinated animals.

A statistically significant increase in RNA synthesis in the lymph glands and spleen ($P < 0.005$) was observed starting from the first week after vaccination, and the decrease in synthesis was significant between 3 and 8 months.

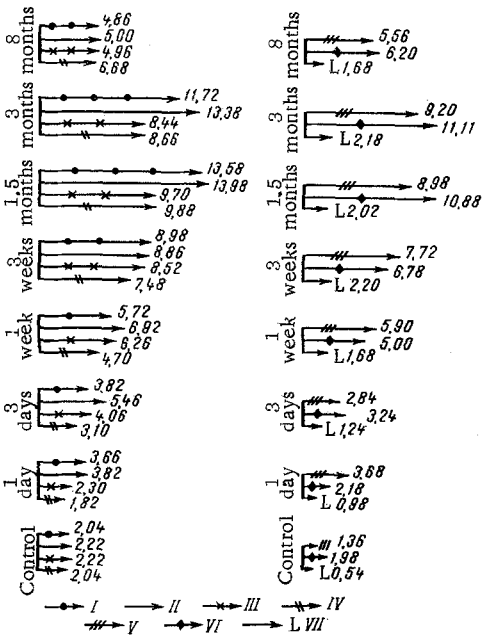

Fig. 3. Dynamics of incorporation of uridine-H^3 into cells of immunocompetent organs. I and II) regional lymph glands, peripheral and central zone respectively; III and IV) distant lymph glands, medullary cords and follicles respectively; V and VI) red and white pulp of spleen respectively, VII) lung

After injection of thymidine-H^3, the activated reticulum cells and blast cells were most intensively and frequently labeled; immature plasma cells, large lymphocytes, and endothelioid cells were less frequently labeled; and small lymphocytes and mature plasma cells rarely incorporated thymidine-H^3.

After injection of uridine-H^3, immature plasma cells, blast cells, activated reticulum cells, large lymphocytes, and endothelioid cells were most frequently and intensively labeled, while small lymphocytes and mature plasma cells rarely incorporated uridine-H^3.

It was noted that the proliferative and synthetic powers of different cells after BCG vaccination (as shown by histoautoradiographic data) frequently did not coincide with results of corresponding histochemical investigation. For example, blast cells stained very weakly by Feulgen's method, while they synthesized DNA actively; the opposite picture was observed when the content and synthesis of DNA in small lymphocytes were compared. The same opposite relationships occurred when synthesis and histochemically determined content of RNA were compared in immature and mature plasma cells. However, this should not be taken to mean that any of the methods mentioned is unreliable or faulty. It is well known that histoautoradiography with labeled precursors gives the quantitative picture of synthesis at one particular moment, while histochemical staining methods demonstrate the total content of a given substance, synthesized previously and obtained from precursor cells or by other ways, in the cell.

The results thus show that activation of nucleic acid synthesis in the cells of immunocompetent organs is a constant component of vaccination resulting in the developement of immunity against tuberculosis.

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