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# RESPONSE OF BROILER CHICKS TO PROBIOTIC (PRONIFER) SUPPLEMENTATION

(With 9 tables & 4 Figures)

By

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## مدي استجابة بداري التسمين لاضافة البرونيفير

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لدراسة أثر اضافة مركب البرونيفيرعلي كفاءة ومناعة الطيور أجري هذا البحث علي عدد ٨٠ كتكوتا (اربوايكر) , قسمت الي أربع مجموعات .غذيت المجموعة الأولي علي العليقة الأساسية بدون أضافة البرونيفير , وغذيت المجموعة الثانية علي العليقة الأساسية مضافا اليها مركب البرونيفير بنسبة (١كجم\طن عليقة) . أما المجموعتين الثالثة والرابعة فقد غذيت علي عليقة مكونة من ( ٩٥٪ من العليقة الأساسية + ٥٪ نخالة القمح ) مضافا اليها مركب البرونيفير بنسبة (١كجم\طن , ٢كجم\طن ) علي التوالي وذلك لدراسة أثر هذه الأضافة عند أرتفاع نسبة الألياف في العليقة في محاولة للتقليل من أسعار تكلفة العليقة. وقد تبين ان اضافة البرونيفير الي عليقة البداري الاساسية ادي الي تحسن في وزن الجسم( ٣,٤٤ %) عن المجموعة الضابطة .بينما وجد ان اضافة البرونيفير(١كجم\طن , ٢كجم\طن ) الي العليقة التي تحتوي علي ٥٪ نخالة قمح سجل اعلي زيادة في وزن الجسم (٤,٨٨٪ , ٦,١٢٪ ) ومعدل التحويل الغذائي ( ٢,٣ , ٢,١) خاصة مع المستوي الأعلي من البرونيفير مقارنة بالمجموعة الضابطة. هذا وبتقدير كمية الميكروبات في الأمعاء كدلالة علي تطهيرها من الميكروبات الضارة وجد ان اضافة البرونيفير الي العليقة ادي الي أنخفاض العد الطبقي الكلي للبكتريا الضارة في امعاء البداري مثل ميكروب الكوليستريديم والميكروبات القولونية والميكروبات الموجبة الجرام وقد أختفي ميكروب الكوليستريديم كلية بعد اسبوعين من اضافة المركب الي العليقة.كما أتضح بدراسة الحالة المناعية للطيور وجود تكثر نسيجي في الكريات البيضاء في الطحال مع وجود زيادة في خلايا البروليمفوسيت , وفي الكريات الحمراء لوحظ زيادة خلايا B - الليمفاوية في المجموعات المعاملة .كما وجد في حويصلة فابريشي زيادة في عدد الكريات مع

زيادة في خلايا البلازما .وأخيرا بفحص صورة الدم لوحظ زيادة في العدد الكلي لخلايا الدم الحمراء والبيضاء مع وجود زيادة ملحوظة في نسبة الخلايا الليمفاوية.


## SUMMARY

The efficiency of a commercial probiotic (Pronifer) as a feed additive on the performance and immunological status of the broiler chicks (Arbor acre) has been investigated. As there is no model currently available which will predict the use of pronifer under practical normal conditions, the product was added at a level of 1Kg/ton diet in an attempt to compare its addition to control one (groups I & II ).In addition pronifer was added at 1Kg & 2Kg/ton diet to groups (III & IV) when wheat bran was replacing 5% of the basal diet.The addition of the pronifer to the basal diet improves the body weight by (3.44%) than the control group. Its effect appeared clearly when added to the diets containing wheat bran with the superiority of the high level (2Kg/ton diet) of supplementation (6.12%) and recorded good for feed conversion (2.2) in comparison with the control group.Pronifer tratment drastically reduced the total viable bacterial count ,total coliform, Escherichia coli with disappearance of Clostridium perfringes after 2 weeks of treatment.The immuno-pathological examination of the spleen revealed hyperplasia of the white pulp with the presence of neumorus prolymphocytic cell proliferation.There was also increase in the number of alkaline phosphatase activated B-lymphocyte in the splenic red pulp. The bursa in the pronifer treated group showed increase in the number of the follicles with presence of neumorus plasma cell reaction in the medulla.The haematological picture showed increase in the number of total erythrocytic and leucocytic cell count with marked increase in the percentage of lymphocyte and monocyte. It was inferred from the present study that the pronifer has brought about benificial effects in the performance and immunological status of broilers and was cost effective.


