

Reactivity of Spleen Germinal Centres in Immunized and ACTH-treated Chickens

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Abstract

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The aim of the study was to estimate the reactivity of spleen white pulp structures in chickens immunized with sheep erythrocytes (SRBC) and remaining under the influence of ACTH. The 11-week-old chickens were divided into four groups. Group I constituted the control, while chickens of groups II and IV were immunized with SRBC. Chickens of groups III and IV were given ACTH. The sections of thymus, bursa of Fabricius and spleen were done and used for histological evaluation. The number of germinal centres of the types I and II was estimated on the determined spleen surface.

It was shown that after SRBC administration in the thymus cortex and in medulla of bursa of Fabricius, proliferation of lymphoid cells was visible. However, after ACTH administration in the discussed thymus and bursa parts, lymphocyte depletion was observed. Morphometric analysis of the spleen showed that after the administration of SRBC and ACTH there is observed the increase in the number of the type II germinal centres.

The results of the study indicated the diversified antigen influence on the morphological picture of the lymphatic organs. They also showed different sensitivity of particular structures to administered ACTH. Moreover, the earlier observations and current results allowed to work out the model of formation and movement of different types of germinal centres in the spleen.

Chickens, spleen, germinal centres, ACTH, SRBC

Numerous environmental factors evoke in an animal organism a set of functional and morphological changes, called stress by H. Selye. These changes most often lead to an adaptation, making it possible for the organism to survive in the changing conditions. According to Puvadolpirod and Thaxton (2000ab), in birds it is difficult to specify a typical stress picture and predict the course of stress. This results not only from the variety of stressors but most of all, from the character of the reaction, in which main co-ordination systems of the organism play a dominant part. An important role is played by the endocrine system and its hormones, especially glucocorticoids which, ensuring the development of adaptation, at the same time contribute to protection against excessive activation of endogenous processes which threaten with self-destruction (Tomaszewska and Przekop 1997; Mitchell and Kettlewell 1998; Goldstein and McEwen 2002). A prolonged activation of the hypothalamic-pituitary-adrenal axis is responsible, among others, for metabolic changes and changes in cells and tissues of the lymphatic system.

It has been shown that both the stressors and parenteral administration of ACTH to birds leads, to clear changes in circulating blood, through activation of the above-

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mentioned axis, besides the release of adrenal steroids, increased level of glucose in the serum and involution of lymphatic organs. This is accompanied by immunosuppression with a simultaneous change of the lymphocyte distribution in peripheral lymphatic tissues, including the spleen (Dohms and Metz 1991; Mashaly et al. 1993; Trout and Mashaly 1994; Piquer et al. 1995; Puvadolpirod and Thaxton 2000cd).

It appears that both the blood picture and the changes of reactivity of spleen structures depend to a high degree on the time of action and kind of factor (Graczyk 1999; Puvadolpirod et al. 2000bc). A particular susceptibility was displayed by germinal centres and periellipsoidal lymphatic tissue (PEL) which are regarded as bursa-dependent structures of the spleen (Graczyk 1994ab; Graczyk and Kuryszko 1995a; Graczyk 1998, 1999).

The above reasons make it advisable to undertake a study, considering the morphology and function of organs engaged in the course of stress.

The objective of the study was to trace the effect of ACTH on the morphology and reactivity of the lymphatic system of chickens immunized with sheep erythrocytes (SRBC). Special attention was paid to the structure of white pulp of the spleen, the organ that plays a basic role in immune response.

Materials and Methods

Female chickens, Hisex Brown hybrids, aged 1 day were used in the study. The birds were kept in accordance with zoohygienic standards for poultry. During all the experiment the birds had access to water, and were fed *ad libitum* a standard feed mixture.

In the 11th week the chickens were randomly divided into four groups, 8 birds in each. Group I constituted the control. Chickens of groups II and IV received an intravenous dose of 0.5 ml 5% SRBC suspension 6 days before sampling the material. Chickens of groups III and IV were given ACTH (Synacthene-Ciba) at a dose of 0.01 mg/100 g body weight (ca 1 IU ACTH/100 g body weight) 24 hrs after administration of SRBC. ACTH administration was repeated two more times, at 2-day intervals.

The samples were collected from birds of each group. Material from birds treated only with ACTH (group III) and from birds treated with ACTH, following SRBC immunization (group IV) was taken on the 6th day after SRBC administration, and 24 hr after the last ACTH injection.

Prior to sampling, body weight of all the experimental chickens was determined. Blood was taken from wing vein of each bird, and blood smears were made. Following staining according to May-Grünwald-Giemsa, leukogram was made by counting 200 consecutive leukocytes (magnification $\times 1000$).

