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STUDIES ON EFFECT OF E. COLI ON OCULAR AND SUBCUTANEOUS TISSUES IN BROILER CHICKENS

(With 14 Figures)

By

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دراسات على تأثير الميكروب القولونى على العين وعلى الأنسجة التى تحت الجلد فى دجاج التسمين

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تم تجميع ٩٠ عينة (كبد – دم – أكياس هوائية) من دجاج بدارى التسمين حى ونافق حديثاً. تم عزل الميكروب القولونى وتصنيفه وتم إجراء عدوى صناعية بالميكروب لكتاكيت عمر ٤ أسابيع عن طريق الحقن تحت الجلد وعن طريق خدش الجلد فى منطقة البطن حيث أدى هذا إلى التهابات تحت الجلد فى منطقة البطن (cellulitis). كما تم أيضاً إجراء عدوى صناعية لكتاكيت عمر ٤ أسابيع عن طريق حقن الميكروب المعزول فى الأكياس الهوائية والذى أدى إلـــى الـــتهابات بالعين وقد تم عمل الصورة الباثولوجية لهذه الحالات والتى أوضحت وجود التهابات محببة فى الجلد والتهابات محببة فى ملتحمة العين والقرنية والتهاب الجفون وكانت الحبيبات مكونة من خلايا وحيدة الخلية وخلايا متعددة الصبغة وخلايا ليفية وخلايا عملاقة.

SUMMARY

Ninety samples (liver, heart, blood and air sacs) were collected from live and freshly dead broilers. *E.coli* was isolated from them and serotyped. Experimental infection of chickens 4 weeks old by the isolated strain subcutaneauslly and by scratching the skin in the abdominal region resulting in the appearance of cellulitis, which appeared as yellow plaque or mass in subcutaneous tissue and made up of cellular debris and abundant fat cells surrounded by granulomas. The later composed of mononuclear cells, heterophils, fibroblasts and giant cells. Inoculation of isolated *E.coli* through air sacs led to ocular lesions which microscopically represented by granulomatous conjunctivitis, dermatitis of eyelids and kertitis .

Key words: *Chickens, E.coli, cellulitis, ocular lesions*

INTRODUCTION

Cellulitis, first reported by Randall *et al.*, (1984) which, refers to inflammation of the subcutaneous tissue and is typically seen in the lower abdomen and thigh and *Escherichia coli* has been reported as the predominant microorganism isolated from the lesions. *E. coli* causes a variety of disease manifestations in poultry including yolk sac infection, omphalitis, respiratory tract infection, panophthalmaitis, swollen head syndrome, septicemia and cellulitis. Colibacillosis in poultry is characterized in its acute form by septicemia resulting in death and in its subacute form by pericarditis, airsacculitis and perihepatitis (Eterradossi *et al.*, (1989). Cellulitis (Sometimes called necrotic dermatitis) results in considerable economic losses through condemnation or down grading of carcasses, which may belong to serogroups O_1, O_2, O_{78} are usually the most consistently isolated bacteria (Glunder, 1990).

There is a strong association between cellulitis and serositis (combined carcass condemnation for airsacculitis, pericarditis, hepatitis, peritonitis and salpingitis) (Goodhope *et al.*, 1992). A fibrinous plaque between the muscle and the subcutis was the most characteristic feature of the cellulitis and there were various degrees of subcutaneous edema, muscle haemorrhages and subcutaneous purulent exudate (Messier *et al.*, 1993). Cellulitis lesions were primarily unilateral and located on the abdominal area, the colour of the skin varied between bright yellow, dull yellow and reddish brown and the skin was swollen at the site of inflammation (Kumor *et al.*, 1998). Recently, this condition has received considerable attention because of an alarming upward trend in condemnation rate of broiler carcasses (Gomis *et al.*, 2002).

The purposes of the present study were: Isolation and identification of *E. coli*, develop a chicken model of cellulitis and ocular lesions. In addition histopathological changes of cellulitis and ocular lesions in experimentally infected chickens were also described.

MATERIAL and METHODS

Material:

1- Specimens:

Ninety samples (liver, heart blood and air sacs) from live and freshly dead broilers collected from different special farms were used for isolation of *E. coli*. Post- mortem examination of them revealed presence of pericarditis, perihepatitis, airsacculitis and congestion of the carcass.

