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Polioencephalomyelitis in Pigs Experimentally Infected with Porcine Enterovirus Isolated in Korea: I. Histopathological Observations

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Enterovirus 감염에 의한 자돈의 Polioencephalomyelit: Ⅰ. 병리조직학적 관찰

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초 록: 국내에서 분리된 enterovirus의 병원성을 관찰하기 위하여 1~2일령의 자돈 10두와 35일령의 유돈 6두에 enterovirus 조직배양부유액을 뇌내 또는 근육내 주사한 후 임상 및 병리학적으로 관찰하였던 바 다음과 같은 결과를 얻었다.

임상적으로는 체온의 상승, 보양창랑, 보행실조, 유약성 마비 및 고도의 삭수가 관찰되었다.

병리조직학적으로는 중추신경계 전반에 걸쳐 수막하 세포침윤, 혈관주위 원형세포침윤, 신경세포의 변성, 미만성과 한국성 gliosis, glial nodule의 형성 등이 관찰되었으며 백질부보다 회백질부에서 다소 심한 경향이었고 배측 신경절염이 전 실험예에서 인정되었다. 한편 hog cholera백신과 enterovirus의 공동주사예에서는 중추신경계의 병변이 enterovirus 단독주사예에 비해 급격히 진행되는 경향이었다.

이상의 소견들을 종합해 볼때 국내에서 분리된 enterovirus는 뇌내 또는 근육내에 접종하였을 때 자돈에서 polioencephalomyelitis를 일으킬 수 있는 병원성이 강한 형으로 추측되며 hog cholera백신과 enterovirus를 공동주사한 예에서 병변의 진행이 급격한 점으로 보아 enterovirus의 자연감염에 hog cholera 백신 등의 stress요인이 작용될 것으로 추측된다.

Introduction

Enteroviruses are widespread in swine as indicated by numerous reports from various countries.[1,6,8,14,16,17,20,23] In Korea, Kwon[10] isolated some pathogenic porcine enterovirus from swine and reproduced porcine polioencephalomyelitis in SPF pigs.

The susceptibility of porcine enterovirus infections was varied by the age of pig, older pigs appeared to be less susceptible to infection and a great number of enterovirus infections occurred at a time when colostral immunity presumably was low.[22] While the

*The isolation and identification of porcine enterovirus were performed by Dr. H.J. Kwon at the Choong Ang Animal Disease Laboratory, Daejeon, Korea.

majority of infections are asymptomatic, porcine enteroviruses have associated with a variety of clinical conditions including polioencephalomyelitis, female reproductive disorders, enteric disease, pneumonia, pericarditis and myocarditis.[8,10-13]

We have done for 3 years(initiated March, 1980) that the brains and spinal cords from all pigs submitted to the Laboratory of Veterinary Pathology, Kyungpook National University were examined, and that enterovirus were isolated from the lung of the diseased pigs.*

This report describes the occurrence of polioencephalomyelitis in pigs with confirmation by isolation of enterovirus and by experimental reproduction of the disease in pigs.

Materials and Methods

Virus: A porcine enterovirus, originally isolated from the lungs of a pigs with polioencephalomyelitis, was obtained from Dr. H.J. Kwon at Choong Ang Animal Disease Laboratory.

The virus had common biological and chemical properties which fullfilled the criteria of porcine enterovirus.[8,10] The virus titer of the inoculum was $10^{7.5}$ $TCID_{50}$/ml.

Experimental animals: 3 groups of conventional pigs were used(Table 1). In experiment I, ten 1 or 2-day-old pigs(colostrum-deprived), in experiment II, two 35-day-old pigs(hog cholera vaccine free), and in experiment III, four 35-day-old pigs(hog cholera vaccine free) were used.

In experiment I, the 5 pigs were infected intracerebrally[14,16] with 0.5ml virus suspension within 48 hours after birth. The other 5 pigs as a contral group were infected with 0.5ml virus free tissue culture suspension. In experiment II, the 2 pigs were infected intramuscularly[24] with 1 ml virus suspension only. In experiment III, the 2 pigs infected intramuscularly with 1 ml virus suspension and inoculated with hog cholera vaccine as a stress factor.[22] The others as a control were inoculated with hog cholera vaccine only.

Examination of the pigs and tissues:

Clinical examination: Rectal temperature and clinical signs were recorded daily.

Postmortem and Laboratory examination: Pigs were euthanatized at late stages of the central nervous system disturbance. Samples of organs including brain and spinal cord from each pig were fixed in 10% formalin, embedded in paraffin, sectioned(5μm thick) and stained with hematoxylin and eosin stain. Thionin stain was also performed to ascertain the degeneration of nerve cells.

Results

Experiment I: Piglets exposed by intracerebral

Table 1. Experimental Design

	Experimental Animal	Route of Inoculation
Experiment I.	10 Pigs ; 1 or 2-day-old ; Colostrum-deprived	5 Pigs-Intracerebral ; 0.5ml virus suspension* 5 Pigs-Intracerebral ; No virus
Experiment II.	2 Pigs ; 35-day-old ; Colostrum taken ; Hog cholera vaccine free	2 Pigs-Intramuscular ; 1.0ml virus suspension*
Experiment III.	4 Pigs ; 35-day-old ; Colostrum taken ; Hog cholera vaccine free	2 Pigs-Intramuscular ; 1.0ml virus suspension* ; Hog cholera vaccination 2 Pigs(Control) ; Hog cholera vaccination ; No virus

*The inoculum contained approximately $10^{7.5}$ $TCID_{50}$/ml.

infection to enterovirus had a febrile response and ataxia at 5 days postinoculation. At 10 to 13 days postinoculation all the piglets developed paralysis in the hind limbs and to a somewhat lesser extent in the forelimbs, and died afterward.