Following decapitation, thymus, bursa of Fabricius and adrenal glands were dissected and weighed. On this basis, the relative weight of these organs was calculated in mg/100 g body weight. Sections of the organs were stained with hematoxylin and eosin (H&E) for histological evaluation.

Spleen was taken from three birds of each group. The number of germinal centres (GC) of type I and II was determined on serial sections stained with hematoxylin and eosin (H&E) and with van Gieson's method. Counts were made on the area of 2.625 mm² including the subcapsular zone and the deep zone of spleen white pulp. The centres were classified according to criteria described in our earlier papers (Graczyk 1994ab; Graczyk and Kuryszko 1995b).

Besides, considering the role of white pulp structures in immunogenesis, especially the avian spleen structure, different from that found in mammals, during histological examination attention was paid to periellipsoidal lymphatic tissue (PEL) and periarteriolar lymphatic tissue (PAL).

Statistical calculations were conducted using StatSoft, Inc. (2001), STATISTICA (data analysis software system), version 6. . Data was compared using one- and two-way ANOVA (Brandt 1997). Significance of differences between treatment means ($P \leq 0.05$ and $P \leq 0.01$) was determined using Tukey's Multiple Range Test.

Results

Haematological studies

The percentage of particular leukocyte types is shown in Table 1. The data indicate that only in chicken injected with ACTH (group III) there was a decrease in the proportion of lymphocytes and increase in heterophils.

Table 1
Percentage of leukocytes in SRBC-immunized chickens after triple ACTH administration (means \pm SD)

	Group I K (n=8)	Group II SRBC (n=8)	Group III ACTH (n=8)	Group IV SRBC+ACTH (n=8)
Lymphocytes - (L)	62.16 ^b ± 14.72	65.33 ^b ± 7.68	56.66 ^a ± 9.75	62.52 ^b ± 10.17
Heterophils - (H)	31.33 ^b ± 13.86	30.5 ^b ± 8.31	40.33 ^a ± 11.63	29.57 ^b ± 11.48
Eosinophils	2.66 ± 2.25	1.33 ± 1.03	0.83 ± 0.98	1.85 ± 1.67
Basophils	3.36 ± 1.21	2.83 ± 1.16	1.83 ± 1.47	6.0 ± 3.26
Monocytes	0.16 ± 0.4	0	0.16 ± 0.4	0
H/L - ratio	0.50	0.46	0.71	0.47

a,b,A,B means in a row with no common superscripts are significantly different: $a,bP \leq 0.05$, $A,BP \leq 0.01$

Body weight and relative weights of organs

No clear differences in the body weight were observed between particular experimental groups, it was shown, however, that the relative weight of thymus and bursa of Fabricius differed (Table 2).

Table 2
Body weight and relative weight of thymus, bursa of Fabricius and adrenal glands in SRBC-immunized chickens after triple ACTH administration (means \pm SD)

	Group I K (n=8)	Group II SRBC (n=8)	Group III ACTH (n=8)	Group IV SRBC+ACTH (n=8)
Body weight (BW)g	1 080.00 ± 147.9	1 166.83 ± 178.03	1 068 ± 104.00	1 081 ± 88.80
Thymus relative weight mg/100g BW	444.24 ^{ab} ± 115.63	585.57 ^a ± 58.56	420.48 ^b ± 88.37	489.38 ^{ab} ± 124.82
Bursa of Fabricius - relative weight - mg/100g BW	232.02 ^a ± 43.09	264.04 ^a ± 74.21	250.18 ^a ± 80.38	354.02 ^b ± 120.49
Adrenal glands relative weight mg/100g BW	9.35 ^a ± 2.11	12.85 ^b ± 1.47	10.93 ^{ab} ± 2.93	11.76 ^b ± 1.61

a,b,A,B means in a row with no common superscripts are significantly different: $a,bP \leq 0.05$, $A,BP \leq 0.01$

In immunized chicken, compared to the control group, the thymus weight was by ca 30% higher while in birds immunized and treated with ACTH there was only an increase tendency in the weight of this organ (Table 2). Administration of ACTH alone led only to a slight decrease in the thymus weight.

Likewise, in immunized chickens and in those that were administered ACTH there was a slight increase in the weight of bursa of Fabricius while immunization and ACTH administration caused an over 50% increase in the weight of the organ (Table 2).

Because of the wide scatter of individual values of the weight of adrenal glands, in spite of some differences between the groups, it was difficult to evaluate unambiguously the results (Table 2).

Morphology of thymus, bursa of Fabricius, spleen and adrenal glands

In the thymus of birds immunized with SRBC the cortex widened, with an accompanying increase in the number of thymocytes of clear mitotic characters.

