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Application of lectin histochemistry to study the formation and reactivity of the marginal transitional zone of the joint capsule

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Key words: Joint Capsule, Marginal Transitional Zone, Lectins.

Aim. To analyze the formation and reactivity of the intermediate part (marginal transitional zone) of joint capsule using lectin histochemistry.

Methods and results. Hip joint of laboratory rats was as an object for present investigation. Method of intrafoetal antigen injection has been taken as a pattern in order to study the reactivity of above-mentioned zone. Fragments of hip joints were fixed in picoformal, decalcinated and dehydrated. We used a standard set of lectins, conjugated with horseradish peroxidase for lectin histochemistry. In the intermediate part, we distinguished regio superficialis and regio profunda. The intensity of expression for lectin receptors in regio superficialis was always higher than in the parietal part of the joint capsule. The intermediate part is distinguishable from the surrounding tissues as early as on the 1st day, meanwhile regio superficialis and regio profunda are discernible from each other only on the 7th day. The intermediate part formation is associated with a decrease in the expression of lectin receptors in regio superficialis and an increase in the content of galactose-, mannose- and glycoconjugates in regio profunda occurring from the 14th day. Changes are more pronounced in regio profunda in comparison with regio superficialis and consist in their premature development, which is accompanied by early expression of a large number of galactoseconjugates in regio profunda on the 7th day, with a subsequent decrease in galactose – and mannose saturation in it and appearance of significant L-fucose residues amount.

Conclusions. Antigenic effect leads to tension in immuno-biological relationship between articular cartilage and joint capsule and following reactive processes affecting lectin receptors glycosylation of intermediate part. Premature glycosylation of regio profunda is aimed on the early formation of the intermediate part of joint capsule having the lectin-mediated barrier, preventing excessive invasion of the joint capsule into the articular cartilage.

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Застосування лектинової гістохімії для вивчення формування та реактивності перехідної частини капсули суглоба

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Мета роботи – за допомогою лектинової гістохімії вивчити формування та реактивність перехідної частини капсули суглоба.

Матеріали та методи. Об'єктом дослідження став кульшовий суглоб лабораторних щурів. Для вивчення реактивності перехідної частини капсули суглоба використовували методику внутрішньоплідного введення антигена щурам на 18 добу антенатального періоду. Фрагменти кульшових суглобів фіксували в рідині Буена, декальцинували, дегідрували та заливали в суміш парафін:віск:каучук. Для дослідження розподілу рецепторів до лектинів використовували стандартні набори лектинів, що кон'юговані з пероксидазою хрому.

Результати. У перехідній частині капсули суглоба виділяли regio superficialis і regio profunda. Встановили, що інтенсивність експресії рецепторів до лектинів у regio superficialis була завжди вищою, ніж у парієтальної частини капсули суглоба. Перехідну частину капсули можна відрізнити від прилеглих тканин суглоба вже в першу добу, а regio superficialis і regio profunda диференціюються між собою лише на 7 добу. У роботі встановили, що формування перехідної частини пов'язане зі зменшенням експресії рецепторів до досліджуваних лектинів у regio superficialis і збільшенням вмісту галактозо-, манозо- та глюкозокон'югатів у regio profunda, що відбувається з 14 доби. Реактивні зміни в перехідній частині капсули суглоба після дії антигенів більш виражені в regio superficialis, ніж у regio profunda й полягають у її передчасному становленні, що супроводжується ранньою експресією великої кількості галактозокон'югатів у regio profunda на 7 добу, з дальшим зниженням у ній галактозо- та манозокон'югатів і появою чималого вмісту залишків L-фукози.

Висновки. Антигенний вплив призводить до напруженості імунобіологічних взаємовідносин між суглобовим хрящем і капсулою суглоба та компенсаторно-приспосувальних змін глікозиляції структур перехідної частини. Передчасна глікозиляція regio profunda спрямована на раннє становлення перехідної частини з метою формування неспецифічного анатомо-фізіологічного лектин-опосередкованого бар'єра задля запобігання надмірній інвазії капсули суглоба в суглобовий хрящ.

Ключові слова: капсула суглоба, перехідна частина капсули суглоба, лектини.

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Применение лектинового гистохимии для изучения формирования и реактивности переходной зоны капсулы сустава

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Цель работы – с помощью лектинового гистохимии изучить формирование и реактивность переходной зоны капсулы сустава.

