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PATHOLOGICAL AND CLINCO-PATHOLOGICAL STUDIES ON THE EFFECT OF INTRODUCING PITUITARY GLAND DNA AS FOREIGN GENES IN A LOCAL CHICKEN BREED

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ABSTRACT :The experiment was conducted as an attempt to introduce DNA of pituitary glands either from broiler chicks (males or females) or turkey at market age into eggs of Montazah chickens (embryo cells) at the third day of incubation. Several hematological and biochemical parameters as well as histopathological changes in different organs were studied on the hatched chicks at different ages.

The results showed that the introduction of foreign DNA had different expression of protein and lipid synthesis. The DNA injected chicks had the highest globulin concentrations ($P<0.05$), while it had insignificant differences in other blood parameters compared to the control group. Moreover, injected pituitary DNA in chicken embryos was capable of increasing not only body weight but also the hyperplastic and proliferative changes in the reticuloendothelial elements in various organs particularly in the bursa of Fabricius and spleen. However, mild inflammatory changes were seen in the various studied organs of the treated chicks compared to control one.

On conclusion, inoculation of foreign DNA into chicken eggs represented a suitable technique by which hyperimmune transgenic chicks could be produced due to increase in the number of lymphocytes in the defense mechanism organs.

INTRODUCTION

The ability to alter the phenotype of intact animal by the insertion of exogenous genetic material had stimulated considerable interest and resulted in the development of improved methods of gene transfer, particularly in domestic animals.

Gene transfer in poultry is usually aimed to improve disease resistance, food utilization or growth **(Shuman and Shoffner, 1982)**. Recombinant DNA and gene transfer will provide means to increase genetic variation, formation of new phenotype that may have increased economic value, permit the transfer of favorable traits, reduce the time, labor and expense of back-crossing **(Freeman and**

Masser, 1985); Hughes et al. (1986) and **Shuman (1991)**.

The present study was conducted to introduce DNA of pituitaries either from turkey or broiler chicken (males and females) into eggs of Montazah chickens (embryonic cells) to determine its effect on chicken performance, hematological, biochemical parameters as well as histopathological changes that would be happed in the different organs.

MATERIALS AND METHODS

I. Isolation and purification of DNA

High molecular weight DNA was extracted from the pituitary glands of the male (M) and female (F) broilers (B) and turkeys (T) at market ages, according to the methods of **Sambrook et al. (1989)** with some modifications according to **Abdel-Fattah (1995)**.

II. Egg treatments

Four hundred eggs of Montazah chicken strain, obtained from poultry station, Sakha, Kafr El-Sheikh Governorate, were used in this study. All eggs were incubated at 37.8 °C in a forced-air incubator. After three days of incubation, clear eggs (122 eggs) were discarded. The remaining fertile eggs (278) were assigned randomly into different groups according to the source of the foreign DNA as follows:

1. **Control group**. There were three types of control groups

   a-Eggs without any treatment (25 eggs).

   b- Eggs drilled and sealed without any injection (20 eggs).

   c-Eggs drilled and injected with TE buffer at levels of 6, 12 and 24 µl (20 eggs each).

2. **Broiler DNA groups.** Eggs were injected with 1, 2 and 4 µg broiler DNA/egg extracted from pituitary glands of either male or female broilers (MB or FB). The number of eggs for MB – DNA was 16, 20 and 20 eggs, respectively and for FB – DNA was 25, 20 and 20 eggs, respectively. Each DNA concentration was dissolved in 250 µl of 1X TE buffer (0.1 mM Tris HCl pH 7.5 and 0.1 mM EDTA)

3. **Turkey DNA groups**. Eggs were injected with 1, 2 and 4 µg turkey DNA/egg extracted from pituitary gland of both sexes and dissolved in 250 µl 1X TE buffer. The number of eggs was 20, 17 and 15 eggs, respectively.

   The surface of egg (shell) was wiped with 70% ethanol, then a hole of 2 mm was drilled over the air-cell of the egg and DNA was injected. The surface of the egg was wiped again, sealed with liquid paraffin and returned to incubator.