***Key words:*** *Broiler chicks-Probiotic-Response*

## INTRODUCTION

While most of the work on probiotics has been done on the ruminant animals, observations that similar effects can be obtained with non-ruminants have also been made (Glade, 1991). As the bacterial populations present in the functional cecum and colon of many non-ruminants are similar to those in the rumen , the possibility for stimulating fermentative digestion in these species was suggested.Currently, considerable attention is being given to the use of probiotics in animal feeding programs.Traditional probiotics are lactic acid bacteria, such as Lactobacillus(L) casei, Lactobacillus acidophilus and

streptococci. Probiotics regulate the microbial environment of the intestines,decrease digestive disturbances, inhibit pathogenic intestinal microorganisms and improve feed conversion efficiency (Windschitl,1992 and Dhingra,1993). As a probiotic agent, it may act through improvement of the balance of the intestinal microflora. It improves health performance and increase growth rates (Bohm and Srour, 1995). Lactobacilli are widely distributed on the surface of plants ,in digestive tract of animals ,and in the environment .Early studies on the intestinal microflora of chicks showed that the crop was the source of the bacillus for maintaining the bacterial balance in the intestines (Fuller and Turvey, 1971). Recently, Fuller (1977) reported that lactobacilli were either bacteriostatic or bactericidal in vitro. As the use of probiotics in broiler diets has revealed conflicting reports concerning growth performances, this experiment was designed to evaluate the effect of commercial probiotics (pronifer) as a feed additive during the growth stage of commercial broiler specially when replacing the expensive raw material with cheaper one in an effort to reduce the cost of the product. Other parameters were also investigated relative to activation of the immune system of the host to overcome growth pathogens as it is well known that lactobacilli are known to be potent immunostimulants which directly activate macrophage functions or by direct interactions with certain immuno-potentiators (Sato, 1984). Miake et al. (1985) reported that L.casei activated the macrophages in vitro and in vivo which judged by the increase in phagocytic or secretion of lysosomal enzymes or by their ability to produce oxygen radicals .

## MATERIALS and METHODS

Fermentation products such as pronifer is made by specific lactic acid fermentation of heat-treated soybean meal and malt ,using a multiple strain mixture of lactobacilli and pediococcus, selected from their natural habitat. It contains: a)viable lactic acid bacteria (L.plantarum ,L.brevis ,L.fermentum ,L.casei and pediococcus acidilacticii). b) lactic acid fermentation metabolities and enzymes (organic acids ,glucosidase and peptidase enz.). c) free (soluble)amino acids and short-chain peptides. ***{from EGGER ,GmbH ,Mitter labill ,Austria }.***

**(A) Chicks and feeding :**

A total of eighty ,day old broiler chicks (Arbor acre) were divided into 4 groups ,20 per each.From 0-6 weeks ,the chicks were floor reared in an experimental room bedded by a layer of chaffed wheat straw and provided

with clean feeders and waterers.Group I received the basal diet described in (table1) and group II fed basal diet supplemented with pronifer at the rate of 1Kg/ton diet ,while group III & IV were assigned to like dietary regimens .This regimen was composed of 95% of the basal diet plus 5% wheat bran .Pronifer was added at the rate of 1Kg/ton diet and 2Kg/ton diet for groups III & IV respectively.The energy/protein ratio of the experimental diets was kept nearly constant (139.1 for starter & 160 for finishing one, table 2).

Chicks in the experiment were fed on the starter diet for the first three weeks and on the grower-finisher diet for the last three weeks .The experimental diets were formulated so that they satisfy the requirements stated in the NRC (1984) .The diets were fed ad-libitum and a fresh clean water was continously available throughout the experimental period which extended for 6 weeks .