Following ACTH administration, in the cortex and medulla of the lobule there was a clear depletion of thymocytes, with vacated places, where macrophages of considerable phagocytic activity appeared (presence of material derived from thymocyte degradation in their cytoplasm). Combined administration of SRBC and ACTH caused a slight regeneration of thymocytes, with single centres of their proliferation. Numerous epithelial cells located in the zone of thymocyte proliferation of the cortex became multiplied.

In bursa of Fabricius of chickens immunized with SRBC a narrowing of cortex of lymphatic follicles was observed, in favour of the widened medulla where fine accumulations of lymphocytes forming secondary follicles were found. Following ACTH administration, in the bursa of Fabricius there appeared the narrowing of the follicular medulla with a slight depletion of lymphocytes.

Estimating the histological structure of adrenal glands it is noteworthy that in chickens, contrary to mammals, cells of the cortical and medullar parts do not form separate layers. They intertwine in the whole gland. The cortical zone is built of polygonal, cylindrical, closely adjoining cells with oval nuclei and light cytoplasm. Next to them there are cells of elongate nucleus and compact chromatin. Cells located in the central part of adrenal glands often stain darker than those located peripherally.

Twenty-four hours after the last, third dose of ACTH in the adrenal glands there was a clear hypertrophy of the fascicular and reticular zone of the organ. The cells of the widened striae were enlarged, with large vesicular nuclei and well-marked nuclear membrane, as well as numerous nucleoli. Chromatin substance, usually displaced to the periphery, adjoined the nuclear membrane in the form of granules. Some cells were in various phases of karyokinesis. Besides the spongy cells there were cells or cell strands with dark cytoplasm of blurred structure. The number of cells with small nuclei and compact chromatin also decreased.

The effect of the described changes in the adrenal cortex was the disappearance of the medulla because of pressure. In the adrenal glands of SRBC and ACTH-treated birds dark cells multiplied more intensely, and the capillary vessels were strongly filled with blood. Within the cortex fine, intensely staining lymphatic follicle appeared.

In the chicken spleen the type I germinal centres located near the pulpal arteries dominated in the picture. They had a form of well-developed spherical structures of lympho-reticular tissue surrounded by fully organised fibrous connective tissue and had no blood vessels. The fibres of the connective tissue surrounding the centres often connect with the fibre system of the outer membrane of arterial vessels (Plate V, Fig. 1). The structure of lympho-reticular tissue has a form of numerous reticular cells with lymphocytes between them.

Germinal centres classified as type II centres are smaller, less numerous and located in the compact zone between the pulpal arteries and the penicilar arterioles (Plate V, Fig. 2). They are built of reticular cells and large lymphocytes accumulated mainly in the central part. They were surrounded by a poorly marked network of connective tissue fibres.

In immunized chickens and ACTH-treated birds the histological picture of spleen did not differ significantly from that found in the control group. Only in ACTH-treated birds type I centres were surrounded by a well developed connective tissue sheath, often with a multi-layered arrangement of fibres which is characteristic of centres that are more advanced in their development.

Periellipsoidal tissue (PEL) of the chicken spleen (Plate VI, Fig. 3) is distributed around terminal portions of arterioles. PEL clusters are built of reticular cells, with intercellular spaces in its internal part filled with concentrically arranged large lymphocytes, and in the external part small lymphocytes.

The periarteriolar tissue (PAL) (Plate VI, Fig. 4) is located around pulpal arteries (corresponding to central arterioles in mammals). The arteries have a characteristic high endothelium and clearly reduced mid membrane. PAL forms large accumulations, mainly of reticular cells and small lymphocytes.

In ACTH-treated chickens a stimulation of cellular structure of periellipsoidal tissue and, to a larger extent, periarteriolar tissue (especially in its central zone) was observed.

Morphometric studies on the spleen

The number of germinal centres of type I and II on the area of 2.625 mm² of the spleen white pulp is presented in Table 3.

Table 3
The number of germinal centres (GC) - all section within 2 625 mm² of white pulp of experimental chicken spleen (means \pm SD)

Item	Group I	Group II SRBC	Group III ACTH	Group IV SRBC+ACTH
Number of measurements	30	30	30	30
GC I and II type	19.27	19.71	18.54	18.62
GC I type	13.24 ^A \pm 1.27	8.74 ^B \pm 1.39	10.15 ^C \pm 0.96	6.28 ^D \pm 0.83
GC Type II	6.03 ^A \pm 1.16	10.97 ^{Ba} \pm 1.22	8.39 ^C \pm 1.04	12.34 ^{Bb} \pm 1.41

^{a,b,A,B} means in a row with no common superscripts are significantly different: ^{a,b} $P \leq 0.05$, ^{A,B} $P \leq 0.01$

However, the analysis of the number of germinal centres of type I and II revealed that in the control chickens (group I) mature centres of type I prevailed. There were fewer type II centres. Following SRBC-immunization the proportion changed in favour of type II centres. In ACTH-treated birds, compared to the control group, with a slight decrease in the total number of centres, there was observed a decrease in the number of type I centres and a slight increase in the number of type II centres. In immunized and ACTH-treated chickens (group IV) the decrease in the number of mature type I centres was accompanied by a clear increase in the number of type II centres. In these birds the increase in the number of type II centres was higher than that found in SRBC-immunized chickens (group II compared to group IV, Table 3).

