Dept. of Pathology and clinical Pathology.
Fac. of Vet. Med. Assiut. University

ETIOLOGICAL AND MORPHOLOGICAL STUDIES ON INFECTIOUS LEG WEAKNESS IN COMMERCIAL BROILER FLOCKS

(with 2 Tables and 12 Figures)

BY

S. Kh. ABD ELGHAFFAR; A. M. ABD-ELGWAD* and HEBAT-All, A. MOHAMED*

*Animal Health Research Institute. Assiut Lab.

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"دراسات سببية ومورفولوجية على حالات ضعف الساق المعدى فى قطعان بدارى التسمين"

ساري خليل عبدالغفار ، عبدالتواب محمد عبدالجواد
هبه الله عبدالحليم محمد

فـــى هـــذا البحـــث تم اختبار عدد ٤٨ من بدارى الدجاج النافقة حديثا والتى كان بها عرج واصـــابات فى الساق جمعت من مزارع حكومية وخاصة بمحافظة أسيوط وخضعت جميعها للفحـــوص البكتـــيرية والفيروسية والميكوبلازما من مفاصل و عظام الأرجل والتى كان بها علامات باثولوجية للعدوى البكتيرية وقد أظهرت الفحوص عدم وجود إصابات بالميكوبلازما والفيروســـات ولكن تم عزل ١٧ حالة إيجابية للبكتيريا (١٤ حالة لميكروب المكور العنقودى الذهبى بنسبة ٢ر٢٩% و ٣ حالات للميكروب القولونى الغير محلل للدم بنسبة ٣ر٦%). هذا وقـــد تم اختيار ميكروب المكور العنقودى الذهبى المعزول لأجراء العدوى الصناعيه به فى بدارى الدجاج عن طريق الحقن فى الوريد وجد أن الأعراض الأكلينيكيه والصفه التشريحيه تشبه الى حد كبير تلك التى لوحظت فى العدوى الطبيعية. وقدتم عزل الميكروب مرة أخري من الطيور المصابة. وقد أظهر الفحص الهستوباثولوجى للطيور المصابه طبيعيا أو تجريبيا وجود التهابات صديديه فيبرينيه حادة أو تحت حادة خاصه فى مفصل الركبه. هذا مع وجود الـــتهابات وتتكرز فى الغضاريف الناميه الموجودة فى نهايات العظام الطويله للأرجل. وقد نوقش التطور المرضى باثولوجيا. وقد تم اختيار ميكروب المكور العنقودى الذهبى المعزول لأجـــراء اختـــبار الحساســـية معمليا ضد أنواع مختلفة من المضادات الحيوية وقد وجد انها جمـــيعا عالـــية الحساســـية لكـــل من السيبروفلوكساسين ، الأنروفلوكساسين ، الجينتاميسين والأميكاسين.

SUMMARY

A total of 48 freshly dead lame broiler (1-7 weeks old) Habard chicken collected from different governmental and private farms at Assiut Governorate. All samples were subjected for mycoplasma, viral and bacterial examination. The microorganisms were cultured from joints and bones of the legs, which showed pathological evidence of infection. All the samples were negative for mycoplasma and viruses but only 17 positive cases for bacteria were isolated (14 cases for *Staphylococcus aureus* with an incidence of 29.2% and 3 cases for Non Hemolytic *E-coli* with an incidence of 6.3%). Experimental infection in broiler chickens via the intravenous (I.V) injection of isolated *Staph.aureus* revealed that the clinical observation and postmortem lesions were similar to a great extent to those of natural infection. Re isolation of inoculated bacteria was also done. The histopathological examination of the joints and bones of the legs from the natural or experimental infected birds revealed acute and subacute fibrinopurulant arthritis especially in the knee joints. The arthritis was associated with osteomylitis and necrosis in the epiphyseal and physeal cartilage at the bony ends. The pathogenesis of these lesions was discussed. The in-vitro sensitivities of the isolated *Staphylococcus aureus* against different antimicrobial agents showed that the examined isolates were highly sensitive to Ciprofloxacin, Enerofloxacin, Gentamycin and Amikacin.

Key word: *Infactious Leg weakness, etiology, Pathology and pathogenesis*

INTRODUCTION

Limb deformities and bony dyschondroplasia were highlighted as the predominant causes of lameness in broilers chickens (Maff, 1981 and FAWC, 1992). Kestin *et al.* (1992) also recorded that leg weakness and lameness in broilers are of major concern to poultry industry.