III. Management

Hatched chicks were wing-banded at hatch day and brooded on floor brooder at Mubark City for Scientific Researches and Technological Applications. The starting temperature was 34 °C for the first week, then decreased 2 – 3 °C every two weeks thereafter. All chicks were fed a commercial diet. Feed and water were provided *ad libitum*.

At 36 weeks of age, the females (42 hens) were inseminated artificially with the semen of males from the same treatment. 500 eggs were collected and incubated to produce the chicks of the next generation (kept until the 8th week of age).

IV. Hematological and biochemical characteristics

Blood samples were taken from the wing vein of all birds subjected for the experiment. Total serum proteins were measured by the Biuret method according to **Armstrong and Carr (1964)**. Albumin concentration was determined according to **Doumas et al. (1977)**. Globulin and total serum lipids were estimated according to **Frings et al. (1972)** and total cholesterol was determined according to **Watson (1960)**. Finally, serum glucose concentration was measured by the method of **Trinder (1969)** using commercial kits – Diamond Diagnostics.

V. Histopathological studies

At 13 weeks of age, 18 birds from all treatments (3 turkey-DNA, 5 MB-DNA, 6 FB-DNA and 4 control) were slaughtered. The various internal organs as well as spleen, bursa of Fabricius, thymus and testes were examined. Tissue specimens from the above mentioned organs of all treated as well as control cases were collected and fixed in 10% neutral buffered formalin and then subjected for the routine technique of paraffin embedding. Sections of 3 – 5 microns, were prepared and stained with hematoxylin and eosin **(Bancroft and Stevens, 1990)**.

VI. Statistical analysis:

Data were analysed by the Student's t-test after **Steel and Torrie (1960)** to determine the significant difference between different groups.

RESULTS AND DISCUSSION

1. Hematological and biochemical changes:

The analysis of results (Table 1) showed insignificant differences among all effects studied for the five traits. The overall mean values of serum glucose, total serum lipids and cholesterol were 179.49 ± 4.81, 417.79 ± 17.99 and 122.44 ± 4.99 mg/100 ml for the treated groups and 176.76 ± 5.30, 399.99 ± 15.33 and 121.04 ± 5.50 mg/100 ml for control groups, respectively. Generally, the mean values of serum glucose obtained in the present study were lower than the corresponding results reported by **Tapper and Kare (1960)** for White Leghorn chicks at the same age. **El-Hindawy et al.**

(1997) showed that the glucose level ranged between 216 to 248 mg/dl for all treatments with broiler chicks. However, the total serum lipids values in the present study were higher than that found by **Diller et al. (1960)** with White Leghorn chicks, while it is in accordance with corresponding results reported by **El-Hindawy et al. (1997)** with broiler chicks. The total serum cholesterol values of the present work are in agreement with the findings of **Weiss (1957)** and **Cadwell and Suydam (1960)** with White Leghorn chicks and cholesterol levels were lower than that reported by **El-Eraky and El-Sadawy (1997)** with broiler chicks. The considerable variation among different investigations may be related to the differences in strains, determination methods, treatments or physiological state.

Table (1): Mean values of serum parameters ± SE* of treated and control groups of Montazah chickens.

Item	Treated group	Control group
Glucose (mg/dl)	179.49 ± 4.81 a	176.76 ± 5.30 a
Total lipids (mg/dl)	417.79 ± 17.99 a	399.99 ± 15.33 a
Cholesterol (mg/dl)	122.44 ± 4.99 a	121.04 ± 5.50 a
Total proteins (g/dl)	3.55 ± 0.10 a	3.79 ± 0.08 a
Albumin (g/dl)	1.93 ± 0.05 a	2.06 ± 0.03 b
Globulin (g/dl)	1.61 ± 0.09 a	1.72 ± 0.06 a

* SE Standard error.

a-b Different letters are significantly different ($P<0.05$).