Media, reagents and solutions:

- MacConkey's agar plates. - Eosin methylen blue agar plates.
- Nutrient agar slopes.
- Semisolid agar (for detection of motility).
- Nutrient broth.
- Kovac's reagent.
- Methyl red reagent.
- Gelatin liquefaction.
- Glucose, lactose, mannitol, sucrose (for sugar fermentation reaction).

Stain:

- Gram's Stain

The different media and reagents used were prepared according to Cruickshank *et al.*, (1975).

Pathogenicity test:

- Fifty, 4 weeks old healthy chickens (hubbard) were used in the experiment, they were obtained from the faculty of Agriculture poultry farm. Five chickens were randomly selected, clinically examined and subjected to bacteriological examination to ensure to be healthy and free from pathogenic *E.coli.*

Methods:

1- Isolation:

Loopfuls from liver, heart blood and air sacs were inoculated into nutrient broth tubes and incubated at 37°C for 24 hours, followed by subculturing on MacConkey's agar plates at 37°C for 24 – 48 hours, suspected colony to be *E.coli* was subcultured on eosin methylene blue agar media and incubated at 37°C for 24 hours. Suspected colonies were picked up and kept on slope agar until further identification to show: colonial morphology, gram's stain and biochemical tests (Indol, vogus proskuer, methyl red and sugar fermentation).

Serological identification of isolates:

Polyvalent I : O_1-O_{11}-O_{20}-O_{157}

Polyvalent II : O_2-O_{10}-O_{55}-O_{142}

Polyvalent III:O_8-O_{22}-O_{12}-O_{127}

Polyvalent VI : O_{87}-O_{86}-O_{114}-O_{109}

Polyvalent V: O_{139}-O_{153}-O_{107}-O_{143}.

Procedure:

- The technique was described by sojka (1965) and Hassanin (1977) using the tube agglutination test. A dense suspension was Prepared From each smooth isolate in sterile saline.

- The suspension was heated at 100°C for 30-60 min. to destroy the k antigen (autoagglutinable suspensions were discarded).
- Double fold dilution of *E.coli* Polyvalent O – antisera (poly I,II,III ,IV,V) were Prepared with normal sterile saline solution starting with dilution 1/50 and ending with dilution 1/200 equal volumes of suspected *E.coli* suspensions were added. The tubes were incubated at 56°C for over night in a water bath.
- Negative control for sera and antigen suspension was prepared.
- If agglutination occurs with one of the Polyvalent 0-antisera the bacterial suspension was tested against the corresponding specific Monovalent O-antisera in double fold dilution starting from dilution 1/10. The tubes were placed in water bath at 56°C for over night.
- Results of the agglutination were read as follow:
- Positive reaction : granular agglutinates on the walls at the bottom of the tube were visible with the naked eye.
- Negative reaction: the bacterial suspension aggregates as a dot at the bottom of the tube. The strains which failed to agglutinate with any of the available antisera were regarded as untypable strains.

2 - Pathogenicity test:

- Fourty-five, 4 weeks old chickens were used, they were divided as follow:

1st group: Ten chickens were inoculated subcutaneouslly with $1x10^6$ CFU, of *E.coli* in the left caudal abdominal region of chickens (Susantha *et al.,* 1997)

2nd group: Five chickens were inoculated subcutaneouslly with normal saline and left as control.

3rd group: Ten chickens were infected by scratching and swabbing the skin on the left side of the abdominal region by the bacterial culture (over night tryptic soy broth culture) (Peighambari *et al.,* 1995).

4th group: Five chickens were kept as control with 3rd group.

5th group: Ten chickens were inoculated via air sacs with 0.5 ml 3.6 x 10^6 C F U of *E. coli* (Nakamura and Abe, 1987)

6th group: Five chickens were kept with 5th group as control.

Gross pathology:

Dead or slaughtered chickens were examined carfully for the existence of skin, subcutaneous and ocular lesions. The other gross pathological changes in carcasses were also reported.

Histopathology:

Skin, subcutaneaus tissue, eyelids, conjunctiva, cornea, sclera, uvea and retina samples obtained from slaughtered chickens were fixed

in 10% neutral buffered formalin. Fixed tissues were dehydrated in a series of alcohols and processed for paraffin embedding technique. Sections were stained with haematoxyline and eosin (HE) (Bancroft & Stevens, 1982).

