HISTOCHEMICAL INVESTIGATION OF THE MUCOSUBSTANCES IN THE BROILER CHICKEN LACHRYMAL GLAND THE FIRST NINE WEEKS AFTER THE HATCHING

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ABSTRACT

One hundred and eighty lachrymal glands obtained from 90 broiler chickens originating from a commercial stock, equal number from both genders at the age of 1, 7, 14, 21, 28, 35, 42, 49 and 56 days were investigated. After the narcosis, decapitation and glandular preparation, using paraffin and frozen sections an evaluation of the gland polysaccharides was carried out following the conventional method – PAS. The combined staining method of Mowry's Alcian Blue followed by PAS was used to reveal the total acidic and neutral mucosubstances. Selective staining of acidic sulfated mucosubstances was done using Alcian Blue at pH 1.0. Acidic nonsulfated (sialylated) glycoconjugates were counted by the differentiation between total acidic and acidic sulfated mucosubstances.

After PAS, cytochemical reactivity was detected for the first time only by the end of the first week (7th day), and afterwards persisted in all age groups. The polysaccharides were a combination of glycogen, mucoproteins, glycoproteins and glycolipids. The combined histochemical methods determined the presence of moderately acidic nonsulfated glycosaminoglycans, and very weak reactivity for sulfated mucins in lachrymal gland's tertiary tubules of 56-day-old broiler chicken.

The analysis of the obtained data showed that the broiler's lachrymal gland in the first nine weeks after hatching has a mucosubstance activity, whose expression and localization is age-dependent.

Key words: lachrymal gland, broiler chickens, histochemistry

INTRODUCTION

All aquatic, amphibian and terrestrial vertebrates possess a lachrymal gland (1). The gland is found in all mammals, although in some species as monkeys, seals, whales, flying mice it is poorly developed whereas in dolphins, deers, Indian elephant, dogs, cats, rabbits – very well developed. Vertebrates from the lower evolutionary hierarchical rows (fish, reptiles, birds) also have lachrymal glands, and among reptiles, they are exceptionally developed in snakes and crocodiles (2, 3). In the eye orbit of birds, the lachrymal gland and the third eyelid (a.k.a. Harderian) gland are the main intraorbital glands. In the intraorbital avian space, the latter is better developed and of greater size (4-7).

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The determination of the purpose of lachrymal gland, its role in the formation of the aqueous medium filling the eye orbit in different animal species, its structural, ultrastructural, species-, gender-, age-related and other specific features has greatly contributed to the substantial body of human knowledge since ancient times with thousands of scientific publications created by researchers who chose it as their object of study. For the first time in 1966, Jones (8) drew the attention of the scientific community to the significance of the lachrymal secretory system, launching a series of publications on the normal histochemical features of the organ and changes occurring after experimental influences. Histochemical and electron microscopy study of salt secreting lachrymal glands in marine turtles was performed by Abel *et al.* (9); Jensen *et al.* (10) investigated histochemically the acinar mucosubstances of human lachrymal gland. Some histological and histochemical features of the lachrymal gland of the Indian buffalo were reported by Singh *et al.* (11); while Millar *et al.* (12) performed a histochemical and immunohistochemical study