The results in Table (1) showed insignificant difference treated and control groups for total serum protein and globulin concentrations. Significant differences were only found betwẽen treated and control groups for albumin concentration. The overall means of serum total protein, albumin and globulin concentrations were 3.55 ± 0.10, 1.93 ± 0.05 and 1.61 ± 0.09 g/100 ml for treated groups and 3.79 ± 0.08, 2.06 ± 0.03 and 1.72 ± 0.06 g/100 ml for control groups, respectively. Generally, the values obtained are in accordance with results reported by **Brand et al. (1951)** with New Hampshire Red chicks, **El-Hindawy et al. (1997)** and **El-Eraky** and **El-Sadawy (1997)** with broiler chicks.

2. Histopathological changes:

The bursa of Fabricius of chickens injected with a dose of 1 μg or 2 μg (M+F) broiler or turkey DNA showed degenerative changes for the epithelial cell covering, subepithelial edema, congestion with a few mononuclear cell infiltration (Fig. 1). In some cases, metaplastic as well as necrotic infolding epithelial cell covering with multiple congested capillaries, edema and cellular infiltration were also noticed (Fig. 2).

While chicken treated with 4 μg DNA (M, F broiler or turkey) showed an extensive lymphocytic cell infiltration in between the follicular wall (pleaca) besides epithelial cell covering degeneration (Fig. 3).

Moreover, bursal lymphoid follicle hyperplasia, metaplastic cystic structure with extensive interfollicular cells proliferation were also noticed besides fibroplasia of the surrounding connective tissue (Fig. 4, 5). No detectable changes showed in control cases (Fig. c). It could be noticed that injected pituitary DNA in embryos is capable of increasing not only body weight (464.8 ± 20.5 vs 565.8 ± 14.5 for treated and control groups) but also the number of lymphocytes, especially in the bursa of Fabricius. Our findings are in close agreement with **Mandour (1996)** who significantly correlated the packed bursa of Fabricius with lymphocytes and improved immunity of transgenic chicken (injected with foreign DNA).

The spleen of chickens treated with a dose of 1 μg (Tur or MB or FB) DNA showed an excessive erythropioesis and hemosiderosis (Fig. 6), while congestion of the subcapsular sinusoids and increase in the thickness of the capsule were also noticed. The chickens treated with 2 μg (tur or MB or FB) DNA showed splenic lymphoid follicle hyperplasia (Fig. 7). The cases treated with 4 μg (tur or MB or FB) DNA showed also splenic lymphoid hyperplasia in addition to reticuloendothelial cell proliferations. The splenic sheathed arteries suffered hyperplasia, endothelial cell proliferation, medial vacuolation as well as hyalinization, accompanied with adventitial proliferation and perivascular edema (Fig. 8, 9). The previously mentioned lesions were in agreement with those reported by **El-Fiky and Mehana (1998)**.

The testes of chickens treated with a dose of 1 μg of turkey DNA, male and female broiler DNA showed spermatogonial layer degeneration represented by vacuolar and hydropic degeneration with presence of some spermatocytic giant cell formation in the lumina of some seminiferous tubules (Fig. 10). Less spermatogenesis could be also seen in other tubules. At a dose of 2 μg of turkey DNA, MB and FB DNA, the most clear changes were in the form of an extensive dilatation of seminiferous tubules, interstitial congestion, fibroplasia as well as mild proliferation of the interstitial Lyedig cells. Finally, the dose of 4 μg of the three types of DNA led to spermatogonial cell hyperplasia with

an extensive tubular dilatation. The testes of the control cases showed only mild degenerative changes of spermatogonial cells with slight interstitial congestion. These results were in parallel line with those reported by **El-Fiky and Mehana (1998)**.

The dose of 1 µg of three tested types of DNA (Tur, MB, MB) led to congested medullary capillaries with perivascular edema, while the dose of 2 µg led to multifocal hyalinized structureless cysts in the thymal medulla. Finally, the dose of 4 µg led to hyperplasia of both lymphoid element and reticuloendothelial cell of the cortex and medulla. The thymic control cases did not show any abnormal changes except congested interfollicular capillaries.