The amount of feed consumed was weekly recorded in each of the different groups .Regarding the development of the body weight ,the birds were individually weighed every week .The growth was measured and expressed in percentage relative to the body weights in order to compare the different groups in relation to its relative rate of growth .The obtained data were statistically analysed according to Snedecor and Cochran (1989).

To potentiate the effect of probiotics ,samples were collected from the experimental chicks for bacteriological and immunological studies.

**(B) Bacteriological study :**

Colonization of Cl.perfriengs and E.coli in the intestinal tract of broiler chicks were examined.The faecal matter samples were collected by sterile forceps in sterile poly-ethylene bags from different group localities at 2,4,6 weeks of age .The samples were delivered directly to the laboratory for bacteriological examination .After through mixing of each sample ,1g was weighed on a sterile watch glass ,then carefully triturated in a sterile morter with 9 ml sterile saline solution before being strained through sterile guaz and the filtrate was collected in a sterile flask. From this basal dilution (1:10),ten fold serial dilutions were obtained by using sterile saline solution .

**1- Aerobic plate count:** The colony forming units (CFU) per gram were carried out according to A.P.H.A.(1985) by using standard plate count agar .

**2- Coliform count:** Enterobacteriaceae were counted in pour plates of violet red bile agar with 1% glucose (Mossel et al., 1962 ; Tabib et al. ,1981 and Baily & Scott, 1994) by inoculating of 0.1 ml of each dilution to double plates. The inoculated plates were incubated at 37°C for 48h. All purplish-red colonies surrounded by a red zone of percipitated bile salts were counted

**3-Escherichia coli count:** E.coli were counted according to Finegold & Boron (1986) and Quinn et.al. (1994).

**4-Clostridium perfringens count:** Vegetatitive and spores of Cl.perfringens were carried out according to Beerens et.al. (1980) .On the other hand,pour plate technique with tryptose sulfite cycloserine agar medium was also used Toply & Wilson's (1991).

**(C) Pathological study :**

At the end of the experiment ,5 birds from each group were slaughtered and specimens from the spleen,bursa ,liver ,intestine and kidneys were taken and fixed in neutral buffer formalin.After fixation ,the specimens were dehydrated ,infilterated and embeded in paraffin .The paraffin blocks were sectioned at 7μ thickness .Tissue slides were stained routinely by haematoxylin and eosin stain for routin histopathological examination Bancroft and Stevens (1977).

**(D) Immunological study :**

**1-Haematological examination :**

Blood samples were collected from the slaughtered birds at the end of the experiment.The samples were used for the detection of :

a-Total erythrocytic count /$mm^3$ blood .

b-Total white blood cells count /$mm^3$ blood.

c-Differential leucocytic count on blood film stained with wrights stain.

**2-Spleen and bursa evaluation :**

Specimens were taken from this organs and fixed in cold aceton ,processed and paraffin infiltrated .The paraffin blocks were sectioned at 7μ .The prepared sections were used for the immunological studies using the following histochemical indices: a-Alkaline phosphatase reaction ,for detection of activated B lymphocyte (Gomeri-calcium method ,1952). b-Non specific estrase activity, for detection of T-lymphocyte (Lojda et.al.,1976).

## RESULTS

The obtained results are summarized in Tables (1-9) and Figures (1- 4).

## DISCUSSION

**(A) Growth study :**

The growth data are shown in Table ( 3 ).In the entire feeding period, addition of the pronifer to the basal diet improves the body weight by (3.44%) than the control group. This agreed with Cho et al. (1992) who found that L.Casei improved body weight by 3.4% to 3.8% . .A numerical

improvement in body weight and feed efficiency was found by Francis et al.(1978) when turkey poults were fed lactobacillus culture. Increased body weight in this experiment with pronifer fed chicks may be due to larger lactobacilli population ,which favorably changed the balance of enteric flora in intestines or the availability of nutrients as recorded by Adler & DaMassa (1980) & McCormick,1984)..More conviencing evidence that lactobacilli affected the balance of enteric organisms and improved weight gain as was mentioned by Fuller & Booker (1974) .Manickam et al. (1994) found that the performance (weight gain and feed conversion efficiency) of broiler chicks given lactobacilli was significant better than of untreated controls.