Материалы и методы. Объектом исследования стал тазобедренный сустав лабораторных крыс. Для изучения реактивности переходной зоны капсулы сустава использовали методику внутриплодного введения антигена крысам на 18 сутки антенатального периода. Фрагменты тазобедренных суставов фиксировали в жидкости Буена, декальцинировали, дегидратировали и заливали в смесь парафин:воск:каучук. Для исследования распределения рецепторов к лектинам использовали стандартные наборы лектинов, конъюгированных с пероксидазой хрена.

Результаты. В переходной зоне капсулы сустава выделяли regio superficialis и regio profunda. Установлено, что интенсивность экспрессии рецепторов к лектинам в regio superficialis была всегда выше, чем в париетальной части капсулы сустава. Переходная зона капсулы отличима от прилегающих тканей сустава уже на 1 сутки, а regio superficialis и regio profunda дифференцируются между собой только на 7 сутки. В работе установлено, что формирование переходной зоны сопряжено с уменьшением экспрессии рецепторов к исследуемым лектинам в regio superficialis и увеличением содержания галактозо-, маннозо- и глюкозоконъюгатив в regio profunda, что имеет место с 14 суток. Реактивные изменения в переходной зоне капсулы сустава после воздействия антигенов более выражены



в *regio superficialis*, чем в *regio profunda* и заключаются в её преждевременном становлении, что сопровождается ранней экспрессией большого количества галактозоконъюгатов в *regio profunda* на 7 сутки с последующим снижением в ней галактозо- и маннозоконъюгатов и появлением значительного содержания остатков L-фукозы.

Выводы. Антигенное влияние приводит к напряжённости иммуно-биологических взаимоотношений между суставным хрящом и капсулой сустава и компенсаторно-приспособительным изменениям гликозилиции структур переходной зоны. Преждевременная гликозилиция *regio profunda*, направленная на раннее становление переходной зоны капсулы сустава с целью формирования неспецифического анатомо-физиологического лектин-опосредованного барьера, предотвращает чрезмерную инвазию капсулы сустава в суставной хрящ.

Ключевые слова: капсула сустава, переходная зона капсулы сустава, лектины.

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At present time the problem of joint morphology research has received considerable attention due to its great importance in general morbidity. It is well-known that the initial signs of joints damage during its inflammation take place at the contact area of the joint capsule and articular cartilage. This area has a key role in maintaining morphological and functional integrity of the joint. A lot of scientists studied this zone using different terminology, namely “переходная зона синовиальной мембраны”, “marginal transitional zone”, “marginal synovium”, “synovium-cartilage junction”, etc [1,5,7,10,11].

We proposed to distinguish the visceral part (*pars visceralis capsulae articularis*), parietal part (*pars parietalis capsulae articularis*) and *pars intermedia capsulae articularis* (intermediate part, generally known term – “marginal transitional zone”). In *pars intermedia* two different zones are discerned: fibrous layer which entwines in the articular cartilage – *regio profunda* and the area of the fibrous layer, which contacts directly with the last one – *regio superficialis* [2,4,9]. Complex immunobiological relationship between the joint capsule and articular cartilage are provided by a number of mechanisms of non-specific immune resistance, in particular carbohydrate-protein factor. Lectin histochemistry are widely used to study the dynamics of carbohydrate residues distribution in organs and tissues. These methods provide an opportunity to assess the formation of non-specific innate protective barrier of the marginal transitional zone of the joint capsule which is aimed to maintain normal physiological interaction between the joint capsule and the articular cartilage and prevent the excessive invasion of the joint capsule into the articular cartilage.

Aim

To study the formation and reactivity of the marginal transitional zone (intermediate part, further in text) of the joint capsule by means of lectin histochemistry.