The prominent histopathological alterations were noticed in the liver of chicken injected with 4 µg DNA. There were pale pink edematous fluid filling the portal area, extravasated RBCs and minute foci of lymphocytic aggregations (Fig. 11). Moreover, extensive hepatocytic degeneration, necrosis, fibroplasia and numerous ectopic lymphoid foci formations were noticed (Fig. 12, 13). No detectable changes were noticed in the control cases except congested hepatic sinusoids. Similar changes were noticed by **Clark and Das (1974), Wood and Richards (1975)** as well as **El-Fiky and Mehana (1998)**. Also, the same changes were detected in hepatocytes of mice carrying human or cattle growth hormone gene **(Wanke et al., 1997)**.

On conclusion to the above mentioned results, it was noticeable that with the exception of some mild inflammatory changes that were seen in the examined organs of treated chickens, obvious hyperplastic as well as proliferative changes were seen in the reticuloendothelial elements in various organs particularly in the bursa of Fabricius and spleen. These changes in the organs responsible for the defensive mechanism are supporting to those results of **Shuman and Shoffner (1982)** and **Mandour (1996)** for the production of hyperimmune transgenic (DNA-injected) chicks.

DESCRIPTION OF FIGURES

Fig. (1): Bursa of Fabricius of chicken, treated with a dose of two µg broiler DNA, showing epithelial cell covering degeneration, subepithelial edema and congestion with a minute mononuclear cell infiltration. (H&E, X250).

Fig. (2): Bursa of Fabricius of chicken, treated with a dose of two µg turkey DNA, showing infolding (A), metaplasia (B) and necrosis (C) of the epithelial cell covering besides subepithelial congestion, edema and cellular infiltration (D). (H&E, X400).

Fig. (3): Bursa of Fabricius of chicken, treated with a dose of four µg turkey DNA, showing extensive lymphocytic cells aggregation in between the internal wall of the bursa (plecea). (H&E, X250).

Fig. (4): Bursa of Fabricius of chicken, treated with a dose of four µg male, female broiler DNA, showing cystic structure (A), bursal lymphoid follicle hyperplasia (B) and interfollicular cells proliferation (C) packed bursa. (H&E, X160).

Fig. (4 A): Bursa of Fabricius of controll chicken showing less lymphocytic cells aggregation between the internal wall of the bursa (plecea). (H&E, X=160).

Fig. (5): Higher magnification of Fig. (4) to show the metaplastic cystic structure. (H&E, X400).

Fig. (6): Spleen of chicken, treated with one µg turkey DNA, showing excessive erythropioesis. (H&E, X250).

Fig. (7): Spleen of chicken, treated with two µg MB DNA, showing splenic follicle hyperplasia. (H&E, X250).

Fig. (8): Spleen of cases treated with four µg FB DNA, showing lymphoid element hyperplasia (A) as well as hyperplasia of splenic sheathed arteries (B). (H&E, X250).

Fig. (9): Higher magnification of Fig. (8) to show medial vacuolation as well as hyalinization of splenic sheathed arteries besides perivascular edema (H&E, X400).

Fig. (10): The testis of chicken treated with a dose of two µg of male broiler DNA showing spermatogonial degeneration represented by vacuolar, hydropic degeneration with spermatocystic giant cells formation in the lumen of seminiferous tubules (H&E X400).

Fig. (11): liver of chicken, treated with four µg turkey DNA, showing pale pink edematous fluid (A) filling the portal area, hemorrhage (B) and minute foci of lymphocytic infiltration (C) (H&E, X250).

Fig. (12): Liver of chicken, treated with four µg turkey DNA, showing an ectopic lymphoid foci (A) and fibroplasia (B) in the interlobular area. (H&E, X250).

Fig. (13): Liver of chicken, treated with four µg turkey DNA, showing diffuse area of necrosis (arrows) (H&E, X250).