Discussion

The results indicate that in chickens a triple ACTH injection leads to changes in the picture of circulating blood. The stress-characteristic shift of percent composition of lymphocytes and heterophils, observed in ACTH-treated birds, is a confirmation of observations of other authors. They showed that stress and increased ACTH level changed the value of H/L ratio, while the level of changes depended on the dose of ACTH (Gross and Siegel 1983; Graczyk 1999; Krasnodępska-Depta and Koncicki 1999; Puvadolpirod and Thaxton 2000b; Zulkifli et al.2000).

Values of H/L ratio, lower than expected, noted in the experimental birds might result, among others, from a small dose of ACTH. The effect of the time between the last injection and blood sampling seems also to be of significance. In earlier studies it has been noted that a single ACTH administration results in maximum changes in the blood picture already after 2-4 hours, and the changes recede gradually during the next 24 hr (Słowik and Graczyk

1984). At the same time, the lack of changes in the blood picture of chickens immunized with SRBC and treated with ACTH, observed in our experiment, indicates a complex mechanism of ACTH effect on the haemopoietic system and blood leukocytes. It can be supposed that a three times ACTH administration leads to short-term, non-cumulative changes in adrenal gland action or, as suggested by Fitko et al. (1992), to an increased activity of adrenal glands.

Another basic objective of our studies was an estimate of morphology of central and peripheral lymphatic organs of the immunized and ACTH-treated birds. Bursa of Fabricius and thymus, being central lymphatic organs and the main source of B and T lymphocytes, in birds function at the same time as peripheral organs. Basic functional units of bursa of Fabricius are bursal follicles in which a medulla and cortex can be distinguished, separated by a strand of undifferentiated epithelial cells.

Using bromodeoxyuridine it has been demonstrated that within an hour ca 1% of all cells inhabiting bursa of Fabricius leave these organs forming a pool of cells, which will invade peripheral lymphatic tissues. The cells emigrate mainly from the cortical part of the lobules (Paramithiotis and Ratcliffe 1994b; Lampisuo et al. 1998). The medulla of bursal follicles contains lymphocytes that are under the effect of stimulation by environmental antigens. The medullar cells of the follicles have typical characters of cells of germinal centres of mammalian lymphatic tissues, facilitating the functioning of the bursa as a peripheral lymphatic organ (Nukkarinen and Sorvari 1982; Ekino 1993).

In chicken thymus, like in bursa of Fabricius, two distinct compartments, favouring T lymphocyte maturation can be distinguished. Intense maturation and selection of thymocytes take place in the cortex. The medulla, of incomplete thymus barrier, contains mainly mature thymocytes, few B lymphocytes and plasma cells or even germinal centres. Both these compartments are separated by a poorly marked corticomedullary border containing Hassal's bodies and a strand of epithelial cells (Olah et al. 1991; Minko and Olah 1996). It turns out that particular parts of thymus and lymphatic follicles of bursa of Fabricius are not stable structures. Changes manifesting as the lymphocyte disappearance have been observed during involution of bursa of Fabricius and thymus, associated with sexual maturation, as well as during viral and bacterial diseases (Milicevic and Milicevic 1993; Ortiz et al. 2001; Milicevic et al. 2002). Lymphocyte depletion, similar to that taking place during infectious diseases, accompanies stress. In the latter case the changes are reversible, after the stress has ceased, the number of lymphocytes returns to its original level (Pope 1991). The widening of the medulla of the follicle, with clear signs of formation of "secondary follicle", observed after chicken immunization, confirms the observations on the functioning of bursa of Fabricius as a peripheral lymphatic organ. However, the lack of changes in the weight of this organ, observed in ACTH-treated chickens, at a simultaneous slight depletion of follicular cells, indicates a high sensitivity of lymphatic structures to the action of adrenal hormones. Such an interpretation is justified by a simultaneous morphological analysis of adrenal glands of these birds. Hyperplasia of the fascicular and reticular zones of adrenal glands in ACTH-treated chickens proves the functional and morphological mobilization of adrenal cells under the influence of the hormone. The picture of adrenal glands of birds administered SRBC and treated with ACTH three times is also interesting. The changes in the fascicular and reticular layer, more intense compared to the birds treated with ACTH only, with a simultaneous appearance of lymphatic follicle in the cortex, suggests a cumulative effect of ACTH and SRBC on the adrenal cell function. SRBC as antigen is also a stress factor which in itself does not evoke distinct changes, but is only a factor allowing and intensifying the action of other factors, in this case ACTH. Similarly acting factors Gruber et al. (1994) are termed "glucocorticoid-increasing factors" which also indicates their immuno-regulating abilities. In the light of our studies it