Materials and methods

We used hip joints of white laboratory rats. Three groups of animals were studied. The first group – intact animals. We injected 0.05 ml of immunoglobulin human normal the second experimental group – antigen-affected rats in order to study reactivity of the marginal transitional zone of the joint capsule. The third group were rats of control group (injected with 0.05 ml of physiological saline). Injections of antigens and physiological saline were given for foetus on the 18th day of their antenatal life per uterus subcutaneously in the interscapular region (the method of Mykola A. Voloshyn (1981) [3,6,9]). Rats were born on the 22nd – 23rd day after conception. Animals were put to death under aether anaesthesia by means of decapitation on the 1st, 7th, 14th, 30th, 60th and 90th days of postnatal life in the afternoon from 13.00 to 14.00 (8 rats in each group, 144 rats

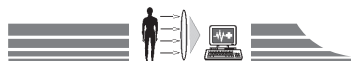
were used in total). We guided by “European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes” Strasbourg, 18.III.1986. Fragments of the hip joints were fixed in microformol, decalcinated in 20 % formic acid solution and dehydrated in alcohols and chloroforms. Pieces were immersed in a paraffin-wax-rubber mix (20:1:1 ratio). Histological specimens 3–5 microns thick were made by microtome. Peanut (PNA–HPR), vicia sativa (VSA–HPR), soybean (SBA–HPR), wheat germ (WGA–HPR), perca fluviatilis (PFA–HPR) agglutinins were used. Control histological sections were incubated in 1 % periodic acid solution for 30 minutes. Lectins binding sites visualization was carried out by diaminobenzidin-hydrogen-peroxide system. Obtained results were processed using semiquantitative analysis (from 0 to +++) by means of $\chi 10$, $\chi 40$ and $\chi 100$ lens magnification. Distribution of lectin receptors was studied in *regio profunda* and *regio superficialis* of the intermediate part of the joint capsule and compared to obtained data of fibrous layer of the parietal part of joint capsule.

Results were processed using semiquantitative analysis (from 0 to +++) by means of $\chi 10$, $\chi 40$ and $\chi 100$ lens magnification with video system “Axiolab” (Carl Zeiss, Germany). Statistical analysis was done by Pearson criterion. Compared data were considered as statistically valid at the difference in 0/+. Obtained data of intact rats and control rats did not differ significantly throughout the whole observation period.

Results of the study and discussion

In newborns intact animals in the parietal part of the joint capsule there were low expression for the peanut (PNA), soybean (SBA) agglutinins: light levels for vicia sativa (VSA) and perca fluviatilis (PFA) lectins. Intensity of benzidine label in *regio superficialis* for studied lectins was less in comparison with parietal part of joint capsule and it is remarkable, that this phenomenon can be observed up to the 90th day (see Table 1). GlcNAc residues were not found in *regio superficialis* till the 14th day, but they could be seen in minimal volume in the parietal part. So, *regio superficialis* is distinguishable from adjoining area of joint capsule because of unequal lectins distribution. Minimal expression of glycoconjugates in *regio profunda* is also determined; it makes this area different from the adjacent articular cartilage. The clear border between *regio superficialis* and *regio profunda* was not defined due to absence of significant difference in lectins distribution in these parts.

On the 7th day in intact rats in *regio superficialis* we revealed appearance of PNA receptors expression with maximum on the 14th day, which allows us to distinguish this zone from *regio profunda*. Expression of VSA lectin receptors started to decrease and remained in the least quantity up to the 90th day.



One must note that fucose residues were not defined in *regio superficialis* from the 7th day till the end of observation. Meanwhile, they could be found in minimal volume in the parietal part (see *Table 1*).

On the 14th day in intact groups there was focus of intense expression of receptors for peanut and soybean lectins in *regio profunda*. From the 30th day strong expression of vicia sativa and wheat germ lectin receptors could be clearly seen in the same zone. These above-mentioned phenomena enable us to discern distinctly *regio profunda* from *regio superficialis* and neighboring articular cartilage as well.

From the 30th and up to the 90th day of observation, expression of receptors for studied lectins in parietal part of the joint capsule was light and in *regio superficialis* was minimal. Along with that, there was pronounced intensity of benzidine labeling for all investigated lectins except perca fluviatilis (PFA) lectin.

Antenatal antigen influence in newborns leads to increased β -D-galactose residues level and reducing of α -D-manose residues number in *regio superficialis* and *regio profunda* as well. The distinct border between *regio superficialis* and *regio profunda* by analogy with intact newborns was not defined. Subsequently, antigenic influence did not affect the dynamics of the lectins receptors distribution in *regio superficialis*. In

antigen-injected rats strong expression of receptors for peanut (PNA) and soybean (SBA) agglutinin in *regio profunda* can be observed as early as on the 7th day in comparison with intact rats. In addition, we observed a significant decrease in quantity both residues β -D-galactose (from the 14th to the 30th day) (*Fig. 1*) and α -D-mannose (from the 30th up to the 45th day) in *regio profunda* and found the appearance of significant quantity of fucoseconjugates (from 30th to 45th day) (*Fig. 2*).

