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HERPESVIRUS ENCEPHALITIS IN PIGEONS

(With 2 Tables and 6 Figures)

By

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الإلتهاب الدماغي بفيروس الهيربس في الحمام

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تضمن البحث إجراء إستبيان لحالات مرضيه ذات أعراض عصبية وإلتهاب العين وإسهالات مخضرة اللون مع وجود إحتقان بالأعضاء الداخلية وغشاء المخ في الطور الحاد وكذلك وجود تنكرز في الكبد في الحالات المتقدمة في برجين من الحمام في نطاق محافظة أسيوط.

كانت معدلات النفوق ٢٠٪، ٣٠٪ في برجي الحمام أ ، ب على التوالي ولم نتمكن من عزل المسببات البكتيرية وكذلك الفطريات.

تم عزل فيروس الهيربس في عدد من الطيور المفحوصة من كلا البرجين وقد أحدث ظهور بثرات على الغشاء الخارجي لأجنة الدجاج المحقونة.

أوضحت الفحوص الباثولوجية للأغشية الجنينية وجود زيادة في عدد خلايا طبقة الأكتوديرم أما أكباد الأجنة فكانت تحوي بؤر تنكرزية عديدة كما أوضحت الفحوص وجود الجسم الأحتوائي قاعدي الصبغة في أنوية كل من خلايا طبقة الأكتوديرم وفي أنوية خلايا الكبد خاصة المجاورة منها لبؤر التنكرز. أما الفحوص الفوقية والتي تمت بإستخدام الميكروسكوب الألكتروني النافذ فقد أظهرت وجود فيروس الهيربس الخاص بالحمام داخل هذه الأجسام الأحتوائية.

قد ثبت أن الفيروس كان حساسًا لكل من الأيثر والكلوروفورم وقد تم القضاء عليه نهائيًا عند درجة حرارة ٥٦ لمدة نصف ساعة وكذلك بإستخدام الفينول (٤٪)، فورمالين (٤٪)، صودا كاويه (٢٪) ولم يتم القضاء عليه عند الرقم الهيدروجيني ٤.

ولقد برهنت العدوى التجريبية للحمام ذوات أعمار ٨ أسابيع عن طريق الحقن تحت الجلد بواسطة المحلول المحضر من الأغشية الخارجية للأجنة المصابة عن الطبيعة الضارية للفيروس.

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SUMMARY

An investigation of a disease with clinical nervous signs, conjunctivitis and greenish diarrhea together with congestion of parenchymatous organs and brain meninges in the acute cases and focal areas of liver necrosis in advanced stages in autopsied pigeons of 2 lofts of pigeons was carried out. The mortality rate was 20% and 30% in loft A and B respectively. All attempts for demonstration of the bacterial agents including chlamydia psittaci or fungi were unsuccessful. Herpesvirus was isolated from a number of birds examined from both lofts. The isolated virus produced pocks on the CAM of chicken embryos. Histopathology of the pock lesions of the CAM revealed areas of ectodermal hyperplasia. The embryonic liver contained several minute necrotic lesions. Single basophilic intranuclear inclusion bodies were observed in the ectodermal cells of the CAM and the hepatocytes surrounding the necrotic lesions of the liver. Ultrastructurally, viral particles typical for pigeon herpesvirus were demonstrated in these inclusions. The virus was sensitive to ether and chloroform and completely destroyed at 56°C for 30 minutes, 4% phenol. 4% formalin and 2 NaoH but not by pH4. The experimental infection of 8-week-old pigeons by inoculation of infected CAM suspension subcutaneously proved the pathogenic nature of the virus.

INTRODUCTION

Several strains of herpesvirus causing contagious fatal nervous disease in pigeons have been isolated before by *VINDEVOGEL* et al. *(1975). MOHAMED* et al. *(1978). TANTAWI* et al. *(1979). KAMIONOKOWSKI (1981) POLLARD and HARAIS (1983) and CARRANZA* et al. *(1986).* In Egypt, herpesvirus was isolated and identified from pigeons by *TANTAWI and HASSAN (1982).* The morbidity rate of the disease was high in most investigated outbreaks. The virus was found to grow on the chorioallantoic membrane (CAM) of embryonated chicken eggs causing death of these embryos and producing pock lesions on their CAM.

In Assiut area, two outbreaks of highly contagious nervous disease characterized by high mortality rate were reported in 2 lofts of pigeons during summer of 1993. A course of antibiotic therapy (oxytetracycline) was given and supportive care continued but this was not effective.

