

# I. EXPERIMENTAL PROBLEMS

## **SPLEEN LYMPHOCYTE BLAST TRANSFORMATION IN THYMECTOMIZED CHICKENS**

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In 1956 B. Glick et al. establish that the surgical remove of Bursa Fabricii in birds tends to a reduction or even stop of antibody-production (6). Later, J. F. A. P. Miller (1961) shows that the thymectomy of new-born mice is the reason for an insufficiency of the lymphocyte population in blood and lymphoid organs (tissues), non-capability of allograft rejection and untimely death due to infection (10).

Consequently certain authors conclude that only birds possess a clear anatomical separation of the central lymphoid organs (thymus and Bursa Fabricii) in relation to T- and B-lymphocyte production (3, 4, 5, 11, 13). Despite that between these two cell types exist definite relations which are the object of many investigations and hypothesis (1, 2, 9).

Concerning the role of T- and B-lymphocytes in the cell immune response, also minding that the thymus is a central lymphoid organ responsible for the T-cell maturity, we have for an object of our study the blast-transformation ability of spleen lymphocytes by phytohaemagglutinin (PHA) in thymectomized chickens. This reactivity towards PHA is characteristic for the T-lymphocyte population expressing its immunologic activity.

### **Materials and methods**

16 non-inbred chickens (breed White Leghorn) from the incubator of the Biological Department at the Higher Institute of Medicine, Varna city, were used in the study. On the first day of their hatch the chickens were divided into two groups: control (8) and experimental (the rest 8); experimental chickens were surgically thymectomized. 6 weeks later the spleens of all chickens were aseptically taken out and placed in a Petri-dish with Medium 199 (Institute Pasteur), enriched with 20% calf serum (IIPD, Sofia), 0.01 ml 20% glucose/ml, penicillin 100 U/ml, streptomycin 50 mg/ml (12). Cell suspension was prepared in a sterile box and left to sedimentation for a period of 30 min at 37° C. Supernatant was removed, alive cells were counted and diluted to a concentration of  $2 \cdot 10^6$  cells/2 ml culture. 2 series of 3 test-tubes each (for any of the chickens) were worked out: the first with PHA (IIPD, Sofia), the second — without PHA; concentration of PHA — 8 mg/ml culture (12). The test-tubes were placed in the incubator at 37° C for 72 hours. After that preparations were stained by using the method of Giemsa. Blast transformation was studied after counting of 1000 cells of each preparation under a light microscope (magn. 1000).

## Results and discussion

Table 1 shows that the PHA — application provides a considerable increase of blast-transformation for the controls (approximately 3.5 times at  $p < 0.001$ ) when compared to thymectomized chickens (approximately 1.8 at

Table 1

Chickens	Number	% of lymphocyte transformation	
		without PHA	with PHA
Control	8	21.04±3.65	75.85±6.23
Thymectomized	8	13.18±3.75	24.29±7.76

$p < 0.01$ ). From the cited results it can be concluded that thymectomy of chickens on the first day after hatch influences the immunological activity of spleen T-lymphocytes. This is confirmed by the fact that neonatal thymectomy decreases the blast index at spontaneous blast transformation approximately 1.6 times ( $p < 0.001$ ), whereas at induced with PHA transformation the decrease is 3 times ( $p < 0.001$ ).

These data prove that there begins a possible change of the interrelations between B- and T-lymphocytes in cultures of thymectomized donors and most of all bursadependent lymphocytes prevail in the mixture; the latter are probably not sensitive towards PHA (7).

Despite the thymectomy was done on the first day after hatch, when the immune system is still not enough mature, certain percent of the lymphocytes are contributed to a blast-transformation. It is acceptable that even during the embryonal development some extrathymus centres of T-cell proliferation are formed, similar to extrabursal centers of B-lymphocyte proliferation, as B. D. Jankovic presumes (8).

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**БЛАСТНАЯ ТРАНСФОРМАЦИЯ ЛИМФОЦИТОВ СЕЛЕЗЕНКИ  
ТИМЭКТОМИРОВАННЫХ ЦЫПЛЯТ**

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**Р Е З Ю М Е**

Селезеночные лимфоциты шестимесячных тимэктомированных цыплят показали более низкую степень бластной трансформации по сравнению с контролями. Несмотря на то, что тимэктомия была сделана в первый день вылупления, когда иммунная система все еще незрелая, часть лимфоцитов тимэктомированных птиц превращалась в бластные клетки. Авторами допускается возможность формирования экстратимусных очагов Т-клеточной пролиферации в течение эмбрионального периода.