of rabbit lachrymal gland cells on histological sections and cell cultures. The localisation of glycosaminoglycans in human lachrymal gland was demonstrated histochemically by Yoshida *et al.* (13). In rabbits, Millar and Koutavas (14) published a histochemical analysis of phosphodiesterase isoenzymes in all eye glands, among which the lachrymal and Harderian glands are the main intraorbital glands. The histology, histochemistry and ultrastructure of lachrymal and nictitans glands in the South American armadillo was investigated by Marcos *et al.* (15). Pinard *et al.* (16) communicated the normal anatomical and histochemical features of lachrymal glands in American bison and calves. Paliwal *et al.* (17) reported the histochemically established sexual dimorphism in peroxidase production by the lachrymal gland of hormonally treated hamsters. Apart the histochemical detection, Paliwal *et al.* (18) succeeded to purify, clone and regulate the levels of acid- lipase-like protein in tears produced by hamster lachrymal gland during lactation. Kleckowska-Nawrot and Dziegiel (19); Shadhast *et al.* (20) studied the morphology and histochemistry of lachrymal gland of porcine fetuses and published the results in a complex report including also the histochemical traits of the gland in an Asian goat breed. All these examples constituting only a negligible part of the huge variety of studies provide evidence for the exceptional diversity of animal species, in which this organ has been histochemically investigated. Nowadays, one of the leading researchers of the lachrymal gland – Walcott (21) was the second after Jones (8) to pose the rhetorical question about gland's significance for the formation of tear film, lining the anterior surface of the eyeball. The significant number of research reports striving at adapting their results from laboratory biological models to finding solutions of severe clinical states of the lachrymal gland in humans made one of the leading research team to warn about the existing structural and functional differences in the organ in laboratory rodents, rabbits and humans (22). Thörig *et al.* (23) were among the first to respect this appeal by comparing the histochemistry and tears in lachrymal glands of rabbits and guinea pigs.

Macromorphology data of avian lachrymal glands could be found mainly in textbooks (4-7), but the first detailed investigation on the anatomy, vascularisation and innervation of lachrymal and Harderian glands of the domestic chicken was performed by Nicolescu (24). First data with respect to micromorphometry and functional traits of lachrymal, Harderian and lateral nasal gland of

the domestic fowl are reported in the PhD thesis of Vasilieva (25). The detailed light microscopy microstructure and histochemistry of the lachrymal gland in chickens and ducks were reported for the first time in a scientific journal by Burns (26). Maxwell and Burns (27) established the ultrastructural presence of glycogen granules in lachrymal and Harderian gland cells in chickens, ducks and turkeys. In his monograph on the physiology and biochemistry of domestic fowl, Freeman (28) provided some scarce information about lachrymal gland histochemistry. The histochemistry of lachrymal, Harderian glands and the pterygopalatine ganglion in native chickens was described in the study of Walcott *et al.* (29).

The review of available literature did not reveal any data about the histochemical features of the lachrymal gland in domesticated fowl species, reared in intensive broiler production systems. This fact, as well as the previous histochemical studies of ours on Harderian glands in broiler chickens (30,31) motivated the performance of the present investigation.

Its aim was to determine the age-related histochemical features of lachrymal glands of broiler chickens during the first 8 weeks of life using conventional histochemistry techniques.

MATERIALS AND METHODS

The histochemical study was performed on specimens obtained from 90 clinically healthy broiler chickens reared in intensive production systems, purchased mainly from poultry farms in Stara Zagora, Haskovo, Razgrad and Kostinbrod. Nine age groups of birds were formed – at 1, 7, 14, 21, 28, 35, 42, 49 and 56 days of age. Each group comprised 10 birds – 5 male and 5 female. After inhalational anesthesia and decapitation, using the method of Aitken and Survashe (32), a pair of lachrymal glands were obtained (left and right), totally 180. The research biological material was collected in compliance with Good Laboratory Practice and Clinical Directive 86/609/EEC/24 of November 1986. Due to the small size and difficult removal of lachrymal glands, a Technival-2 stereomicroscope (Karlzeiss Jena, Germany) was used in age groups of 1-, 7-, 14- and 21-day-old birds, and glands were removed via microdissection.

The major part of obtained lachrymal glands were immediately placed in fixative solutions as 10% neutral formalin, formal saline, Carnoy's and Bouin's fixatives. A certain part was left in native state for elaboration of frozen

histological sections. Using conventional histological techniques, a large number of single and serial histological sections up to 6 µm thick were elaborated by means of sliding and rotational microtome (Reichert Jung, Austria).