Lameness in broilers may be associated with pathological lesions in the growth plate of the ends of the femur, which may be either infectious or non-infectious in origin (Throp *et al.*, 1993). In a recent study, a high prevalence of bacterial infection of the growth plate in the ends of the femur was observed histopathologically in broilers with clinical evidence of lameness due to joint lesions. Reece (1992) recorded that staphylococci are not only the bacteria contributing to proximal femoral osteomyelitis, but *E.coli* and salmonellosis has been reported in

some affected flocks. kirkskeeles (1997) found that *Staphylococcus aureus (Staph. aureus)* infections are common in poultry, the most frequent site being bones, tendon sheaths and joints of legs.

McNamee *et al.* (1998) isolated 12 *Staph. aureus* isolates from lame broilers chicken with a percentage of 27.3 % and Non Haemolytic *E-coli* (NHEC) was isolated from 6 (13.6%) of the same lame birds. Rizk and Bkhiet (2001) revealed the isolation of *Staph. aureus* at a ratio of 8.87% in native and foreign broiler chickens at Bohaira Governorate. So the work reported in this paper was undertaken to give an idea about the following:

A-Isolation and identification of the associated infectious agents which contributed to leg weakness in a representative samples of lame commercial broilers.

B-Experimental infections with the isolated organisms in broiler chicks by I.V. route of inoculation.

C-The main pathological lesions in leg joints and bones of the lame broilers in natural and experimental infections and its pathogenesis.

D-In vitro sensitivity test of the isolated Staph.aureus strains against different antibiotics.

MATERIALS and METHODS

Materials:

1- A total of 48, freshly dead and lame broiler chickens (1-7 weeks old) were obtained from different governmental and private farms at Assiut Governorate. Those birds were suffered from swollen joints, lameness and retardation in growth. Those chickens were fed balanced ration. The leg joints and bones as well as lungs, liver and spleen were examined for bacteria, virus and mycoplasma.

2- Experimental birds:- Fifteen (3 week old) Habard chicks obtained from faculty of agriculture farm, Assiut university were used for pathogencity study.

Methods:

1- Bacteriology:

Swabs from the joints and bones head of the legs were inoculated into one broth tube and other loopfull from liver, lung and spleen were also inoculated into other broth tube. The broth tubes were incubated at 37c° for 18-24 h. followed by subculturing on solid media as:-

-Baird-Parker agar media at 37c° for 48h.as a selective medium for staphylococci (Baird - Parker 1962).

-blood agar and Macconkey's agar media at 37c° for 24h. The obtained colonies were picked up and stored in semisolid agar for further identification morphologically, microscopically and biochemically according to Crusckshank *et al,*. (1975) and Baird - Parker (1979).

2- Mycoplasma examination:

Swabs from the joints and head of the leg bones were collected and cultured by standard techniques (Fery *et al.*, 1968, Ball *et al.*, 1994).

3- Virus isolation:

Bacteria free suspensions (10% w/v) were made in phosphate buffered saline containing antibiotics from the joints and head of the leg bones. Samples were centrifuged at 4000 rpm for 20 minutes. Five chicken embryos (8 day old) were inoculated via allantoic sac with 0.1ml of bacterial free supernatant fluid. All embryos were incubated and held for 6 days. Embryos were candled daily and fluids were harvested and tested for haemagglutination activity (Yamaguchi *et al.* 1981).

4-Pathogencity study:-

Fifteen healthy broilers chicks, free from pathogenic infection used in this study were observed for one week. Ten chicks were inoculated I.V with 0.5 ml of 24 h. Trypticase soy broth containing 1×10^5 *Staph.aureus* / chick (Griffiths *et al.* 1984). The other five chicks were left as a control without inoculation. All chicks left for 30 days under observation.

2- Histopathology:

For histopathological examination the leg joints (hip and knee joints) including head of the bones (femur and tibiotarsal bones) were taken from the naturally and experimentally infected cases, then fixed in 10% neutral buffered formalin before decalcification in nitric acid / formalin solution. Paraffin embedded sections were then prepared, stained with haematoxylin and eosin and examined. To appreciate fully the extent and nature of histopathology, interrupted serial sections were prepared.

6- Sensitivity test:

The paper disc technique was carried out after Finegold and Baron (1986) using identified *Staph. aureus* isolate and 15 chemotherapeutic discs produced by Oxoid Basingstake, Hampshire, England in order to determine their antibiogram. Discs are including: Gentamycin (10 µg), Kanamycin (30ug), Streptomycin (10µg), Amoxacillin (25µg), Neomycin (30µg), Ampicillin (10µg),

Erythromycin (15μg), Ciprofloxacin (5μg), Penicillin (10μg), Amikacin (30μg), Cloxacillin (5μg), Enerofloxacin (5μg), Lincospectin (15μg), Tobramycin (5μg).