RESULTS

Bacteriological examination revealed isolation of rose-pink colonies on MacConKey's agar, on eosin methylene blue agar media the colonies were mettalic sheen in colour and some isolates were motile and others were non motile.

Gram's stain showed gram negative bacilli.

Biochemical reactions revealed that suspected isolate of *E. coli* was indole and methyl red positive, ferment glucose, Lactose, mannitol and sucrose, while vogus proskauer, gelatin liquefaction, urea, citrate and H_2S production were negative.

Serotyping of the isolate were 0_2 and untypeable.

Pathogenicity test:

1st group showed 70% mortality between day 2 and 7 post inoculation (p-i) and 90% of birds developed cellulitis represented by presence of subcutaneous yellowish thin, tand, tough plaque. The underlying muscles were congested (fig. 1). All birds developed peritonitis, fibrinous pericarditis, airsacculitis and perihepatitis (fig. 2, 3) in different degrees between day 2 and 15 p-i. Microscopically, the yellow plaque found in subcutaneous tissue made up of cellular debris, abundant fat cells surrounded by granulomas composed of mononuclear cells, heterophils, fibroblasts and giant cells (fig. 4, 5).

No death was occurred in 3rd group but 100% of birds showing cellulitis represented by the presence of yellow mass under the scratched area and congestion of underlying muscles (fig. 6) in different degrees between day 7 and 18 p-i. There was focal ulceration of the skin with or without presence of crusts. 50% of birds developed pericarditis, airsacculitis and perihepatitis (fig. 7). Microscopically, cellulites was of severe degree than that observed in 1st group. Skin showed epidermal hyperplasia, hypodermal granulomas composed of necrotic center surrounded by mononuclear cells and fibrous capsule. The subcutaneous tissue was infiltrated with mononuclear cells (fig. 8, 9).

5th group showed ocular lesions between 50% of inoculated birds represented by swelling of the eyelids, presence of serous exudate and corneal opacity (fig.10, 11). The ocular lesions were mostly bilateral.

These birds also showed pericarditis, airsacculitis, Perihepatitis and peritonitis. Microscopically the conjunctiva showed subepithelial granulomas composed of mononuclear cells, heterophils, fibroblasts and giant cells (fig. 12). Eyelids showed epidermal and dermal hyperplasia with hypodermal granulomas. The subcutaneous tissue of the eyelids were congested and infiltrated with leucocytes (fig. 13). Cornea showed epithelial hyperplasia. The corneal stroma was edematous and infiltrated with mononuclear cells. The stroma of the iris infiltrated with lymphocytes and heterophils (fig. 14).

There were absence of lesions or deaths in any bird in groups 2,4 and 6. Reisolation of *E.coli* from materials of cellulitis, liver, heart blood and air sacs in experimentally infected birds was succeeded.

DISCUSSION

Inflammation of the subcutaneous tissue occurs below the dermis and a few foci of necrotic debris were occasionally found in the dermal layer in addition to those in the subcutis, therefore "cellulitis" would be a more appropriate name for this condition as it refers to an infectious process that might lead to the presence of a purulent (caseous) exudate in the subcutaneous tissue (Peighambari *et al.*, 1995 and Susantha *et al.*, 1997).

In the present study birds subcutaneouslly inoculated with isolated *E.coli* developed cellulitis between day 2 and 7 p-i at rate of (90%),while mortality rate of inoculated birds due to septicemia was 70%. Moreover all birds revealed pericarditis, perihepatitis and airsacculitis. Our result is somewhat similar to that obtained by Susantha *et. al,* (1997) where they observed that (98%) of birds developed cellulitis and (29%) of them died from septicemia between day 1 and 6 p-i and 80 - 100% of birds developed pericarditis. airsacculitis and perihepatitis between day 2 and 14 p-i.

Also cellulitis could be induced by scarification of the skin and rubbing the area with a broth culture of *E.coli* and (100%) of birds developed cellulitis (between day 7 and 18 p-i) with no deaths, also pericarditis, airsacculitis and perihepatitis were developed in 50% of infected birds, our result is in agreement with that observed by Peighambari *et al.*, (1995) but they noted that the signs of inflammation begane within 24 hours p-i and the lesions occurred in (86%) of infected birds.