The present investigation was undertaken to:

- Describe the clinical picture of the disease.
- Isolate and identify the causative agent.
- Study the pathogenicity of the virus for pigeons under experimental conditions.

MATERIAL and METHODS

Two outbreaks of nervous disease in pigeons of 160 and 200 birds over 2 months-old were investigated. Twenty-one clinically diseased birds were received for post-mortem examination. Five of those pigeons died after a short period of illness and the remainder, still-alive, were killed for laboratory examination.

Bacteriology:

Listeria and salmonella:

Cultures from heart blood, brain, liver, spleen and intestine were streaked on sheep blood agar and MacConkey's agar plates for evidence of listeria or salmonella growth.

Chlamydia:

Impression smears were prepared from airsacs, spleen, liver and kidney stained with Giemsa examined for the presence of chlamydial agent. Isolation attempts from these tissues were carried out by:

a) Intraperitoneal inoculation of 4-weeks-old mice. The mice were kept under observation for 14 days before necropsy.
b) Yolk sac inoculation of 6-days-old chicken embryos. Both tissues of the mice and yolk sac were examined for the presence of chlamydial intracytoplasmic inclusion bodies.

Mycoplasma:

Swabs from nasal cavity, trachea and air sacs were inoculated onto frey's media and incubated at 37°C for 5 days, transferred into plates and incubated at 37°C for 5 days and examined for the presence of mycoplasma organism.

Mycology:

Swabs from trachea, lungs and air sacs were streaked onto sabouraud dextrose agar and incubated at 37°C for 3 days.

Moreover swabs from liver, spleen, intestine and lungs were streaked onto glucose agar, incubated at 30°C for 3 days, after which the plates were examined for the growth of Aspergillus and Cryptococcus, respectively.

Virology:

Virus isolation: An amount of 0.2 ml of antibiotic treated 10% tissue suspension from brain, liver, spleen, kidney and pancreas was inoculated onto the CAM of 10-days-old chicken embryos. The inoculated eggs were incubated at 37°C. The CAM of the dead embryos were collected and kept at -20°C for further investigation.

Virus assay: Virus infectivity titers were determined by inoculating CAM of chicken embryos of the same age used for virus isolation. The inoculated eggs were examined 72 hours postinoculation and the occurrence of infection was indicated chiefly by the presence of pock lesions. The titers were calculated and expressed as EID_{50} by *REED and MUENCH (1938).*

Effect of ether and chloroform:

The effect of e ther and chloroform on virus infectivity was measured by methods described by *ANDREWES and HORSTMANN (1949)* and *FELDMAN and WANG (1961).*

Effect of temperature:

The thermal effect on virus infectivity was studied at 56°C for 30 minutes. The residual virus infectivity was then measured by inoculating it into the CAM.

Effect of pH:

The effect of pH4 on virus infectivity for one hour was determined.

Effect of disinfectant:

The effect of 4% phenol. 4% formalin and 2% sodium hydroxide on the virus infectivity was studied.

Haemagglutination:

The haemagglutinating ability of the isolated virus was tested against 1% suspension of pigeon, chicken, turkey, duck, geese and quail RBCs.

Serological investigation:

Haemagglutination inhibition (HI) test was carried out by microtechnique on sera collected from naturally infected pigeons in both lofts against Newcastle disease virus (NDV).

Blood and faecal examinations:

Blood and faecal samples from sick pigeons were examined for blood parasites and parasitic ovae, respectively.

Histopathology:

The chicken embryos together with their membranes were removed from the infected eggs and examined for the presence of gross lesions. Samples from the pock lesions of the CAM and the livers were fixed in 5% cacodylate buffered glutaraldehyde. Fixed tissues were trimmed to approximately 1x1x2 mm blocks. These blocks were postfixed in 1% osmic acid, dehydrated, and embedded in epon. Semithin sections were stained with 0.25% toluidine blue for light microscopy.

Transmission electron microscopy:

Representative fields were chosen for TEM and ultrathin sections were cut with a diamond knife, mounted on copper grids, and contrastained with urany acetate and lead citrate. The ultrastructural investigation was carried out with electron microscope Jeol 100 CXII at 80 Kv.