RESULTS

I- Bacteriology:

According to morphological and biochemical characters. 14 isolates (29.2%) were identified to be *Staph. aureus*.These isolates were characterized by its typical growth of coaggulase positive, black colonies surrounded by faint yellow zone on Baird-Parker agar media and its hemolytic activity on blood agar media. Non Hemolytic *E-coli* were also isolated from 3 (6.3%). The results for isolation and identification of bacterial agents were summarized in table (1). The greatest incidence of total bacterial isolates (*Staph. aureus*) occurred between 3-4 weeks of age.

II- Virology:

No viral agents were recovered from the examined specimens.

III- Mycoplasma results:

No mycoplasma was isolated.

IV-Pathogencity study:-

Two weeks post inoculation, the inoculated birds appeared stunted when compared with the control birds. They also had ruffled feathers, reluctant to walk and sitting on their knee (Fig. I). The knee joints were swollen and warm compared with the control (Fig. 2). Bumble foot was also observed (Fig. 3). Some birds had only one leg affected, the others were bilaterally affected. Osteomylitis and necrosis were grossly observed in bones in some birds after scarification at the end of the experiment (Fig. 4). The kidneys appeared swollen and the ureters were filled with urates.

Staph. aureus was reisolated from the leg joints and bones, liver, spleen and heart blood of all experimental infected chickens.

V-Histopathological study:-

The pathological examination of the affected joints and bones revealed similar lesions both in the experimental and in the field cases.

The joints (in the younger bird 1-3 week old) suffered from acute fibrinopurulant arthritis manifested by:

a-Heterophilic cellular reaction associated with congestion of the blood vessels and fibrinous exudation at the dermis (dermatitis) and subcutis (cellulitis) (Fig. 5 and Fig. 7).

b-Heterophilic cellular reaction associated with congestion of the blood vessels and fibrinous exudation at the synovial membrane (synovitis) and articular surface lead to their adhesion (Fig.6 and Fig. 8). Minimal degeneration in the articular cartilage was also observed (Fig. 8).

The joints (in the older birds 4 -7 week old) suffered from subacute fibrinopurulant arthritis manifested by:

a- Thickening of the synovial membrane due to cellular hyperplasia and hypertrophy associated with fibrinopuralant reaction on their surface (Fig.9).

b- The vascular canal at the epiphyseal cartilage of the bone was occluded with fibrinopurulant □xudates associated with necrobiosis of the vascular wall as well as thrombosis (Fig. 10).

c- The epiphyseal and physeal cartilage of the bone showed either focal or diffuse necrosis of the chondrocytes with presence of eosinophilic debris (Fig. 11 and Fig. 12).

d- Destruction on the articular surface of the bone was also noticed in some severe cases (Fig. 12).

VI- Sensitivity test:

The effect of different antibiotics on the isolated *Staph.aureus* isolates is illustrated in table 2.

DISCUSSION

Poultry meat is considered one of the most important source of animal protein in Egypt. Leg weakness is a problem or disease condition and that causes economic losses among broiler chickens. The birds in the present study were selected for postmortem on the basis that lameness was likely to be caused by a lesion in the legs joints and bones.

In this study, seventeen of 48 samples yielded bacteria, of which 14(29.2%) were Staph.aureus and 3(6.3%) were NHEC. A nearly similar finding was mentioned by Thorp *et al.* (1993), McNamee *et al.* (1998) who isolated Staph.aureus with a percentage of 30.3 and 27.3% respectively from the proximal femora of the birds.

With respect to NHEC, our results of isolation was much higher than that reported by Thorp *et al.* (1993) 1.5% and much lower than McNamee *et al.* (1998) 13.6%. Staphylococci are not the only bacteria contributing to femoral osteomylitis; E.coli and Salmonellosis have been reported in some affected flocks (Reece 1992).

The data obtained from the present cases revealed that neither virus nor mycoplasma was isolated. Many authors reported the positive isolation of viruses (Vander Heide 1981, Yogaratham 1995 and

McNamee *et al.* (1998) recorded that no mycoplasma were isolated from lame broiler chicken.