In the present study, ocular lesions represented by conjunctivitis. inflammation of eyelids and keratitis could be induced by

inoculation of birds by isolated *E.coli* in the airsacs. In fact, the avilable literatures concerned with ocular lesions caused by *E.coli* infections in chickens were relatively little except that observed by Nakamura and Abe (1987) and it was more or less similar to our finding.

Therefore it is concluded that the damage to the surface of the skin appears to be requirement for initiation of the infection. Also factors other than skin scratches may be important as predisposing factors for cellulitis such as closer contact among birds in the barn and litter quality may related to opportunity for skin injury. Also some birds in the present study developed other complications of *E.coli* such as pericarditis, airsacculiti, and perihepatitis, indicating that the bacteria entering through the skin sometimes found their way into the blood circulation and caused infections in different organs. This is similar to occurrences in some field cases and it is not clear what environmental or genetic factors allow *E. coli* to cause both cellulitis and other lesions

REFERENCES

Bancroft, D. and Stevens ,A (1982): Theory and Practice of Histological Techniques 2nd Edn. Churchill livingstone (Edinburg, London, Melbourne).

Cruickshank, K.; Duguid, J.P. and swain. R.H. (1975): Medical Microbiology 12th Ed. Es. Livingston Limited Edinburgh and London.

Eterradossi, N.; Doulin, P.; Bennejean, G.; Lahellec, C.; Picault, J.P; Wyers, M. and Toux, J.Y. (1989): Necrotic dermatitis in broiler chickens: Pathological epidemiological findings and experimental reproduction proc.world Veterininary Poultry Association Meeting, Brighton, England. P P. 87- 88.

Glunder G., (1990): Dermatitis in broilers caused by Escherichia: isolation of *Escherichia coli* from field cases, reproduction of the disease with *Escherichia coli* O_{78}:K_{80} and conclusions under consideration of predisposing factors. Zentraibl. Veterinamed. Reihe B 37 : 383 - 391.

Gomis, S.; Amoako, K.; Ngeleka, M.B.; Elanger, L.; Althouse, B.; Kumor, L.; Waters, E.; Stephens, S.; Riddell, C.; Potter, A. and Allan, B. (2002): Histopathologic and Bacteriologic evaluations of Cellulitis detected in legs and Caudal abdominal regions of turkeys Avian. Dis. 40: 192 - 197.

Goodhope, R.S.; Riddel, C. and Allon, B. (1992): A descriptive study of cellulitis in Saskatchewan broiler chickens. Proc.129th Annual Meet Am. Vet. Assoc., Boston.

Hassanien, Z.A. (1977): Colisepticeamia in chickens with Particular reference to the role of K antigens. M.V.SC. thesis (poultry) fac.Vet Med., Cairo Univ.

Kumor, L.W.; Olkowski, A.A.; Gomis, S.M. and Allan, B.J. (1998): Cellulitis in broiler chickens: Epidemiological trends, meat hygiene and possible human health implications. Avian Dis., 42: 285- 291.

Messier, S.; Quessy, S.; Robinson, Y.; Devriese, L. and Hommez, J. (1993): Focal dermatitis and cellulitis in broiler chickens. Bacteriological and Pathological Findings. Avian Dis. : 37 839-844.

Nakamura, K. and Abe, F. (1987): Ocular lesions in chickens inoculated with *Escherichia coli*. Candian J. Vet. Res., 51 (4) 528-530.

Peighambri, S.M.; Julian, R.J.; Vaillancourt, J.P. and Gyles, C.L. (1995): Escherichia coli cellulitis experimental infections in broiler chickens. Avian Dis 39: 117 – 134.

Randall, C.J.P.A.; Meakins, Harris M.P. and Walt D.J. (1984): A new skin disease in broilers. Vet.Rec. 114:246.

Sojka. W.J. (1965): E.coli in domestic animals and Poultry 1st Edi Common Wealth Agriculture Bureau Farnhan, Royal Bucks, England.

Susantha M. Gomis, Trent Watts, Craig Riddell, Andrew, A. Potter and Bremda, J. Allan (1997): Experimental reproduction of *Escherichia coli* Cellulitis and septicemia in broiler chickens. Avian Dis. 41: 234 – 240.