The following histochemical tests were performed on histological sections: 1. test for detection of polysaccharides (glycogen, mucoproteins, glycoproteins and glycolipids) through PAS reaction; 2. test for detection of acid mucopolysaccharides with Alcian blue staining after Stidman; 3. combined staining with Mowry's Alcian blue/PAS for simultaneous detection of acid and other mucins at pH 2.5; 4. selective Alcian blue staining at pH 1.0 for acid sulfated glycosaminoglycans; 5. selective Alcian blue staining at pH 2.5 for acid non-sulfated glycosaminoglycans. All histochemical tests and respective controls were performed as per

Pearse Everson (33), Lillie (34) and Kiernan (35). After light microscopic analysis of all permanent and non-permanent histological preparations, findings were microphotographed on a universal light microscope (NU-2, Karlzeiss Jena, Germany). The degree of detected cytochemical and histochemical reactivity was qualitatively scored using one or several (+) and presented in tables.

RESULTS

The routine PAS histochemical testing for carbohydrates (glycogen) did not reveal any tissue reactivity in lachrymal glands of broiler chickens at hatching (1 day of age). The first positive results from this test were detected in lachrymal glands of 7-day-old chickens and the cytochemical reactivity persisted in all other studied age groups with different extent and localisation (**Table 1**).

Table 1. Results from the histochemical reactivity of the broiler chicken lachrymal gland after histochemical test for polysaccharides

Test	Age (day)	Connect. tissue	Acinus		Tertiary tubules		Secondary tubules		Primary tubules	
			Epithelium	Lumen	Epithelium	Lumen	Epithelium	Lumen	Epithelium	Lumen
	1	–	–	–	–	–	–	–	–	–
	7	–	+++	+++	+++	+++	+++	+++	+++	+++
	14	–	++	++	++	+	++	+	++	++
	21	–	++ (+++)	++ (+++)	++ (+++)	+	++	+	++	++
PAS	28	–	++	++	++	++	++	++	++	++
	35	–	++ (+++)	++ (+++)	++ (+++)	++ (+++)	++	++	++	++
	42	–	++	++	++ (+++)	++ (+++)	++ (+++)	++ (+++)	++ (+++)	++ (+++)
	49	±	++	++	++	++	++	++	++	++
	56	±	++ (+++)	++ (+++)	++ (+++)	++ (+++)	++	++	++	++

PAS – Perijod acid / Shif

(–) There is not reactivity, (+) Low reactivity, (++) Middle reactivity, (+++) Good reactivity

Light microscopy analysis demonstrated that connective tissue structures of the organ (capsule, interlobular and interstitial connective tissue) were PAS-negative in almost all age groups. The study showed weak PAS reactivity only in single, mainly blast cells in the interlobular and less frequently, the interlobular interstitial connective tissue of the gland during the last 2 weeks of the studied age period (49–56 days of age). The detected PAS positive reactions in one week-old lachrymal gland was manifested by purple red staining of acini, tertiary, secondary and primary interlobular secretory tubules. Apart

the bodies of glandular cells, the lumen of aforementioned microstructural glandular units were filled with glandular secretion of the same colour, which had a foamy appearance in areas with large amounts of it (**Figure 1**).

The glandular epithelium lining the acini and tubules of 7-day-old lachrymal gland of broiler chickens was strongly PAS-positive and staining intensity was uniform. At higher magnifications, the purple red staining of these structures was found to be due to the large number of fine strongly PAS-positive granules, densely and diffusely situated into glandular cells (**Figure 2**).

Figure 1. 7th day broiler chicken lachrymal gland – Acinus, tertiary, secondary, primary secretory tubules, the lumen and covered main lobular duct epithelium with good PAS reactivity. PAS – 125X.

Figure 2. 7th day broiler chicken lachrymal gland – Acinus and parts of the interlobular secretory tubules contain granules with good PAS reactivity. PAS – 650X.

The PAS reactivity of 2-week-old lachrymal gland was moderate and uniform in all epithelial-glandular structures of the organ. Similar histochemical reactivity was present in broilers at 28 and 49 days of age.