The addition of pronifer to the diet containing 5% wheat bran increased the body weight by (4.88%, 6.12%) with the superiority of the high level of pronifer (2Kg/ton diet). Possibly, the improvement in the growth of chicks fed on the diet supplemented with high pronifer level may be due to modification of the fermentation processes in the hindgut of birds similar to its effects in the ruminants as it increased feed utilization and reduce excretion of endogenus nitrogen (Stockland,1993).

Regarding the feed intake (Table ,4) ,diets with 5%wheat bran seemed to be bulky for the chicks than the basal diets as feed consumption was noted to be decreased ,but the groups fed on this diets recorded good feed conversion (2.2 ,2.3) in comparison with the control group (2.6), (Table ,5). This may be due to increased dry matter and protein digestion as was recorded by Wiedmeier et al (1987) ,beside the increased fibre digestion by probiotics supplementation as found by (Gomez-Alarcon et al. (1990).

**(B) Bacteriological study:**

Data presented in Tables ( 6,7,8 ) showed that pronifer tratment drastically reduced the total viable count, Cl.perfringes ,total coliform and *E.coli*. It was revealed that Cl.perfringes was completely absent after two weeks of treatment.

However, the total viable bacterial count was highly inhibited and the percentage of inhibition reach up to 99.6% after the 6th week of prnifer addition.The reduction of this bacteria were linerally increased throughout the exp. The total coliform were inhibited by 91.4% during the 6th week of the exp., while, *E.coli* was inhibited by 91.1%.

Concerning the antimicrobial effect of pronifer, there is no available enough literature but it was attributed to increase the immune status of the host, through activation of the macrophages, increase phagocytic activity or secretion of lysosomal enzymes or by their ability to produce oxygen radicale (Miake et al., 1985; Sato , 1984). Recently, it has been reported that

lactobacilli produce some compounds as $H_2O_2$,organic acids (as lactic and aceticacids) and other complex materials as lactolin, bulgarican, acidophilin, lactocidin, acidolin and nisin. All these substances are powerful antibacterial agents specially against gram -ve bacteria. Moreover, $H_2O_2$ is a part of peroxidase enzyme which play a role in rising the host immunity and protect the host against infection.

**(C)Histopathological and immunological studies :**

The histopathological examination of the pronifer treated group revealed no pathological changes in the liver or kidney. However , in the intestine showed slight lymphocytic cell infiltration at the lamina propria. The histo-pathological examination of the spleen revealed hyperplasia of the white pulp with presence of neumorus prolymphocytic cell proliferation (Fig.1). There is also an increase in the number of alkaline phosphatase positive activated B-lymphocytes in the splenic red pulp (Fig. 2).T-cell reaction (esterase +ve) was similar as control. Alkaline phosphatase and non specific estrase reaction was used as indicator for activated B-lymphocyte and T-lymphocytes respectively (El-sherry et al.,1994 and Inoue et.al.,1988).The immuno-pathological examination of the bursa in the pronifer treated group showed an increase in the number of the follicles (Fig.3) with presence of neumorus plasma cells reaction in the medulla (Fig. 4)

The haematological picture (Table 9) showed an increase in the total erythrocytic and leucocytic cells count with a marked increase in the percentage of lymphocytes and monocytes. From the immunopathological view, these results indicating that pronifer have an enhancement effect to the humoral immune response as it increase the number of activated B-lymphocytes and plasma cells in the spleen and bursa of the treated birds.This conclusion was agreed with Sato (1984) and Miake et al. (1985) whom stated that lactobacilli are potent immunostimulant through enhancement of humoral and cellular immune response.