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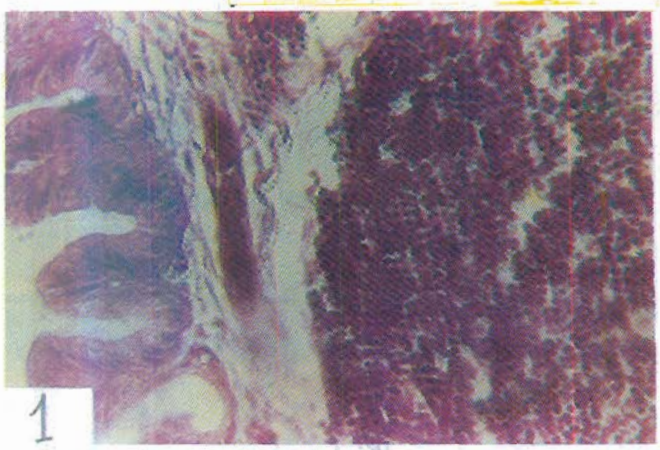
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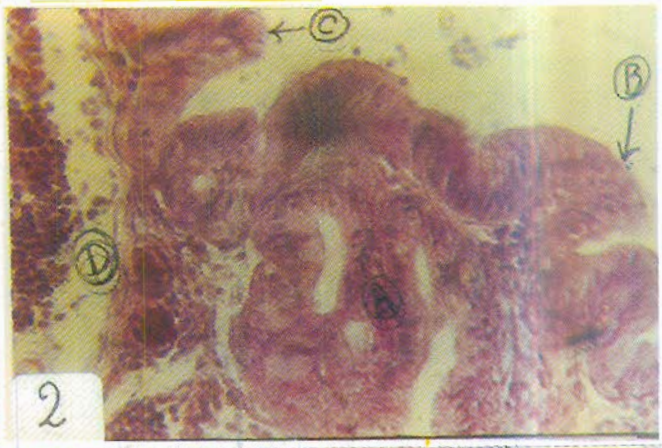

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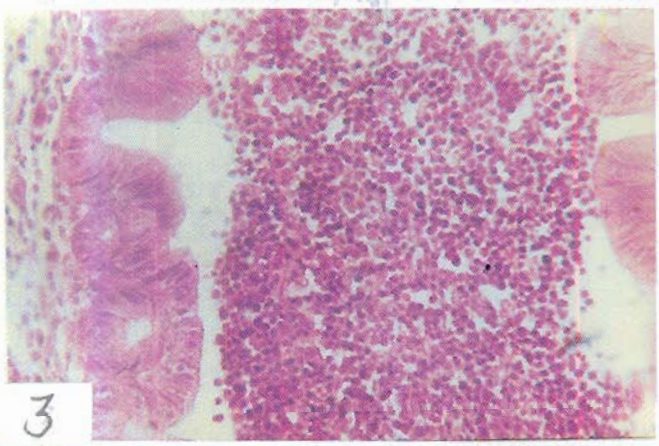
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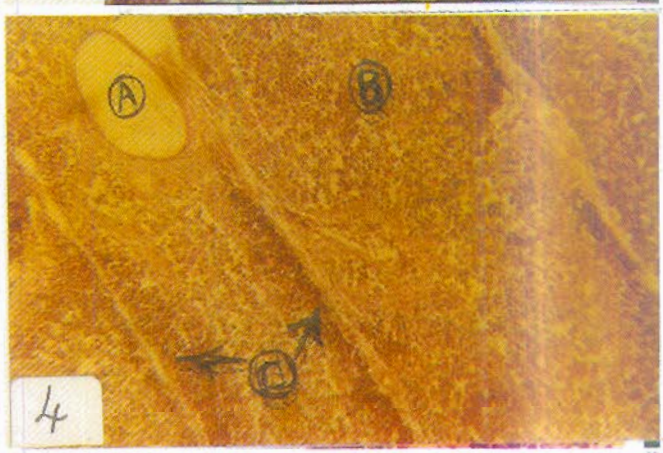