The other 4 age groups of broiler chickens (21, 35, 42 and 56 days of age) exhibited a cytochemical reactivity of variable extent and localisation. In the lachrymal glands of 3-week-old (21st day) and 5-week-old (35th day) chickens, glandular lobules with moderate PAS reactivity were the most numerous. At the

same time, in one-third of glandular lobules of most studied lachrymal glands, the acini and tertiary tubules had a pronounced PAS-positive reactivity (**Figure 3**).

The surface acini in glandular lobules in 42-day-old chickens showed mainly an intermediate PAS reactivity. The PAS cytochemical reactivity of the complex interlobular system of tertiary, secondary and primary secretory tubules was well expressed (**Figure 4**).

Figure 3. 35th day broiler chicken lachrymal gland – Secretory tubules and acinus in the glandular lobulus with middle PAS reactivity, but in the same lobulus a little lateral glandular part demonstrated very good PAS reactivity. PAS – 250X.

Figure 4. 42 day broiler chicken lachrymal gland – All glandular lobules in the periphery consisted acinus with middle PAS reactivity and central localized micro structural units with good and very good PAS reactivity. PAS – 40X.

During the last week of the experimental period (56th day), the lachrymal gland lobules exhibited a mixed reactivity pattern. Most of them were of intermediate PAS positivity, but it was pronounced in the acini and tertiary tubules in the remaining lobules. In this age group, the staining intensity of acinar and tubular epithelial cells of lobules with mixed cytochemical reactivity was different. The red staining of luminal content of acini and tertiary tubules was more intensive as compared to that of secondary and tertiary tubules (**Figure 5**).

The PAS tests performed in each age groups and parallel incubation of control sections

showed that the carbohydrates were bound to other substances. On the basis of our results it could be suggested that lachrymal glands of broiler chickens from post hatch day 7 to 56 days of age was an organ with pronounced polysaccharide secretory activity. It was due to epithelial-glandular microstructural component of the gland, its extent and localisation was age-dependent and secreted polysaccharides were mainly in a bound state.

After detection of results from control histological sections in all age groups of chickens, histochemical staining tests for glycosaminoglycans (acid mucopoly-

saccharides) were performed with Alcian blue. Two histochemical tests were conducted in

each age group – Alcian blue at pH 1.0 and pH 2.5 (**Table 2**).

Figure 5. 56th day broiler chicken lachrymal gland – Lobular part with mixed PAS reactivity – secretory tubules with middle and glandular acinus with good PAS reactivity. PAS – 250X.

Table 2. Results from the histochemical reactivity of the broiler chicken lachrymal gland after histochemical tests AB pH 1.0 and AB pH 2.5

Test	Age (day)	Connect. tissue	Acinus		Tertiary tubules		Secondary tubules		Tertiary tubules	
			Epithelium	Lumen	Epithelium	Lumen	Epithelium	Lumen	Epithelium	Lumen
AB ₁	1	–	–	–	–	–	–	–	–	–
		–	–(+)	–(+)	++	+	+	+	+	+
	7	–	–	–	–	–	–	–	–	–
		–	++	++	+	++	++	++	++	++
AB ₁	14	–	–	–	–	–	–	–	–	–
		–	–(+)	–	++	+	+	–(+)	+	+
	21	–	–	–	–	–	–	–	–	–
		–	–(+)	–	+	+	+	+	+	+
AB ₁	28	–	–	–	–	–	–	–	–	–
		–	++	++	+	+	+	+	++	+
	35	–	–	–	–	–	–	–	–	–
		–	++	+	++	++	+	+	+	+
AB ₂	42	–	–	–	–	–	–	–	–	–
		–(+)	++	++	+	+	+	+	+	+
	49	–	–	–	–	–	–	–	–	–
		–(+)	+	+	+	+	++	++	++	++
AB ₂	56	–	–(+)	–	–(+)	–	–	–	–	–
		–(+)	++	++	++	++	++	++	++	++