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## LIST OF FIGURES

**Table (1): Physical composition of the starter & grower-finisher diets:**

| Item (%) | Diets | |
|---|---|---|
| | Starter | Grower-finisher |
| Yellow corn,ground | 67.40 | 73.40 |
| Soybean meal | 20.80 | 16.67 |
| Fish meal | 4.00 | 4.00 |
| Meat meal | 6.50 | 4.45 |
| Limestone,ground | 0.75 | 0.83 |
| Dicalcium phosphate | 0.20 | 0.35 |
| Common salt | 0.08 | 0.14 |
| DL-methionine | 0.10 | 0.01 |
| Premix* | 0.15 | 0.13 |
| $MnSo_4$ | 0.02 | 0.02 |

*Pfizer broiler premix :Furnishing the following ingredients per Kg of feed :Vit.A 12000 IU vit.$D_3$ 2000 IU,vit.E 10 mg ,folic acid 1 mg ,niacin 20 mg ,pantothenic acid 10 mg ,vit.K 2 mg ,vit.$B_1$ 1 mg, vit.$B_2$ 4 mg ,vit.$B_6$ 1.5 mg ,vit.$B_{12}$ 10µg ,biotin 50µg ,iron 30mg , copper 10mg ,zinc 55mg ,Mn 55mg ,iodine 1mg ,Se 0.1mg ,choline chloride 500mg. This premix is instructed to be added at the rate of 2.5Kg/ton diet.

**Table (2):Chemical composition of the experimental diets .**

| Item | Basal diets | | 95% basal diet + 5% wheat bran | |
|---|---|---|---|---|
| | Starter | Grower-finisher | Starter | Grower-finisher |
| ME (Kcal/Kg) | 2997 | 3059 | 2854 | 2913 |
| Crude protein (%) | 21.54 | 19.12 | 20.51 | 18.21 |
| C/P ratio | 139.1 | 160.0 | 139.1 | 160 |
| Fibre (%) | 3.03 | 2.86 | 3.43 | 3.27 |
| Calcium (%) | 1.01 | 0.90 | 0.97 | 0.86 |
| Phosphorus (%) | 0.46 | 0.41 | 0.45 | 0.40 |
| Sodium (%) | 0.15 | 0.15 | 0.15 | 0.147 |
| Manganese (mg/Kg) | 60 | 59 | 62.6 | 61.7 |
| Methionine (%) | 0.50 | 0.38 | 0.48 | 0.37 |
| Lysine (%) | 1.20 | 1.02 | 1.17 | 1.00 |

**Table 3: Body weight development (g) of chicks during the experimental period.**

| Weeks | Groups | | | |
|---|---|---|---|---|
| | I* | II | III | IV |
| 0 | 42±3.52 | 41±4.00 | 45±2.63 | 45±3.87# |
| 1 | 160±4.47 | 180±3.07 | 199±4.01 | 204±5.02** |
| 2 | 300±13.48 | 325±13.83 | 339±10.96 | 347±7.17 |
| 3 | 550±24.51 | 570±17.64 | 598±15.21 | 617±11.87 |
| 4 | 835±29.56 | 851±30.59 | 868±36.86 | 880±35.00 |
| 5 | 1142±35.12 | 1153±37.52 | 1197±42.53 | 1215±41.85 |
| 6 | 1454±40.88 | 1504±49.76 | 1525±50.04 | 1543±39.03 |

*Group 1 was considered as a control. **Significant at < 0.01.
# Data are means ± standard error.

**Table 4: Weekly feed intake (g ) during the experimental period .**

| Weeks | Groups | | | |
|---|---|---|---|---|
| | I | II | III | IV |
| 0-1 | 145 | 140 | 130 | 135 |
| 1-2 | 380 | 370 | 274 | 250 |
| 2-3 | 470 | 455 | 446 | 355 |
| Total (0-3) | 995 | 965 | 850 | 740 |
| 3-4 | 694 | 664 | 628 | 633 |
| 4-5 | 938 | 916 | 827 | 882 |
| 5-6 | 1050 | 1032 | 1092 | 1012 |
| Total (3-6) | 2682 | 2612 | 2547 | 2527 |
| Total (0-6) | 3677 | 3577 | 3397 | 3267 |

**Table (5) :Feed conversion ratio for chicks during the experimental period .**

| Item | Groups | | | |
|---|---|---|---|---|
| | I | II | III | IV |
| Feed intake (g) | 3677 | 3577 | 3397 | 3267 |
| Weight gain (g) | 1412 | 1463 | 1480 | 1498 |
| Feed conversion | 2.60 | 2.45 | 2.30 | 2.18 |