appears that the cumulative effect of ACTH and SRBC does not pertain only to the stimulation of endocrine system. The appearance of lymphatic follicles in adrenal glands of immunized and ACTH-treated chickens suggests a possibility of paracrine stimulation of lymphatic system components located in adrenal glands. Any changes of this kind were observed in birds treated with SRBC only.

In most bird species devoid of developed lymphatic glands, the white pulp of the spleen plays a significant part in the antigen response. In the white pulp, following penetration by antigen, an array of functional and morphological changes takes place. Especially conspicuous changes are those in the germinal centres, which are among the most variable structures in the spleen (Graczyk 1994b). As it was shown, the process of formation of germinal centres is complex, depending not only on antigen but also on the effect of central lymphatic organs, which are the source of the germinal centre lymphocytes, and also of spleen neighbouring structures (PAL and PEL) (Graczyk 1994b; Graczyk and Kuryszko 1995b).

Ogata et al. (1981) demonstrated that in immunized birds the appearing germinal centres differ in the class of produced antibodies. Based on the suggestions of these authors, in our earlier studies concerning the behaviour of germinal centres in bursectomized birds, thymectomized chickens and immunized birds, it has been shown that the functional diversity of spleen germinal centres includes also morphological differentiation (Graczyk 1994b; Graczyk and Kuryszko 1995b; Graczyk 1999). Based on this, two types of centres have been distinguished. Type I, represented by germinal centres located mainly close to pulpal arterioles, surrounded by a well-developed connective tissue sheath, predominating in the histological picture of control birds, are probably mature structures after a completed process of clonal proliferation of lymphocytes and differentiation of cells of high antigen-affinity. In the light of our studies, germinal centres of type I appear to be more stable structures compared to type II centres. As has been already mentioned, newly formed type II GC are less numerous in control birds. They become numerous following stimulation of the lymphatic system by antigens, which is evidenced by a clear increase in their number following immunization with SRBC. These centres are mostly located near arterioles adjacent to areas of periellipsoidal lymphatic tissue which suggests their origin in PEL and gradual migration within PAL so that, with maturation, they reach the position described for type I centres. The results of the previous studies as well as the present observations made it possible to propose a scheme illustrating kinetics of these centres in chicken which is presented in Fig. 5 (Plate VII).

The fact that increased level of corticoids in the serum leads to increased values of H/L index has been well documented. It has also been shown that this is accompanied by changes in the distribution of lymphocytes in lymphatic tissues (among others spleen) where optimum conditions enhancing the antigen sensitivity and the antigen processing (Mashaly et al. 1993; Godfrey et al. 2000). Adrenal corticoids control cytokine production. An increased corticoid level causes a decrease in secretion of the main cytokines (IL2, IFN-gamma), increasing secretion of IL4 (Harbuz and Lightman 1992; Mashaly et al. 1993).

Probably, ACTH release by activated leukocytes is a critical event in the early lymphocyte redistribution, facilitating the initiation of immune response.

The over twofold increase in the number of newly-formed type II GC, observed in immunized chickens, is a morphological evidence for the dependence between the structure and function of lymphatic tissues and the effect of endocrine system function. Based on this we postulate that the ACTH-caused change in the level of corticoids and the consequent change of cell interaction in the white pulp leads to activation of structures associated with early phase of immunogenesis (PEL, PAL, type II GC).

Mature structures (type I GC) where probably a later phase of specific response is effected (clonal proliferation and selection of cells of high antigen affinity) disappear at that time. This is all the more convincing, since administration to chickens of ACTH only did not evoke such clear changes.

Whether or not the newly formed type II GC is fully prepared to participation in specific response is still an open question. Our studies do not provide a direct answer. However, a fragmentary analysis of anti-SRBC agglutinins indicates that ACTH administration during the antigen response causes the level of agglutinins decrease thrice compared to the level observed in birds not treated with ACTH.

Assuming that in birds the spleen is the main peripheral lymphatic organ, responsible for antibody production, and considering the earlier suggestion that type II GC transform into mature type I centres with progressing immune response, an increase rather than decrease in the level of antibodies could be expected. However, bearing in mind that the material was sampled on the 6th day after immunization, the decreased quantity of antibodies may be interpreted as an effect of immuno-suppressive action of adrenal hormones, or as a result of interaction of central and peripheral lymphatic organs, disturbed by ACTH administration. The relation between the described changes in the thymus and bursa of Fabricius, and also perhaps the resulting predominance of immature structures in the spleen (type II centres), would be an expression of this phenomenon.