LEGEND OF FIGURES

Fig. 1: A chicken's carcass infected with *E. coli* through subcutaneous injection showing congestion of muscles and presence of yellowish material.

Fig. 2: A Carcass of chicken infected with *E.coli* through subcutaneous injection showing peritonitis.

Fig. 3: A chicken's carcass infected with *E.coli* through subcutaneous injection showing severe fibrinous pericarditis.

Fig. 4: Subcutaneous tissue of a chicken showing presence of cellular debris. (H&E X200).

Fig. 5: Subcutaneous tissue of a chicken showing abundant fat cells surrounded by granuloma composed of mononuclear cells, heterophils, fibroblasts and giant cells (H&E X400).

Fig. 6: A chicken's carcass infected with *E.coli* through scratching and swabbing the skin showing presence of subcutaneous yellow mass.

Fig. 7: A chicken's carcass infected with *E. coli* through scratching and swabbing the skin showing fibrinous pericarditis and perihepatitis.

Fig. 8: Skin of a chicken showing hypodermal granulomas composed of necrotic center. mononuclear cells and surrounded by fibrous capsule (H& E X200).

Fig. 9: Skin of a chicken showing epidermal hyperplasia. The subcutaneous tissue infiltrated with mononuclear cells (H&E X200).

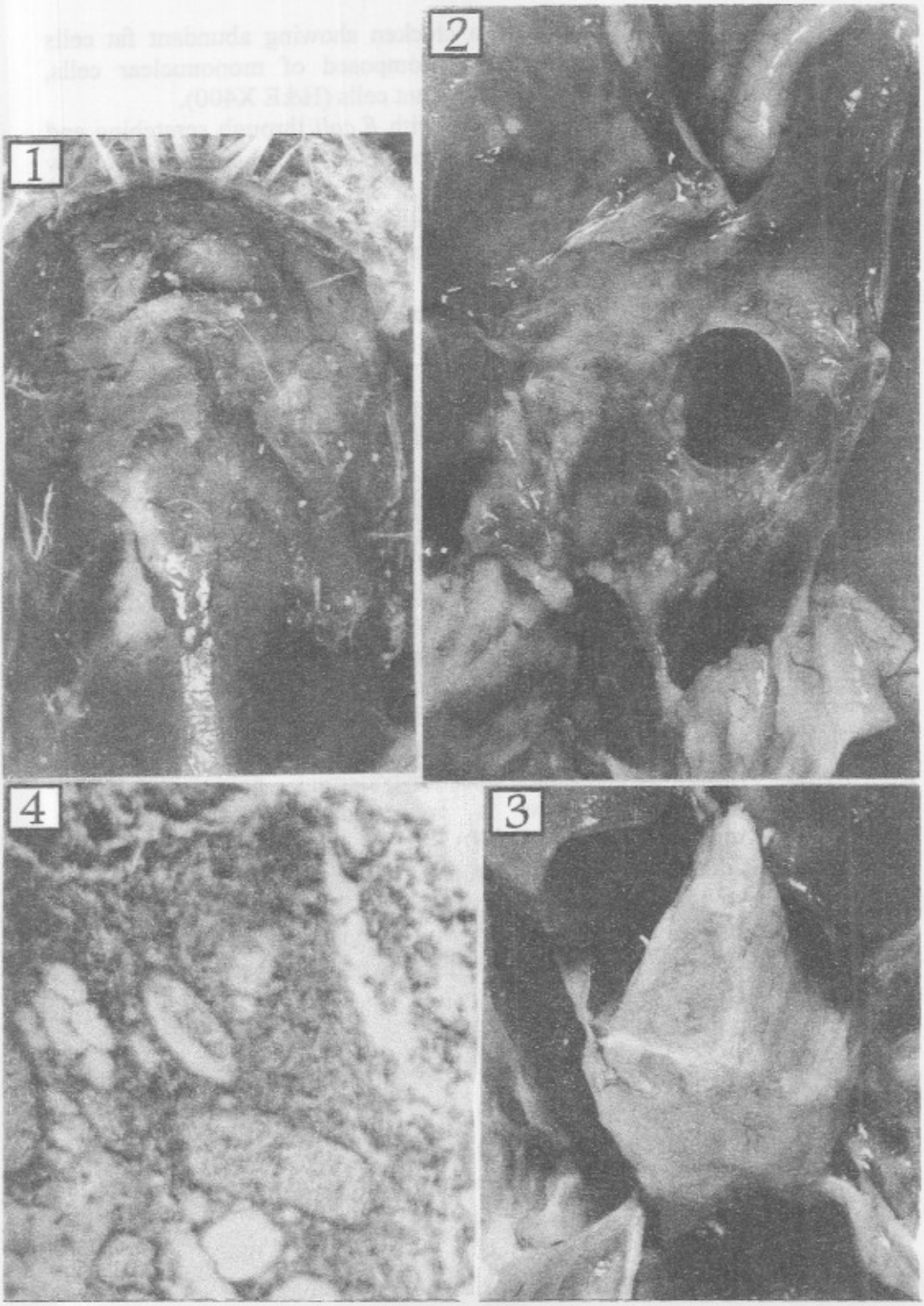
Fig. 10: Eye of a chicken infected with *E. coli* through injection in air sacs showing swelling of the lower eyelid with presence of serous exudates