Bacterial arthritis and osteomylitis in the proximal and distal ends of the femur were the predominant and important causes of lameness in broilers with clinical evidence of joints problem (Thorp *et al.*, 1993). In this study it was found that the pathology of experimental and of field cases of septic arthritis and osteomylitis appear similar. This finding was also recorded by Thorp, *et al.*, 1993).

The pathogenesis of this problem in growing broilers began with bacterial wounds infection due to trauma or mosquito bites in naturally infected cases (Hinshow and McNeil 1952) or through intravenous inoculation of staphylococci in experimental study (Daum *et al.*, 1990). This lead to constant bacteraemia. That can cause septic arthritis and osteomyelitis (Emslie *et al.*, 1983). Staphylococci can adhere to (Nade and speers, 1987) and may be tropic for avian growth plate cartilage (Emslie *et al.*, 1983; speers and Nade, 1985; Alderson and Nade, 1987). Cartilage vascular canal enables the bacteria to spread from the metaphysis to physis and epiphysis (Alderson *et al.*, 1986). This leads to their necrosis. Staphylococcal necrosis of the head of the femur in broiler chickens is a syndrome known as femoral head necrosis (Griffiths *et al.*; 1984).

The degeneration in the walls of the blood vessels in the vascular canal of growing cartilage leads to their thrombosis and subsequently degeneration and necrosis of the epiphysial and physial cartilages and articular surface. Tibiotarsal infarcts was noticed in the turkeys with isolation of staphylococci and E.coli from the lesions (Mutalib and Maslin, 1996). So the lesions began with arthritis followed by extension of infection upward to the subcutis and dermis of the skin leading to diffuse cellulitis and dermatitis and down ward to the articular surfaces and the growing cartilage of the long bones leading to osteomylitis and necrosis

In vitro sensitivity testing of the isolated Staph.aureus strains to 15 antimicrobial agents revealed that the isolates examined were highly sensitive to Ciprofloxacin, Enexofloxacin, Gentamycin, Amikin and moderately sensitive to Rifampcin, Streptomycin, Ampcillin, Tobramycin while Amoxocillin, Linco-spectan are weakly sensitive but penicillin, Erythromycin, Cloxacillin, Kanamycin, Neomycin had no effect at all. Our results agree to some extent with those reported by Carucappa *et al.*, (1991), Abd-El-Motelib and El-Zanaty (1993) and

Abd- El-Hafeez (2002) but to less extent with those reported by Nabila (1982), Ashgan (1988) and Ayhan and Aydin (1991).

Finally, it may be concluded that infectious leg weakness in broilers chicken is of special significance and its pathogenesis is well established. So we advise the addition of the highly sensitive type of antibiotics to the ration once the occurrence of any signs of lameness on the broilers flocks.

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LEGEND OF FIGURES

Fig. 1: Inoculated chickens could not walk and sitting on their hocks.

Fig. 2: Swollen of the knee joint of infected bird (upper) compared with normal joint in control (lower).

Fig. 3: Bumble foot in infected bird (upper) compared with normal footpad (lower).

Fig. 4: Osteomylitis and necrosis in the proximal end of the femur.

Fig. 5: Skin and subcutaneous tissue above the knee joint of infected birds showing diffuse purulant dermatitis and cellulitis at the dermis and subcutis with severe heterophelic cellular reaction. H&E. 10x10.

Fig. 6: knee joint of infected birds showing purulant synovitis. The synovium is thickened due to congestion edema, and heterophilic cellular infiltration. H&E. 10x10.

Fig. 7: Higher magnification of skin and subcutis above the knee joint showing diffuse extensive heterophilic cellular infiltration as well as edema and congestion of the vasculature. H&E. 10 x 40.

Fig. 8: knee joint of infected bird showing sever congestion of the Synovial
blood vessels with extensive purulant synovitis and evidences of degeneration of epiphyseal cartilage (arrows). H&E 10x 40.

Fig. 9: The synovium from a knee joint of infected birds is thickened due to synovial hypertrophy and hyperplasia in association with fibrinopurulant reaction on the surface (arrows). H&E . 10 x 25.

Fig.10: Epiphyseal cartilage from the head of the femur of infected bird.The epiphyseal vascular canal is occluded with fibrinopurulant exudate with necrosis of the vascular wall and thrombosis (S). H&E. 10 x40

Fig. 11: Epiphyseal (E) and physeal (p) cartilage from the head of the femur of infected birds. Note a multiple foci of necrosis in the chondrocytes leaving eosinophilic necrotic debris (N).H&E . 10x 40.