**Table (6) :Viable count/g of faecal matter after two weeks of the pronifer treatment.**

| Count of variable | Control | Pronifer treatment | |
|---|---|---|---|
| | | Count | % of inhibition |
| Aerobic plate count | $1.6\times10^{9}\pm2.3\times10^{8}$ | $6.4\times10^{8}\pm4.2\times10^{6}$ | 60 |
| Total coliform count | $4.5\times10^{8}\pm1.7\times10^{7}$ | $1.6\times10^{8}\pm1.6\times10^{7}$ | 64 |
| E.coli count | $4.2\times10^{7}\pm7.3\times10^{6}$ | $2.5\times10^{7}\pm2.9\times10^{6}$ | 40.5 |
| Cl.perfringenes count | $6.2\times10^{2}\pm7.1\times10$ | $4.8\times10^{2}\pm1.6\times10$ | 22.6 |

**Table (7) :Viable count/g of faecal matter after 4 weeks of the pronifer treatment.**

| Count of variable | Control | Pronifer treatment | |
|---|---|---|---|
| | | Count | % of inhibition |
| Aerobic plate count | $1.9\times10^{9}\pm2.5\times10^{8}$ | $6.1\times10^{8}\pm1.1\times10^{7}$ | 66.1 |
| Total coliform count | $2.4\times10^{7}\pm5.2\times10^{4}$ | $2.1\times10^{6}\pm3.7\times10^{4}$ | 91.3 |
| E.coli count | $2.3\times10^{7}\pm4.4\times10^{3}$ | $1.8\times10^{6}\pm6.2\times10^{4}$ | 92.2 |
| Cl.perfringenes count | $1.4\times10\pm0.2\times10$ | 0 | 100 |

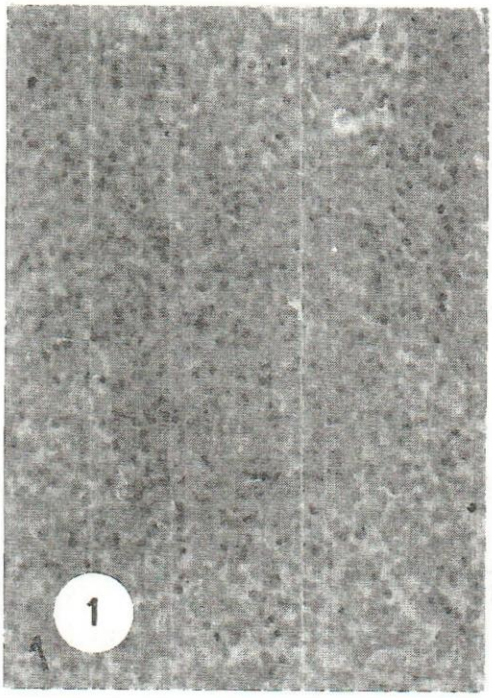
**Table(8):Viable count/g of faecal matter after 6 weeks of the pronifer treatment.**

| Count of variable | Control | Pronifer treatment | |
|---|---|---|---|
| | | Count | % of inhibition |
| Aerobic plate count | $8.2\times10^{8}\pm7.4\times10^{6}$ | $3.2\times10^{6}\pm2.1\times10^{6}$ | 99.6 |
| Total coliform count | $7.8\times10^{7}\pm2.8\times10^{5}$ | $6.7\times10^{6}\pm3.3\times10^{4}$ | 91.4 |
| E.coli count | $6.2\times10^{7}\pm1.2\times10^{3}$ | $5.5\times10^{6}\pm1.7\times10^{4}$ | 91.1 |
| Cl.perfringenes count | $1\times10\pm0.82$ | 0 | 100 |