Experimental infection:

Forty apparently healthy pigeons, 8-weeks-old, proved to be free from pathogenic microorganims, were divided into 2 groups 1 and 2 of ^{20} birds each and kept separately, pigeons of group 1 were inoculated by subcutaneous (S/C) route, each bird with 0.5 ml of infected CAM suspension (10^6 EID_{50}). The other group was served as non infected control. pigeons were kept under observation for 4 weeks.

RESULTS

The investigated outbreaks of the disease involved pigeons over 2 months-old and occurred during summer months of 1993. In both lofts. The only clinical sign common to the majority of cases was nervous manifestations in the form of head tremors. circling movement and torticollis (Fig. 1). The other signs included depression. anorexia. conjunctivitis (Fig. 2) and inability to fly. Paresis or paralysis of the extremities was observed among few birds. Greenish diarrhea and deviation in the normality of caruncles colour to yellow grey were noticed during the course of the disease. The severity of natural signs varied considerably among the birds of the 2 lofts. The observed morbidity rates were 40% and 50% while mortality rates were 20% and 30% in the two infected lofts A and B respectively. The most consistent lesions in the majority of examined birds were distension of gall bladder and marked

congestion of parenchymatous organs. including liver. spleen. pancreas. kidney as well as brain meninges and intestinal blood vessels. The presence of focal liver necrosis was observed in the advanced cases. Bacteriological and mycotic examinations for listeria. salmonella. chlamydia. Mycoplasma. Aspergillus and Cryptococcus were negative. Haemagglutinating ability for tissue samples and HI titers for sera of naturally infected pigeons were proved negative against paramyxo and ND viruses. Examinations for blood parasites and parasitic ovae revealed negative results. The herpesvirus was isolated from 13 cases. the details are shown in table (1). The virus produced numerous pocks on CAM inoculated with the tissue suspensions. Embryonic deaths occurred 3-5 days pI. The dead embryos were stunted with congestion covering the whole body and minute grayish foci in the livers.

Histopathology:

Light microscopical investigation of the CAM revealed foci of ectodermal and endodermal cell proliferation. Their nuclei were swollen and contained basophilic intranuclear inclusion bodies surrounded by a clear halo. There was peripheral margination of the nuclear chromatin (Fig. 3) The mesodermal blood vessels were congested. In the liver. The grossly observed minute grayish foci were areas of necrosis of the hepatocytes with pyknosis of their nuclei (Fig. 4). The hepatocytes at the periphery of the necrotic areas were in variable stages of degeneration. They had enlarged nuclei which frequently contained a single basophilic intranuclear inclusion body. These inclusions were surrounded by a clear halo with peripheral chromatin margination (Fig. 4).

Transmission electron microscopy:

Ultrastructural investigation of the CAM and liver revealed that these inclusions composed of aggregates of viral particles (Fig. 5). The virion composed of capsomers surrounding a central core of granular electron dense aggregated particulate material (Fig. 6). There was peripheral margination of the nuclear chromatin and blebbing of the outer nuclear membrane (Fig. 5). Tests indicated that the virus was ether and chloroform sensitive. destroyed at temperature of 56°C for 30 minutes and by exposure to 4% phenol or formalin and 2% NaoH but not by exposure to pH4.

The observed clinical signs in experimentally infected pigeons were greatly similar to those mentioned in natural infection. Moreover. a marked paleness in the colour of the liver was observed. particularly in the acute stage. The

nervous signs developed 9-12 days PI and 40% of diseased pigeons died after a short period of illness as shown in table (2). The virus was successfully reisolated from dead infected pigeons.

DISCUSSION

Nervous symptoms in pigeons are mostly attributed to infection with paramyxoviruses *(WILSON. 1986 and EL-ZANATY et al., 1988).* herpesvirus *(TANTAWI and HASSAN. 1982 and CARRANZA et al., 1986).* Chlamydia. Aspergillus *(WHITEMAN and BICKFORD. 1989)* and cryptococcus *(EL-BADRI and SHAHATA. 1988).*

In the present work. The involvement of NDV or any other paramyxoviruses was completely ruled out by the non haemagglutinating activity of the isolated agent to pigeon. chicken. turkey. duck. geese and quail RBCs. Listeria. salmonella. chlamydia Mycoplasma. Aspergillus and Cryptococcus were ruled out via culturing on artificial media or inoculation of chicken embryos or mice.