Reaktivita germinálních center ve slezině u imunizovaných kuřat po podání ACTH

Cílem studie bylo stanovit reaktivitu struktur bílé dřeně sleziny u kuřat imunizovaných ovčími erytrocyty pod vlivem ACTH. Kuřata ve věku 11 týdnů byla rozdělena do čtyř skupin. Skupina I byla kontrolní, kuřata skupin II a IV byla imunizována ovčími erytrocyty. Kuřatům skupin III a IV byl podán ACTH. Řezy thymu, burzy Fabriciovy a sleziny byly zhotoveny pro histologické vyhodnocení. Počet germinálních center I a II byl stanoven na povrchu sleziny. Po podání ovčích erytrocytů nastala proliferace lymfoidních buněk v kůře thymu, ve dření burzy Fabriciovy. Po podání ACTH však v thymu a burze došlo k depleci lymfocytů. Morfometrická analýza sleziny ukázala, že po podání ovčích erytrocytů a ACTH došlo k vzestupu počtu germinálních center typu II.

Výsledky studie ukázal odlišný vliv antigenu na morfologii lymfatických orgánů. Ukázaly také různou citlivost sledovaných struktur vůči podanému ACTH. Naše dřívější a nynější výsledky tak umožňují vypracovat model tvorby a pohybu typů germinálních center ve slezině kuřat.

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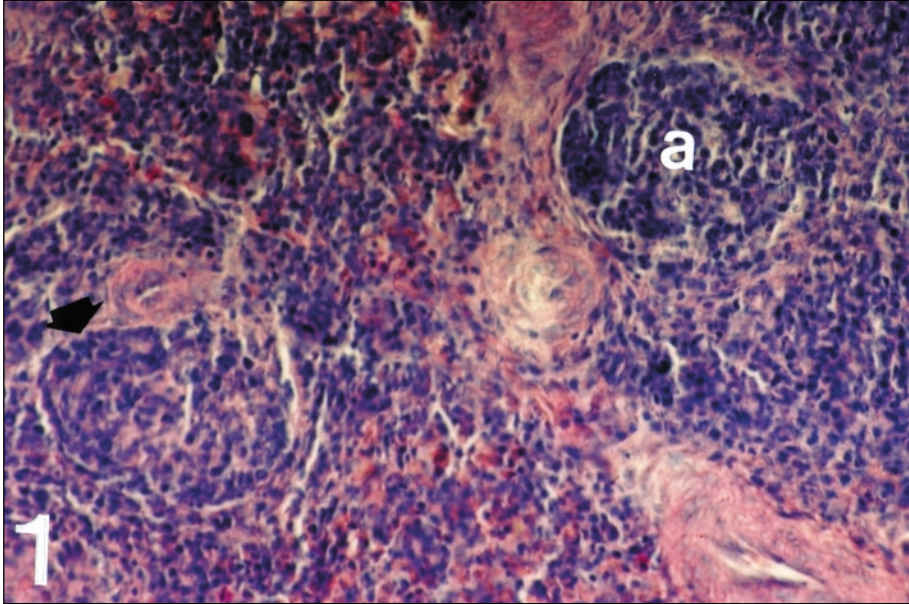


Fig. 1. Type I germinal centre (GC-I) (▶) and a degenerating germinal centre near trabecular artery (a). Fusion of collagen fibres of the centre with the artery visible. H&E $\times 250$