Thus, in the contact area of the joint capsule with articular cartilage there is zone, which is different from the adjacent part of the articular cartilage and the parietal part of the joint capsule as well. This difference derived from the distinction in lectin receptors distribution in it. Such changes in carbohydrate residues saturation are aimed for organization of, so-called, non-specific anatomical and physiological wall to regulate complex immunobiological interactions of joint capsule and articular cartilage, being as bar for immune cells. *Regio superficialis* formation passes against the background of decreasing lectin receptors expression in it. Meanwhile, *regio profunda* formation is accompanied by pronounced expression for all studied lectins but perca fluviatilis (PFA) lectin (see *Table 1*). It should be noted, that during the formation of *regio profunda*, there first occurred galactosylation of its matrix. From the 14th day one can observe the formation

Table 1

Comparative lectin histochemical description of formation of certain parts of the joint capsule

Parameters		Parietal part of the joint capsule (fibrous layer)		Intermediate part of the joint capsule (marginal transitional zone)			
				regio superficialis		regio profunda	
day	lectins	norm	antigen influence	norm	antigen influence	norm	antigen influence
1 st	PNA	from 0/+ to +	from + to ++	0	+	0	+
	SBA	from 0/+ to +	from + to ++	0/+	0/+	from 0/+ to 0	0
	VSA	from + to ++	0/+	+	0/+	0/+	0
	WGA	0/+	0/+	0	0	0	0
	PFA	+	+	+	+	0/+	0/+
7 th	PNA	from + to +/++	++	+	+	0	+++
	SBA	+	++	0/+	0/+	from 0/+ to 0	+++
	VSA	+	+	0/+	0/+	0	0
	WGA	0/+	0/+	0	0	0	0
	PFA	0/+	0/+	0	0	0	0
14 th	PNA	from ++ to +	from ++ to +	++	++	+++	0
	SBA	from ++ to +	from ++ to +	0/+	0/+	+++	+++
	VSA	+	+	0/+	0/+	0	0
	WGA	0/+	0/+	0	0	0	0
	PFA	0/+	0/+	0	0	0	0
30 th	PNA	from + to 0/+	from + to 0/+	0/+	0/+	+++	0
	SBA	+	+	0/+	0/+	+++	+++
	VSA	+	+	0/+	0/+	+++	0
	WGA	+	+	0/+	0/+	+++	+++
	PFA	0/+	0/+	0	0	0	0
60 th and 90 th	PNA	from + to 0/+	from + to 0/+	0/+	0/+	+++	+++
	SBA	+	+	0/+	0/+	+++	+++
	VSA	+	+	0/+	0/+	+++	+++
	WGA	+	+	0/+	0/+	+++	+++
	PFA	0/+	0/+	0	0	0	0

Notes: 0 – absence of binding; 0/+ minimal lectin binding; + light lectin binding; ++ moderate lectin binding; +++ intensive lectin binding. Abbreviations: PNA – peanut agglutinin, VSA – vicia sativa agglutinin, SBA – soybean agglutinin, WGA – wheat germ agglutinin, PFA – perca fluviatilis agglutinin.