In the present investigation. The main picture of the outbreaks among sick pigeons included nervous signs. *AL FALLUJI et al. (1979). TANTAWI and HASSAN (1982). POLLARD and HARAIS (1983) and CARRANZA et al. (1986)* obtained similar results in outbreaks of herpesvirus in pigeons. The obtained data in our study revealed that the mortality rate of the disease was 20-30% among naturally infected pigeons. These are similar to previously mentioned data by *AL FALLUJI et al. (1979). TANTAWI and HASSAN (1982) and CARRANZA et al. (1986).* Moreover. certain results of the present investigation about age susceptibility over 2-months-old are in accordance with those reported by *SMADEL et al. (1945). CORNWELL et al. (1967). LEHNER et al. (1967). CALLINAN et al. (1979) and TUDOR (1991).* The gross pathological findings including congested parenchymatous organs. together with brain meninges. intestinal blood vessels and liver necrosis in acute and advanced cases respectively. are nearly similar to those described by *CORNWELL and WRIGHT (1970). AL FALLUJI et al. (1979) and CARRANZA et al. (1986).* Histopathology of the CAM revealed that the pock lesions were formed of areas of etodermal hyperplasia with the presence of basophilic intrannclear inclusions. The lesions of embryonic liver were minute focal necrosis of the hepatic cells. Their nuclei contain basophilic intranuclear inclusions. These findings were consistent with other reports on herpesvirus infection *(CORNWELL and WEIR. 1970. TANTAWI and AL-SHEIKHLY. 1980).* Ultrastructurally, these inclusions were similar to

those described by *CALLINAN* et al *1979 and SAIK* et al., *1985.* The viral particles were typical for pigeon herpesvirus. The causative virus was isolated from 13 out of 21 examined freshly dead and sick pigeons. The virus showed a close relationship to the herpesvirus group on the basis of its morphology. ether and chloroform sensitivity. Its infectivity was totally destroyed for 30 minutes at 56°C. Moreover. the observed gross lesions in naturally and experimentally infected pigeons. pock lesions in the CAM of inoculated chicken embryos and characteristic intranuclear inclusion bodies are similar to those previously reported by *CORNWELL* et al. *(1967). TANTAWI* et al. *(1979) and CARRANZA* et al. *(1986).*

Concerning experimental infection. the inoculated chicken embryos died 3-5 days pI. *CORNWELL and WEIR (1970). AL SHEIKHLY* et al. *(1980) and TANTAWI and HASSAN (1982)* obtained somewhat similar results. On the other hand these results disagree with those reported by *JYLLING (1967)* who reported that lethal effect of isolated virus to the embryos occurred 6-9 days pI.

The concentration of 4% phenol or formalin and 2% NaoH was effective in disinfection of the contaminated articles. *TANTAWI* et al. *(1980)* obtained nearly similar results.

The results of the present investigation proved that pigen herpesvirus is pathogenic to young pigeons after administration by s/c route. inducing the same clinical observations of naturally infected pigeons. *CALLINAN* et al. *(1979) and CARRANZA* et al. *(1986)* observed paleness of the colour of the liver of both infected pigeons and inoculated embryos. This finding coincided with our observations in experimentally infected pigeons.

Finally. the present work denoted the presence of a disastrous nervous disease caused by herpesvirus among pigeon population in upper Egypt. Therefore. further studies on vaccine preparation and evaluation will be tried in the near future to control such outbreaks.

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LEGENDS

Fig. 1: Nervous signs of naturally, infected pigeons with herpesvirus.

Fig. 2: Eye lesions of naturally, infected pigeons with herpesvirus.

Fig. 3: Photomicrograph of the embryonic liver showing focal areas of necrosis (n). The surrounding hepatocytes showing nuclear swelling and contain basophilic inclusions ( ). Their cytoplasm contain fat globules ( ) (Toluidine blue stain 560X).

Fig. 4: Photomicrograph of the CAM showing areas of ectodermal and endodermal cell proliferation (P). The mesoderm is congested and edematous (E). (Toluidine blue stain 560X).

Fig. 5: Electron micrograph showing aggregations of PHV particles in a nucleus of hepatocyte forming an inclusion body. Notice margination of nuclear chromatin (C) and
blebbing of the outer nuclear membrane ( ). (uranyl acetate and lead citrate X22.000).