AB₁ – Alcian blue at pH 1.0; AB₂ – Alcian blue at pH 2.5

(–) There is not reactivity, (+) Low reactivity, (++) Middle reactivity, (+++) Good reactivity

The light microscopy showed absence, weak or moderate reactivity for both tests (the lack of reactivity made us repeat the tests several times on paraffin sections made of biological samples fixed in different fixatives, but the results were identical to those presented in the

table). The connective tissue component (capsule, interlobular and interstitial connective tissue) of the lachrymal gland microstructure in broiler chickens did not exhibit any cytochemical reactivity in almost all age groups. Only chickens from the last

three groups (42, 49 and 56 days of age) exhibited single cells in the interstitial, subepithelial and interlobular loose connective tissues with weak Alcian blue reactivity at pH 2.5 (probably, these were young synthesising cells of the loose connective tissue, including young plasmocytes). The Alcian blue staining at pH 1.0 provoked a very weak alcianophilia only in few acini and tertiary secretory ducts in 5-week-old broiler chickens, whereas in all other age groups, no cytochemical reactivity was observed. Alcian blue test at pH 2.5 showed a positive reactivity in lachrymal glands from the nine age groups with various intensity (from weak to moderate) and localisation. In newly hatched chicks, weak alcianophilia was detected only in some acini and tertiary secretory ducts but it was present in all secondary and primary interlobular secretory tubules. The one-week-old lachrymal gland had a moderate reactivity in all epithelial glandular microstructures of the organ, whereas 2- and 3-week-old glands had Alcian blue reactivity similar to that in day-old chickens. The acini in 28- and 42-day-old lachrymal glands exhibited a moderate reactivity in the test, but in all glandular tubules types in these age groups it was very weak. In 35-day-old broiler chickens, the alcianophilia of acini and tertiary secretory tubules was moderate, whereas in the other categories tubules it was weak. By the 49th day, the secondary and primary interlobular

glandular ducts had an intermediate reactivity, while the acini and tertiary tubules – weak cytochemical reactivity. At the end of the study (56th day), all epithelial glandular microstructural elements of broiler's lachrymal gland exhibited a moderate alcianophilia.

The results from the two differentiating histochemical tests demonstrated that during the first 8 weeks after the hatch, the lachrymal gland of broiler chickens produced mainly moderately acidic non-sulfated glycosaminoglycans. A very weak reaction for sulfated glycosaminoglycans was exhibited only in few glandular acini as well as in single tertiary interlobular secretory tubules.

For verification of the results from both histochemical test, a combined staining with Mowry's Alcian blue (MAB) followed immediately by PAS staining was performed for simultaneous staining of neutral and acid glycosaminoglycans (**Table 3**). The light microscopy analysis of stained histological sections showed that connective tissue units of lachrymal glands – capsule, interlobular and interstitial connective tissue did not react in almost all age groups. Only single cells or single groups of cells, as well as single fibres in chickens from the groups aged 42, 49 and 56 days, exhibited a weak PAS-positive reaction or alcianophilia.

Table 3. Results from the histochemical reactivity of the broiler chicken lachrymal gland after histochemical test MAB/PAS

Test	Age (day)	Connect. tissue	Acinus		Tertiary tubules		Secondary tubules		Tertiary tubules	
			Epithelium	Lumen	Epithelium	Lumen	Epithelium	Lumen	Epithelium	Lumen
	1	–	+	+	+	+	+	+	+	+
	7	–	++ (+++)	++	+++	+++	+++	++	+++	+++
	14	–	++	++	++	++	++	+	++	++
	21	–	++	+	++	++	++	++	++	++
MAB/ PAS	28	–	++	++ (+++)	++	++	++	++	++	+++ (++)
	35	–	++ (+++)	++	++ (+++)	++ (+++)	++	++	+++	++
	42	– (+)	++	++	++	++	++	++	++	++
	49	(+) (+)	++	++	++	++	+++ (++)	+++	+++	+++
	56	(+) (+)	+++ (++)	++	+++ (++)	++	+++ (++)	+++	+++	+++