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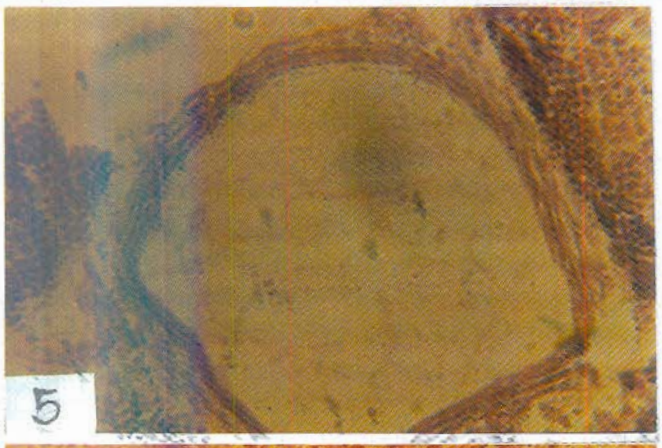
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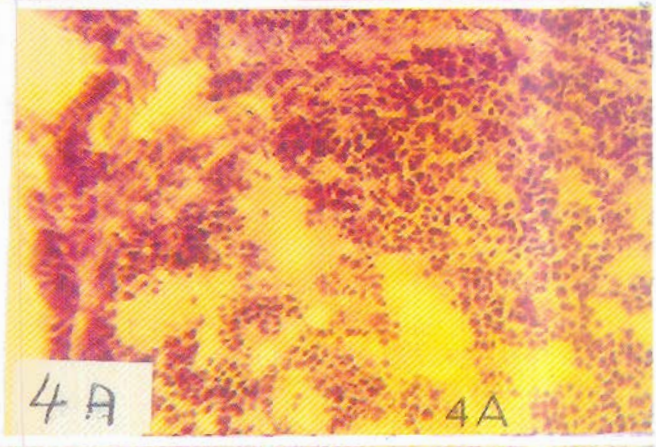