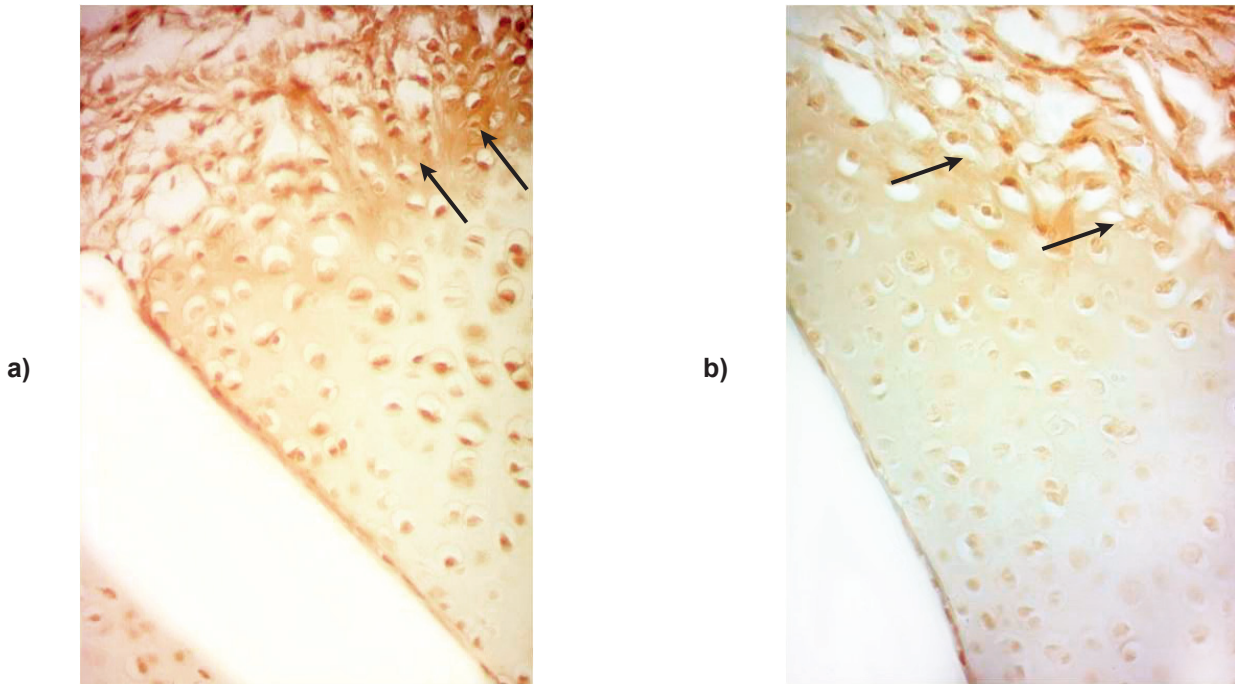


Fig. 1. Fragment of intermediate part of hip joint capsule (showed by arrows) in acetabulum: a) intact rat; b) immunoglobulin-injected rat. 14th day. Histochemical reaction with peanut agglutinin (PNA–HRP), $\times 40$ lens magnification. Absence of peanut agglutinin expression in the intermediate part of the joint capsule after antigen exposure.