Fig. 12: Epiphyseal and physeal cartilages from the head of the femur. Note the extensive necrosis of the cartilage in the femoral head (V) with destruction of the articular surface (arrows). H&E. 10x 40.

Table 1: Frequency and percentage of isolated Staph. aureus and NHEC isolates.

No. of examined samples	*Staph.aureus* isolates		NHEC* isolates	
	No	%	No	%
48	14	29.2	3	6.3

*NHEC = Non Hemolytic *E-coli*

Table 2: Results of sensitivity of *Staph.aureus* isolates.

Antimicrobial agents	Sensitivity of Staph.aureus isolates
Ciprofloxacin	+++
Enerofloxacin	+++
Gentamycin	+++
Amikacin	+++
Rifampein	++
Streptomycin	++
Ampcillin	++
Tobramycin	++
Amoxcillin	+
Linco-spectan	+
Pencillin	-
Erythromycin	-
Cloxacillin	-
Kanamycin	-
Neomycin	-

+++ = Highly sensitive
++ = Moderate sensitive
+ = Weakly sensitive
- = Resistant


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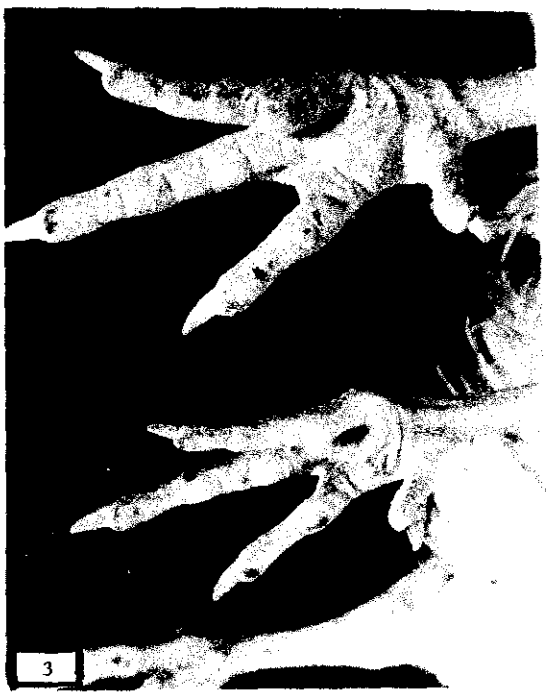
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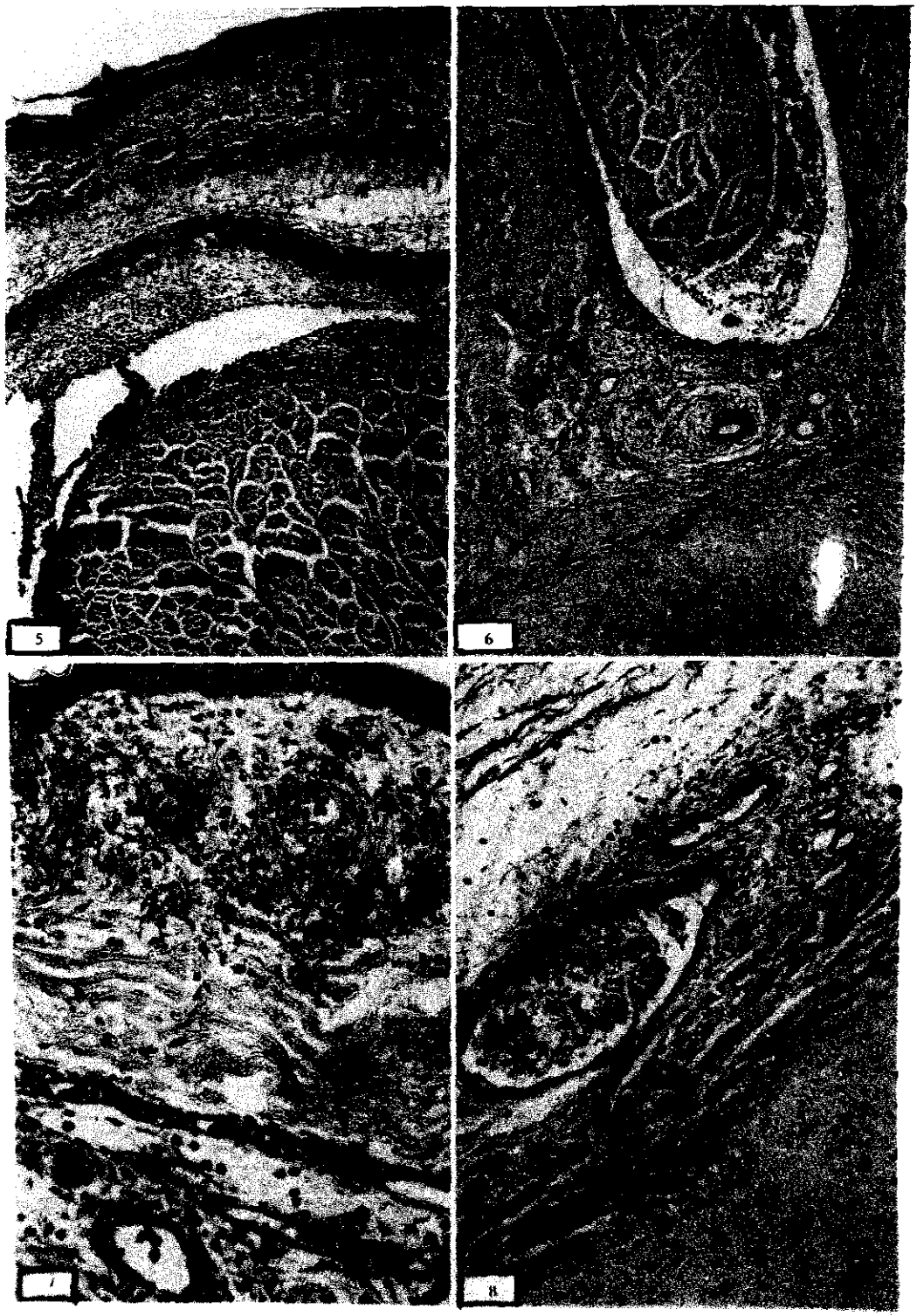
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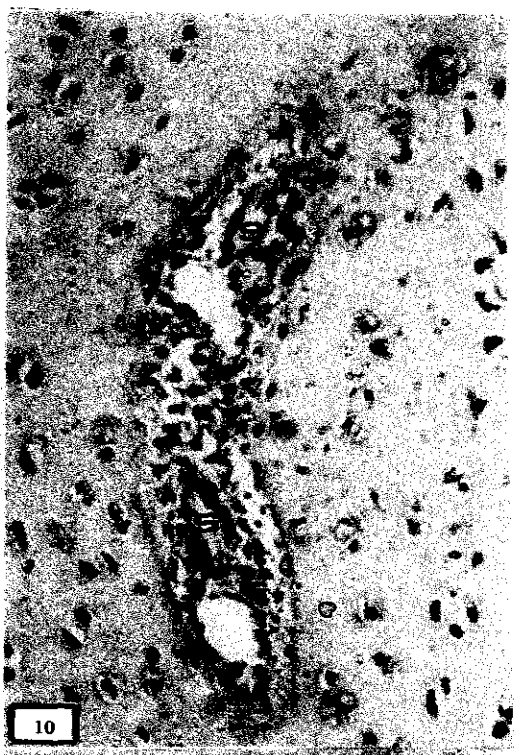
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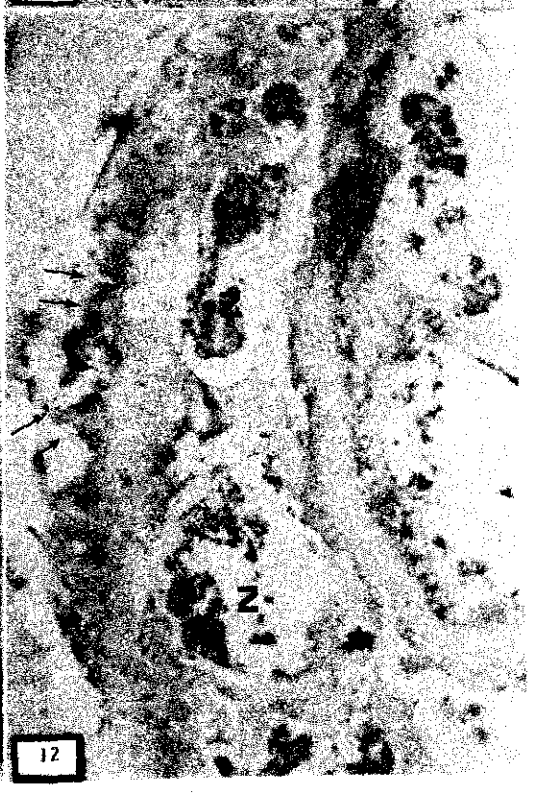
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