**Table (9):Haematological picture in the control and pronifer treatment groups***

| Blood cell | Control | Pronifer treatment |
|---|---|---|
| Total erythrocyte ( $\times10^{6}/\mu l$ ) | 3.12 ±0.06 | 3.15 ±0.03 |
| Total leucocyte ( $\times10^{3}/\mu l$ ) | 12.1 ±0.11 | 12.7 ±0.09 |
| Heterophil (%) | 31 ±0.15 | 25 ±0.16 |
| Lymphocyte (%) | 55 ±0.32 | 59 ±0.91 |
| Monocyte (%) | 10 ±0.08 | 12 ±0.69 |
| Eosinophil (%) | 4 ±0.03 | 3 ±0.01 |
| Basophil (%) | rare | 1 ±0.002 |

*Mean value ±SE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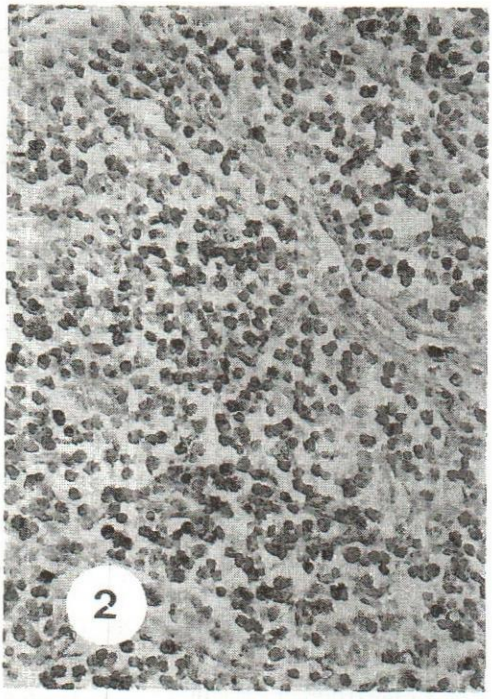
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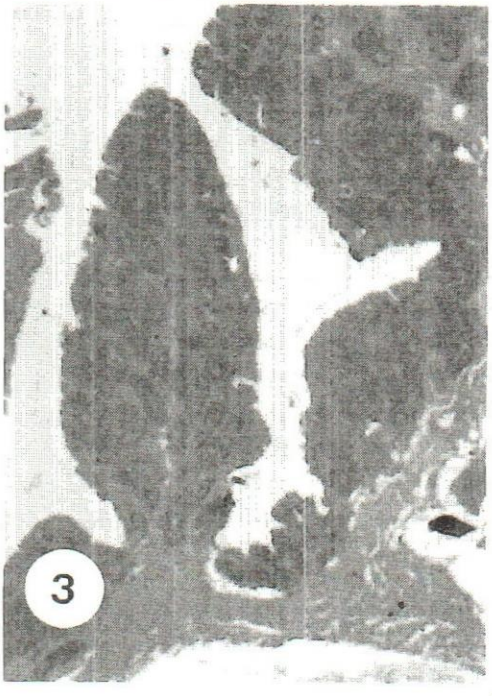
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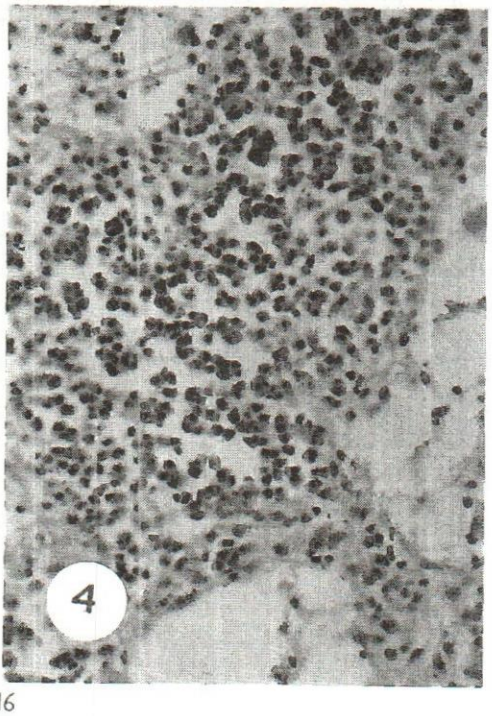
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