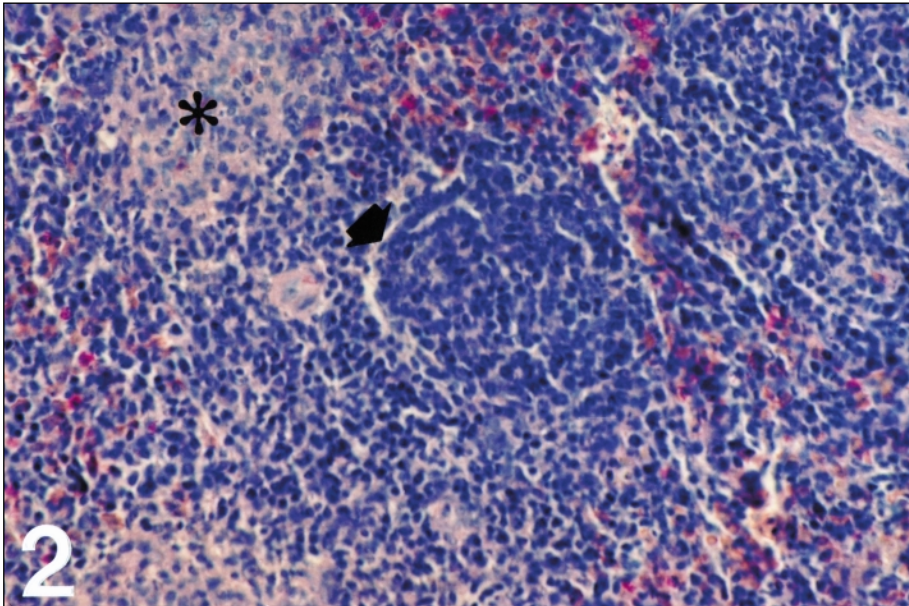


Fig. 2. Fully developed type II germinal centre (GC-II) (▶) Appearing network of connective tissue fibres surrounding the centre visible. Near the centre PEL visible (*). H&E $\times 400$

Plate VI

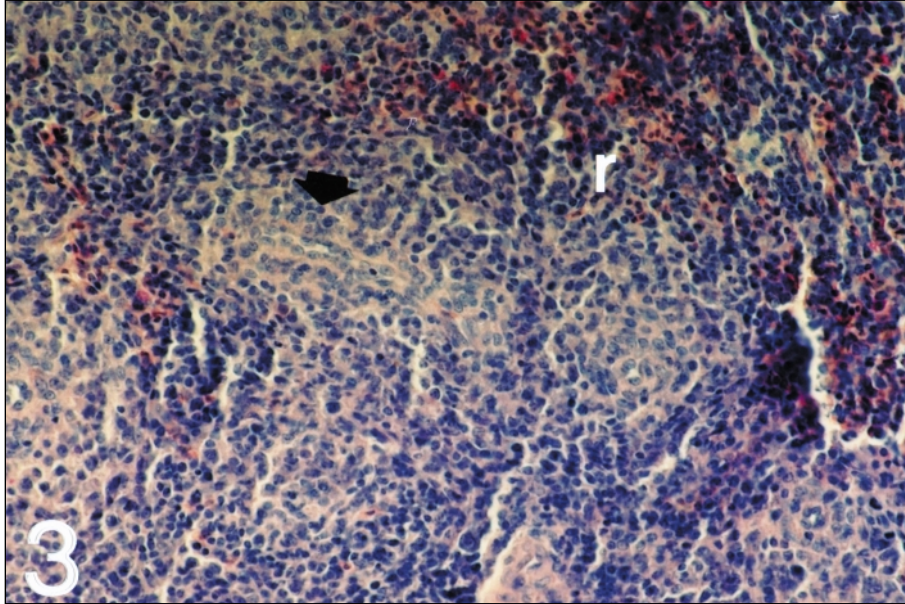


Fig. 3. Periellipsoidal lymphatic tissue (PEL) (▶) with an arterial vessel in the centre, lined with a characteristic high endothelium. Around PEL aggregation normally organised red pulp (r). H&E $\times 400$

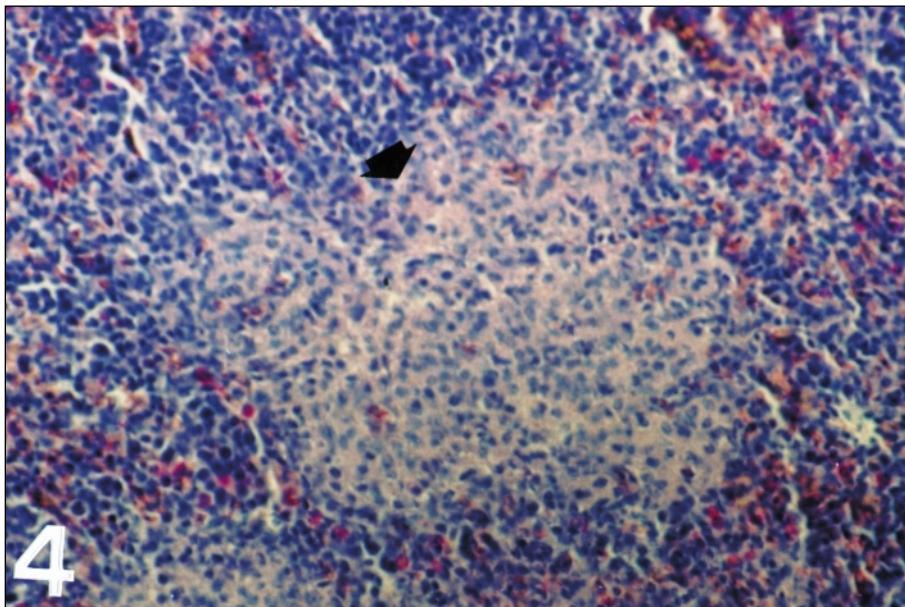


Fig. 4. Periarteriolar lymphatic tissue (PAL) (▶). In the centre arterial vessels with high endothelium; red pulp normally developed. H&E $\times 400$