MAB – Mowries alcian blue; PAS – Perijod acid / Shif

(–) There is not reactivity, (+) Low reactivity, (++) Middle reactivity, (+++) Good reactivity

In all groups, the epithelial-glandular component of acini and ducts exhibited alcianophilia of various intensity and localisation, whereas PAS positivity was not

detected. The structural units of the lachrymal gland in day-old broiler chickens had a weak alcianophilia, attaining a moderate expression only in several secretory tubules (**Figure 6**).

Figure 6. One day broiler chicken lachrymal gland and section through the beginning of the draining glandular duct (to the right part of the figure) – All categories tubules, lumen, covered epithelium and acinus demonstrated low, middle and good alcianophilia after MAB/PAS. MAB/PAS – 40X.

Figure 7. 35th day broiler chicken lachrymal gland – All epithelial components in the glandular lobules demonstrated middle and good alcianophilia. MAB/PAS – 100X.

The one week-old lachrymal gland and that of 35-day-old chickens demonstrated moderate alcianophilia in acini, which, in few lobules attained a good level of expression; in the same lobules, the three categories of secretory tubules showed a strong histochemical reactivity (**Figure 7**). In four age groups – 14, 21, 28 and 42 days of age – the reactivity of lachrymal glands in the MAB/PAS test was

identical. The alcianophilia was of moderate intensity comprising the acini, tertiary, secondary and primary tubules of the gland, with the exception of some primary secretory tubules of 28-day-old gland, where several lobules exhibited strong histochemical reactivity and intensive blue staining. The light microscopy analysis of the lachrymal gland in 49-day-old chickens revealed a mixed type of

histochemical reactivity of glandular acini. Acini and tertiary secretory tubules in those lobules showed a moderate reactivity in the MAB/PAS and of less intensive colour, while the primary and secondary tubules within the same lobules had strong cytochemical reactivity and intensive emerald blue colour

(Figure 8). During the last week of the study period (56th day), the lachrymal gland of broilers was actively secreting, and all its epithelial-glandular structural components – acini and all secretory duct types – exhibited a strong alcianophilia and emerald blue colour (Figure 9).

Figure 8. 49th day broiler chicken lachrymal gland –The periphery located lobular structural elements showed middle alcianophilia, but centrally situated demonstrated good stain reactivity after MAB/PAS. MAB/PAS – 100X.

Figure 9. 56th day broiler chicken lachrymal gland – All micro structural lobular secretory elements, lumen, covered and secretory draining duct epithelium and lumen showed very good alcianophilia. MAB / PAS – 100X.

The results from the histochemical MAB/PAS test confirmed that the secretion of the lachrymal gland of broiler chickens during the first eight weeks of age contained moderately acidic mucopolysaccharides.

DISCUSSION

In her PhD thesis on the morphology and functional traits of the lachrymal, Harderian

and lateral nasal glands of domestic fowl, Vasilieva. (25) established a positive histochemical reactivity for glycogen only in the lachrymal gland, without specifying the structures. In the view of Burns (26) lachrymal glands of Shavers layer hens and adult Pekin ducks exhibited different cytochemical reactivity in the PAS test. The author found out that the epithelium of chicken lachrymal gland

was strongly PAS-positive, and that acinar and tubular epithelial cells were of similar staining intensity, whereas in ducks, the reactivity of acini and tubules was different. The cells of acini were weakly stained after PAS, whereas not all cells in tubules were of positive histochemical reactivity. The luminal content of tubules in both avian species had a stronger PAS reactivity than that of glandular acini. By light microscopy, Soloveva *et al.* (36) discovered a strong histochemical reactivity for glycogen in the lachrymal gland but extremely weak reactivity of Harderian gland in a local turkey breed. Maxwell and Burns (27) proved ultrastructurally the presence of glycogen granules in the lachrymal gland of turkeys, ducks and chickens.