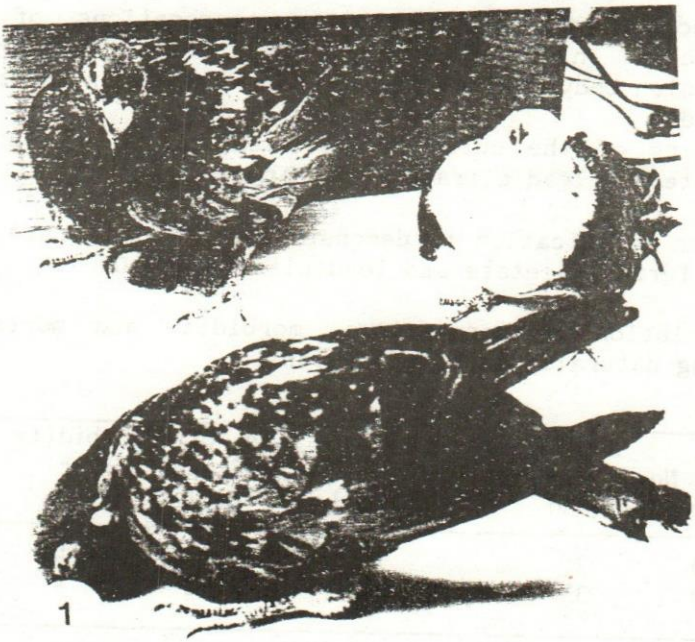
Fig. 6: Higher manification to demonstrate the viral particles ( ). (uranyl acetate and lead citrate X88.000).

Table 1: Isolation of herpesvirus, morbidity and mortality among naturally infected pigeons.

Loft	Total No. of pigeons	No. of examined cases: dead	No. of examined cases: diseased	No. of positive: Total	Morbidity rate	Mortality rate
A	160	2	7	5/9	40 %	20 %
B	200	3	9	8/12	50 %	30 %

Table 2: Pathogenicity of pigon herpes encephalitis virus to young pigeons.

Group No.	No. of birds	age of birds	inoculated material	Dose	Route of inoculation	Diseased Total	%	Dead Total	%
1	20	8 weeks	infected CAM	0.5ml ($10^6 EID_{50}$)	S/C	16/20	80%	8/20	40%
2	20	8 weeks	-	-	-	0/20	0 %	0/20	0 %



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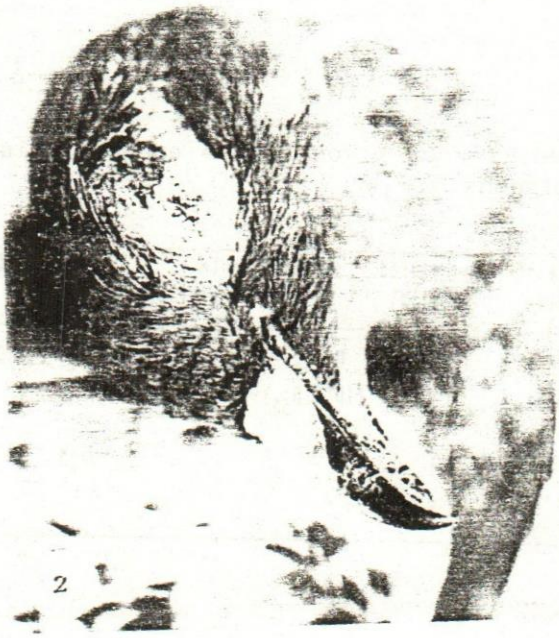