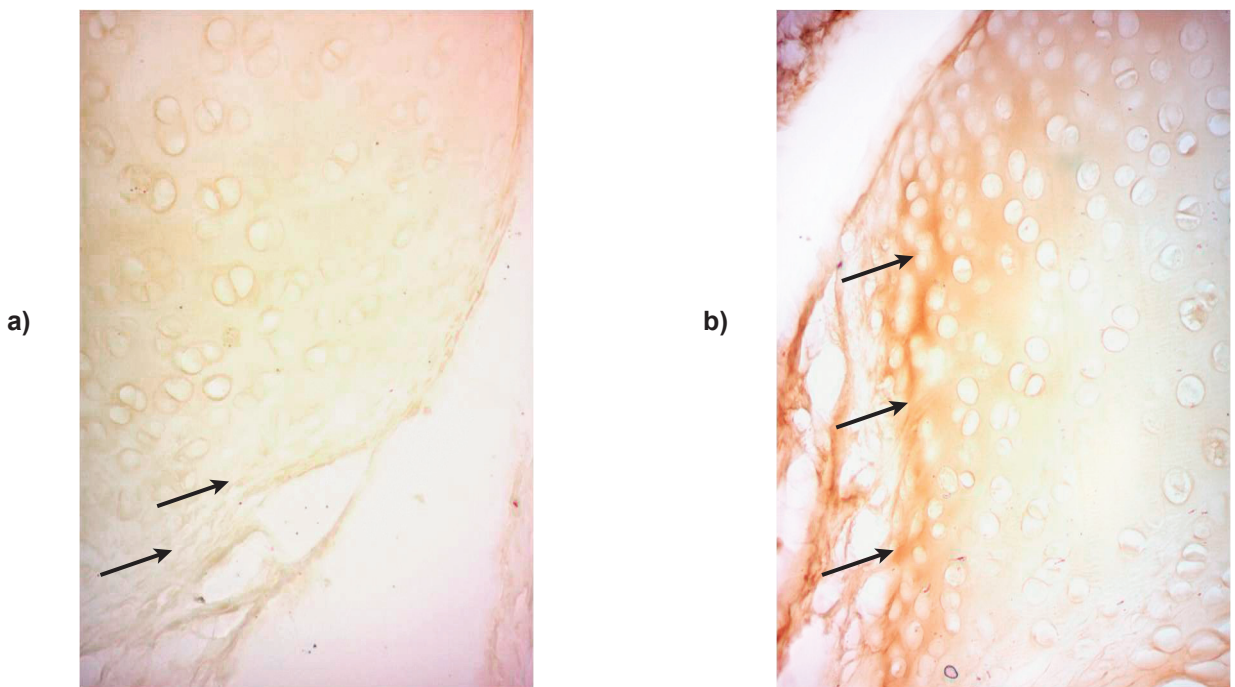


Fig. 2. Fragment of intermediate part of hip joint capsule (showed by arrows): a) intact rat; b) immunoglobulin-injected rat. 30th day. Histochemical reaction with percafluviatilis agglutinin (PFA–HRP), $\times 40$ lens magnification. Appearance of residues β -L-fucose excessive amounts in the intermediate part after antigen exposure..

of double lectin-mediated barrier, consisting in reducing the lectin receptors expression in the one zone and appearance of strong lectin receptors expression in the other zone. It can be common biological feature of organs which are not recognized by immune cells in norm. Such phenomenon has been described in placenta [6]. Considering the obtained data, it is advisable to distinguish two zones in *pars intermedia capsulae articularis*, namely *regio superficialis* and *regio profunda*.

Antigen influence leads to a significant increase in the number of immune cells in the organs, in particular in joint capsule, which affects the rate and terms of organs formation [3]. Increased number of lymphoid cells in the joint capsule is the immunological risk due to possible breaching the barrier between the cartilage and the capsule and further penetration of joint capsule into the articular cartilage with subsequent development of immune inflammation in it.



This leads to the development of compensatory and adaptive reactive processes concerning quantitative and qualitative changes in glycosylation of components of marginal transitional zone. These phenomena are strongly pronounced in *regio profunda*, in contrast to *regio superficialis*, that probably indicates *regio profunda* as most vulnerable structure. So, the premature appearance in *regio profunda* increased amounts of β -D-galactose residues can be considered as early formation of joint capsule intermediate part.

On the other hand, there are a number of matrix components in the joint capsule which serve as non-specific resistance factors because of having good adsorbing properties to stop activated lymphocytes, macrophages, etc., for example, fibronectin. Glycosylation apparently plays crucial role to provide biological function of matrix. Fibronectin has in its structure all above-mentioned carbohydrate residues, except for α -L-fucose. We can presume that the lack of saturation of fibronectin molecules with residues of galactose and mannose, occurring on the 30th day is substituted by residues of fucose, but it is necessary to notify that fucose-saturated fibronectin is a product of fetal or neoplastic tissues [8]. Residues of β -D-galactose and α -D-mannose are part of a number of biopolymers, its deficiency can lead to negative effect on physical and chemical properties of intercellular substance. These data allow us to substantiate high frequency of joint pathology appearance in children with antenatal antigenic exposure.

Conclusions

1. Thus, *pars intermedia capsulae articularis* (joint capsule marginal transitional zone) is a structure that is morphologically different from the surrounding tissue and includes “*regio superficialis*” and “*regio profunda*”. Glycosylation of structures of the marginal transitional zone provides the formation of lectin-mediated barrier between the joint capsule and the articular cartilage that ensures the integrity of the joint as a whole. Formation of *pars intermedia* is accompanied by the decrease in expression of receptors to all investigated lectins in *regio superficialis* and the appearance of significant galactose-, mannose- and glucoseconjugates quantity in *regio profunda*.

2. Antenatal antigens influence leads to tension in immunobiological relationship between joint capsule and articular cartilage resulting in premature galactosylation of components of *regio profunda*, subsequently being accompanied by a decrease in the number of residues of galactose and mannose and appearance of excessive amounts of residues α -L-fucose.

Aspects of further investigations. In the future some regularities of lectin receptors distribution in other components of joint will be also studied.

Conflict of Interest. All authors disclose no financial nor personal relationships with other people or organizations that could potentially and inappropriately influence (bias) their work and conclusions.

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