Fig. 11: Eye of a chicken infected with *E. coli* through injection in air sacs showing swelling of the eyelids and corneal opacity.

Fig. 12: Conjunctiva of a chicken injected with *E.coli* through air sac showing subepithelial granulomas composed of mononuclear cells. heterophils. firoblasts and giant cells (H & E X 400)

Fig. 13: Eyelid of a chicken injected with *E.coli* through air sac showing dermal hyperplasia and hypodermal granulomas. The subcutaneous tissue is congested and infiltrated with lymphocytes (H & E X 200).

Fig. 14: Cornea of a chicken injected with *E coli* through air sac showing epithelial hyperplasia. The corneal stroma is edematous and infiltrated with mononuclear cells. The stroma of the iris containing lymphocytes and heterophils (H&E X200).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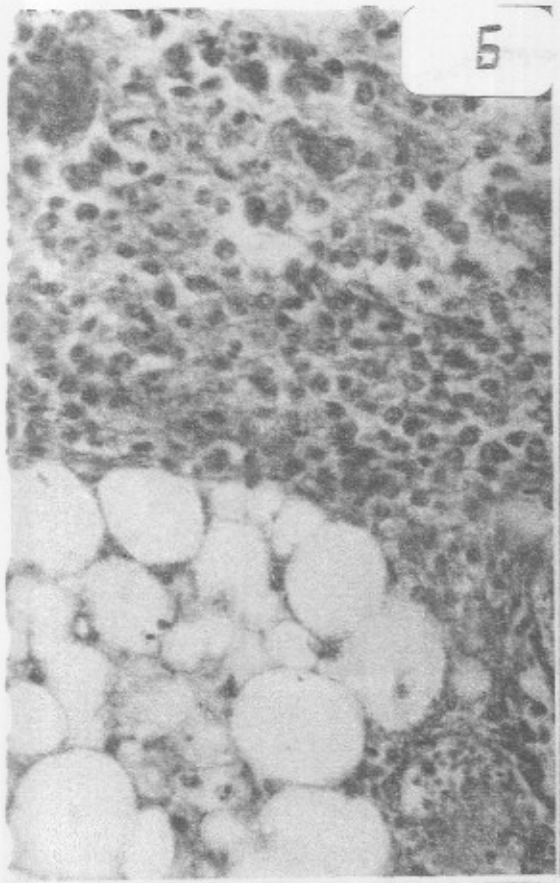
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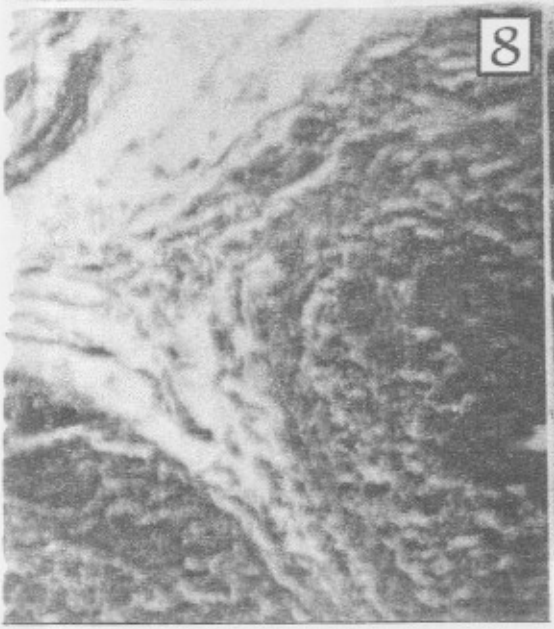
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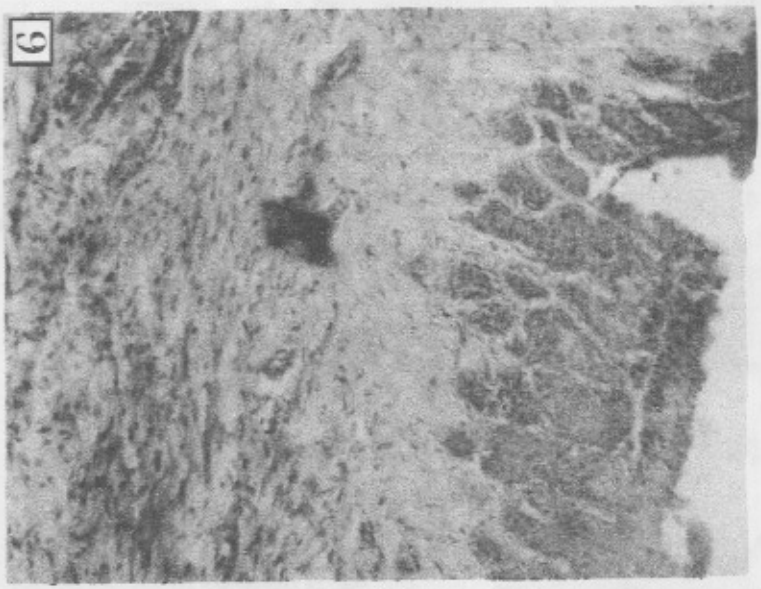
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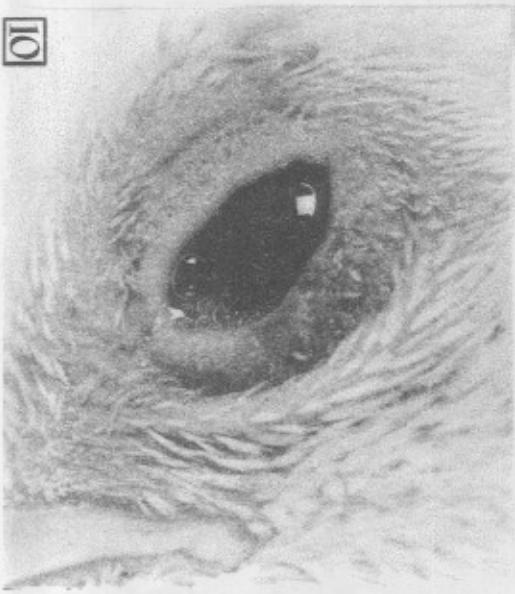
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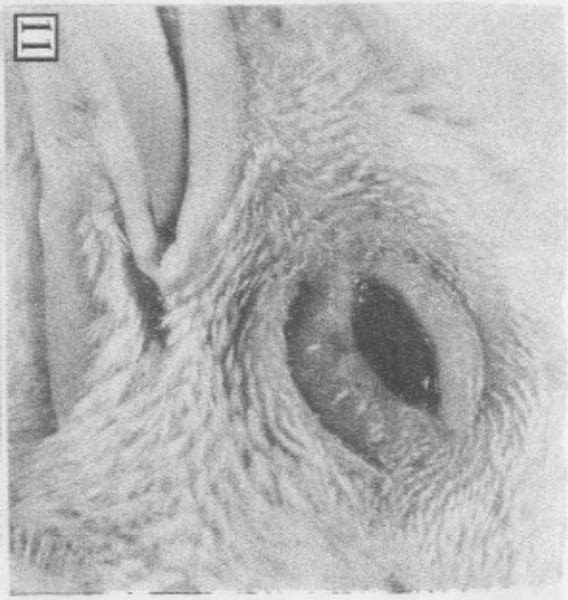
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