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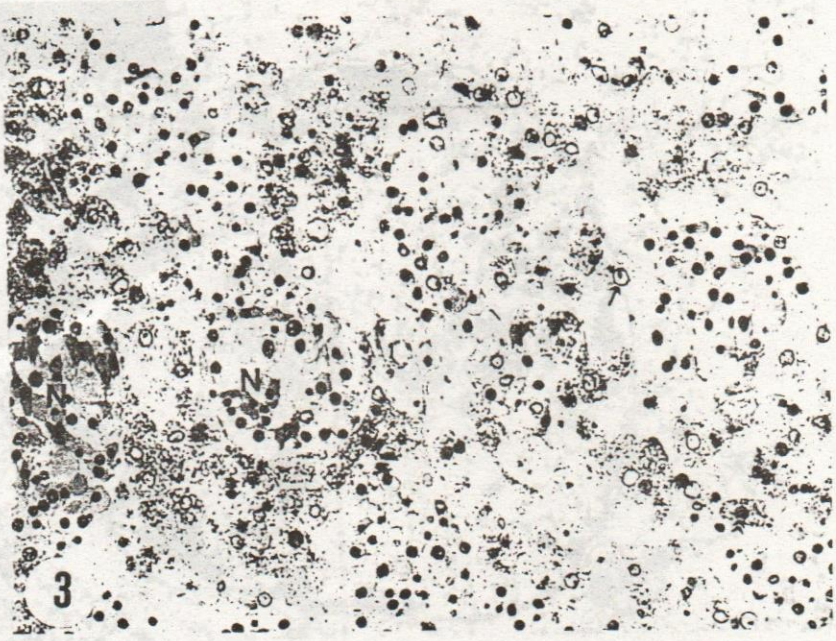
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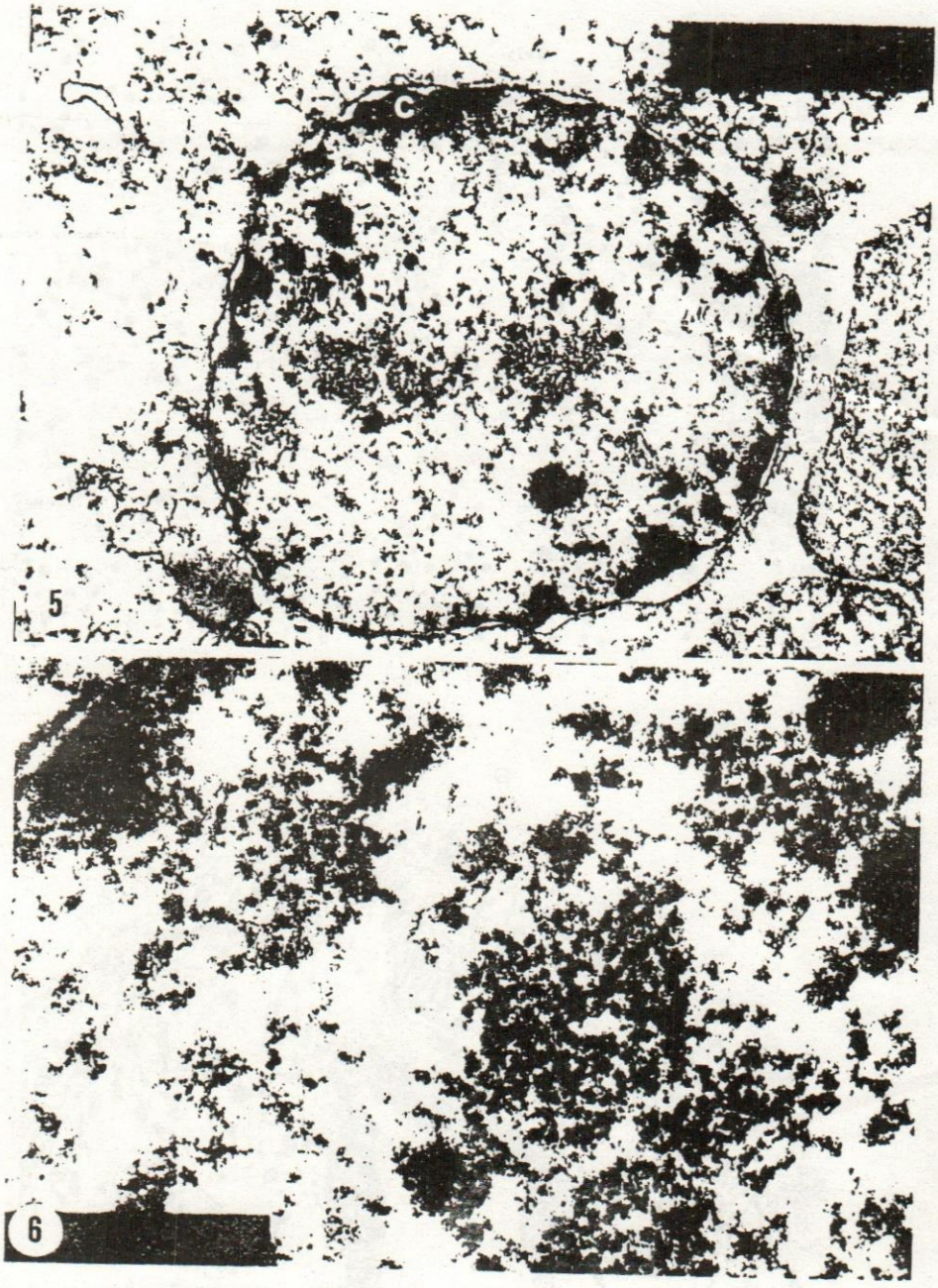
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