The results from our investigation on lachrymal glands of broiler chickens confirmed at a considerable extent data of cited authors in layer hens, domestic fowl and turkeys. The observed PAS-positive cytoactivity was absent in newly hatched broiler chickens, appeared at the end of the first week (day 7) but was detected in the organ during the subsequent eight weeks. In all age groups, connective tissue structures of the lachrymal gland were negative in the PAS tests; only single cells of 49- and 56-day-old glands located mainly in the interlobular and more rarely in the interstitial tissue, demonstrated weak PAS-positive reaction. This age pattern of cytochemical reactivity could be explained by the differentiation of tissues. The connective tissue in the lachrymal gland of broiler chickens at 49 and 56 days of age contained mature cell types (fibrocytes, plasmocytes etc.) which usually exhibit positive cytochemical reactivity in the PAS test.

Similar to layer hens, domestic fowl and turkeys, the lachrymal glands of broiler chickens from all age groups positive in the PAS cytochemical test, was stained in a purple red colour. The light microscopy analysis demonstrated that regardless of the localisation and intensity of staining, PAS reactivity was due to numerous fine, compact coloured granules diffusely situated within the glandular cells. Control tests proved that similar to domestic fowl and layer hens, the carbohydrates detected in the lachrymal gland of broiler chickens during the first 8 weeks of life were mainly in a bound state and that the organ exhibited a marked polysaccharide activity, whose extent and localisation was age-related.

According to Burns (26) the epithelium in the acini and tubules of lachrymal glands in Pekin ducks stained intensely blue in the Alcian blue test at pH 2.5, whereas the content of acinar and tubular lumens – moderately blue. The author reported that this test gave a weak reaction in the lachrymal gland of Shivers layers as glandular cells of acini and tubules assumed a very pale blue colour. Also, the author affirmed that the Alcian blue test at pH 1.0 resulted in moderate staining intensity in glandular cells of acini and tubules of Pekin duck's lachrymal gland while in layer hens, the test revealed a weak reactivity.

The results from the two Alcian blue tests (at pH 1.0 and pH 2.5) in all age groups were partly comparable to those in layer hens – absent, weak or moderate extent of cytochemical reactivity in each age group. This allowed us affirming that during the first 8 weeks of life, the lachrymal gland of broiler chickens produced moderately acidic non-sulfated glycosaminoglycans. A very weak reactivity for sulfated glycosaminoglycans was exhibited only by several, few acini and tertiary tubules of the 56-day-old lachrymal gland.

As per Burns (26) when the Alcian blue test was followed by PAS test, the cells of acini and tertiary tubules of lachrymal glands of layer hens stained intensely red, whereas those in secondary and primary tubules – blue.

The MAB/PAS test of our study confirmed partly the findings of Burns (26). The red colour in glandular cells of acini and tertiary tubules was absent, but the absence, moderate or strong alcianophilia of the epithelial-glandular component responsible for the emerald blue colour of different intensity in all age groups was observed in all age groups after the first week of life. The results from the combined tests confirmed that the secretions of the lachrymal gland of broiler chickens during the first 8 weeks after hatching consisted of moderately acidic polysaccharides.

CONCLUSION

The results from the present histochemical study allowed us affirming that from the end of the 1st week to the 56th day of life, the lachrymal gland of broiler chickens was an organ with marked polysaccharide secretory activity, which exhibited an age-dependent intensity and localisation. The secreted polysaccharides were mainly in a bound state.

During the first weight weeks of its post-incubation development, the lachrymal gland

of the broiler chicken produced mainly moderately acidic non-sulfated glycosaminoglycans (mucopolysaccharides). Only few acini and tertiary tubules of the gland in 56-day-old chickens exhibited a very weak reaction for sulfated mucopolysaccharides.

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