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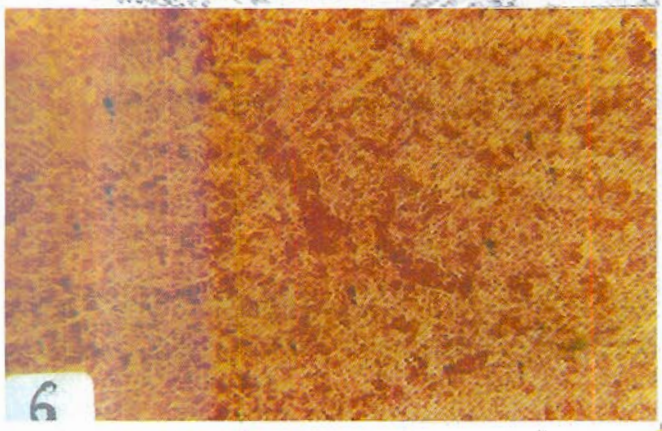
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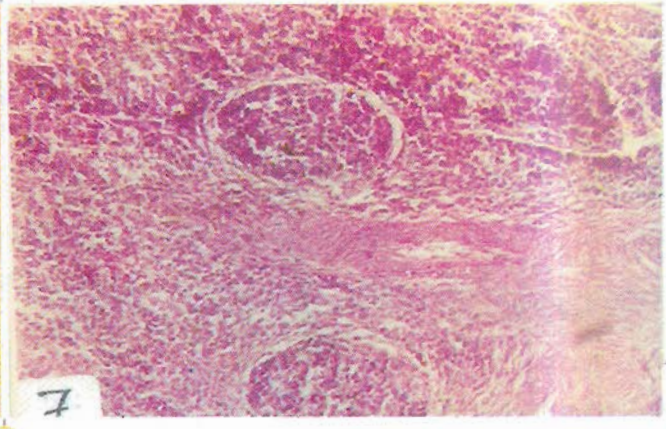
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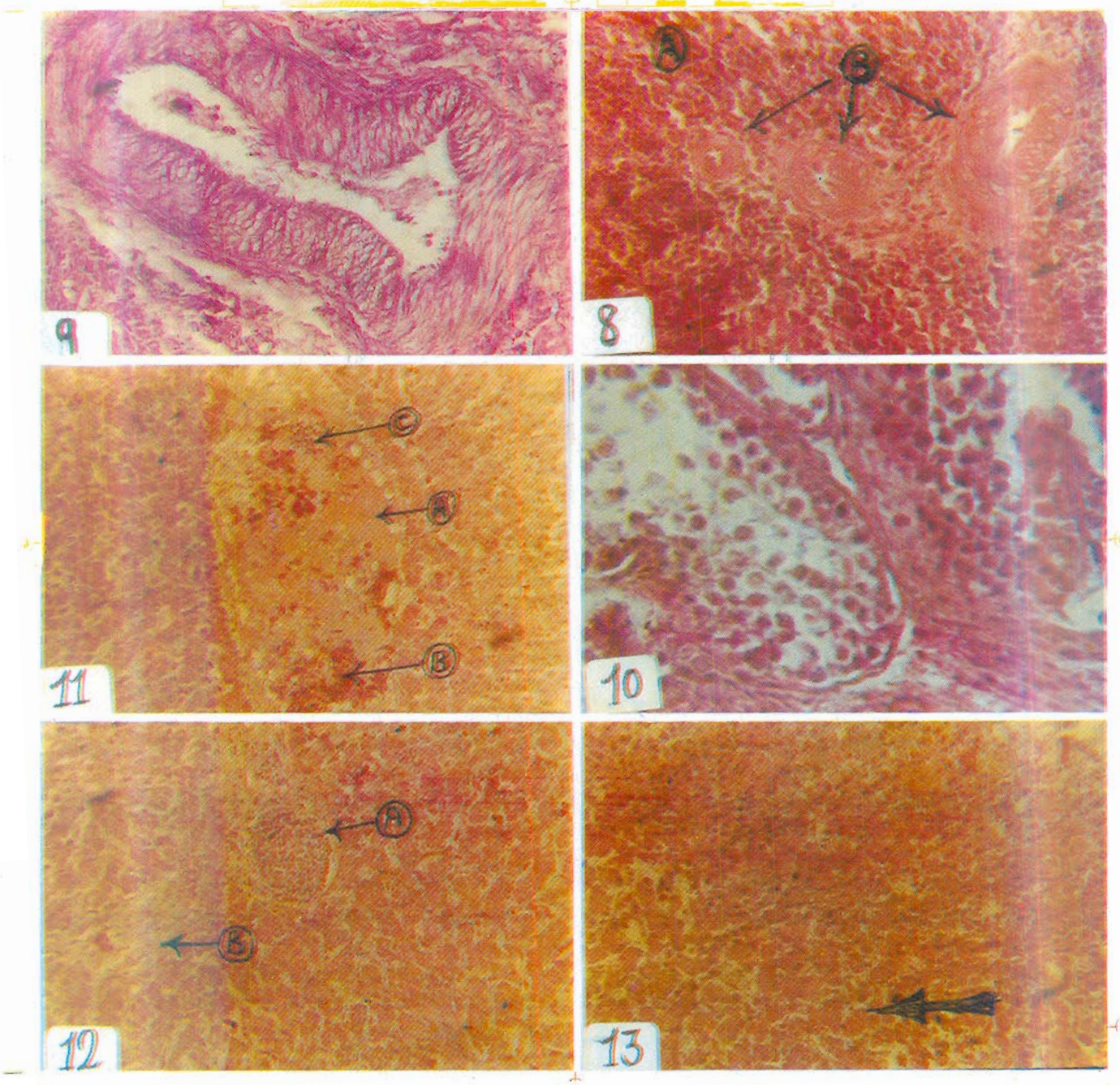
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