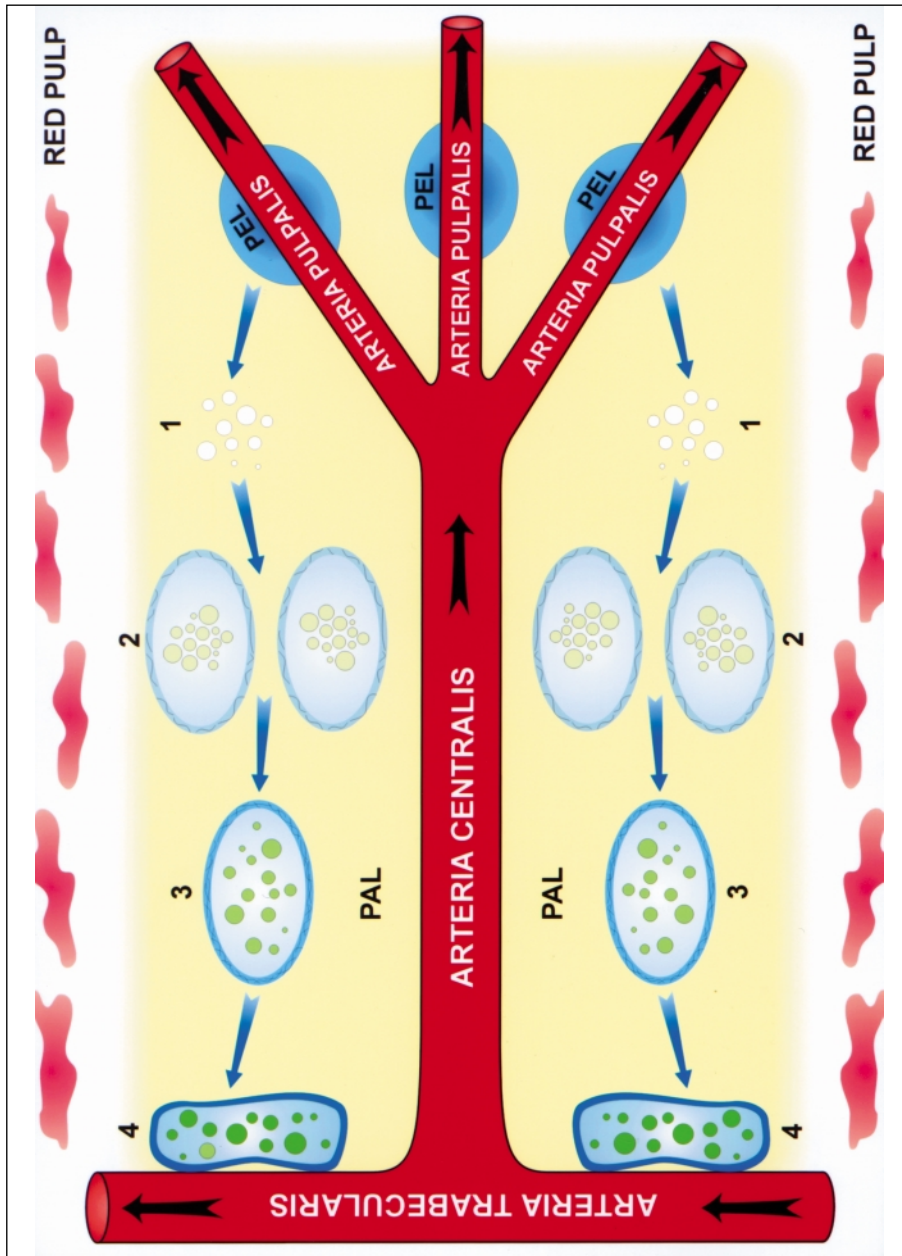


Fig. 5. Proposed scheme of formation and movement of germinal centres within white pulp of chicken spleen. Lymphatic cells emigrating from PEL form loose aggregations within PAL (1). The aggregations are surrounded by a delicate net of connective tissue fibres, and form type II germinal centre (2). Type II germinal centres, gradually isolated from the rest of white pulp by a distinct connective tissue sheath keep functioning as germinal centres type I (3). Fibres of connective tissue surrounding type I centre, connected with vessel external membrane fibres, cause movement of germinal centres towards vessel wall where they degrade (4). **PEL** - periellipsoidal lymphatic tissue, **PAL** - periarteriolar lymphatic tissue