Grossly piglets necropsied at 13 days postinoculation had severe catarrhal enteritis.

Histopathologically, meningeal infiltration(Fig. 3) and focal gliosis(Fig. 4) coupled with perivascular lymphocytic infiltration of scattered vessels was more prominet in cerebrum and cerebellum than medulla oblongata and spinal cord(Fig. 16). Ganglionitis of the dorsal root ganglia was frequently observed(Fig. 17).

Experiment Ⅱ. Pigs intramuscularly infected with enterovirus developed a rise in rectal temperature (Fig. 1) and diarrhea at 8 days postinoculation and gradually followed incordination, ataxia, flaccid paralysis, emarciation and recumbency at 9 to 23 days postinoculation(Fig. 2).

The pigs inoculated with enterovirus revealed the histopathological changes of nonsuppurative polioencephalomyelitis, which consisted of degeneration of nerve cells, neuronophagia, diffuse gliosis, glial nodules and perivascular lymphocytic infiltrations. The lesions were present in both gray and white matter at all levels of cerebrum(Fig. 5, 6, 7) cerebellum(Fig. 9, 10, 11), medulla oblongata(Fig. 13, 14) and spinal cord(Fig. 18, 19), though usually more severe in the gray matter. The severe damage of the spinal cord was always found in the ventral horns, though to a much lesser degree, in the dorsal horns of gray matter(Fig. 18). Ganglionitis and degenerative changes of cerebellar Purkinje's cells were also recognized(Fig. 11, 19).

Experiment Ⅲ: Pigs inoculated with enterovirus and hog cholera vaccine showed similar clinical signs observed in Experiment Ⅱ, furthermore central nervous system disturbance was rapidly progressed than the pigs inoculated with enterovirus only. Two pigs became recumbent at 12, 14 days postinoculation(Fig. 1).

No gross lesions were found at necropsy without severe catarrhal enteritis.

Histopathologically, diffuse proliferation of microglia

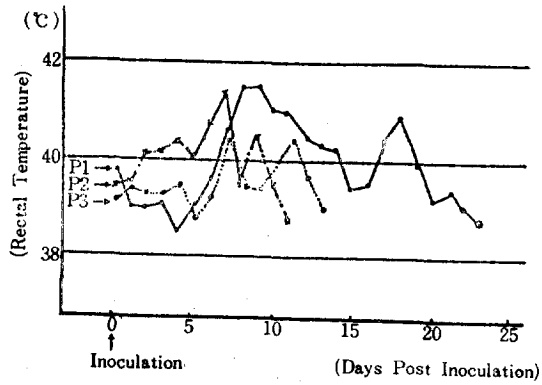


Fig. 1. Body temperature of pigs inoculated with porcine enterovirus only(P1) and both porcine enterovirus and hog cholera vaccine (P2, 3)

occurred at all the levels of central nervous system (Fig. 8, 12, 15, 20). Meningeal infiltration of lymphocytes was recognized in cerebrum. Ganglionitis of the dorsal root ganglia was also recognized (Fig. 20). It was suggested that usual outbreak of enterovirus infections in the weanling pigs should be associated with a certain stress factor such as hog cholera vaccination.

Discussion

Porcine enteroviruses have been associated with a variety of clinical conditions including polioencephalomyelitis,[1,5,6,8,15,16,23] female reproductive disorders,[5,15] enteric disease[9,18] and pneumonia,[5,15] which the majority of infections are asymptomatic, and also a syndrome of wasting and arrested growth in immature pigs.[11,21,22]

Clinical and pathological features of this condition closely resembled those of diseases reported from England,[6] Canada,[1] America,[8] Japan,[16] and France.[23] They all represent a porcine polioencephalomyelitis and are similar histopathologically. In Korea, experimental reproduction of polioencephalomyelitis in only SPF pigs with enteroviruses isolated from natural enzootics of the disease was reported previously.[10] Our isolates, however, was capable of producing porcine polioencephalomyelitis not only in colostrum-deprived pigs, which were not taken the maternal antibodies by the colostrum, and but also

in conventional weanling pigs at a time when colostral immunity presumably was low.

Experimental transmission to pigs had generally been found to be successful by the intracerebral, intranasal, and oral routes,[5,14] but occasionally positive results had been reported following intramuscular routes.[24] Especially Yamanouchi *et al.*[24] reproduced polioencephalomyelitis by the routes of intramuscular inoculation of virulent enterovirus (EI strain), but could not produced the disease by the intramuscular injection of less pathogenic E_4 strain, although a viremia was demonstrated in some of the pigs. Long *et al.*[14] described that porcine enterovirus isolated from feces of a healthy herd failed to produce lesions of polioencephalomyelitis in germ-free pigs orally exposed. When this virus was given intracerebrally, however, it produced polioencephalomyelitis in germ-free pigs. It was possible to draw out the conclusions which the virus, although neurotropic, was unsuccessful in reaching the brain and spinal cord from the intestinal tract and adjacent lymphoid tissue and thus was unable to complete this phase of the cycle of infection. In this paper, all the pigs inoculated with enterovirus either intramuscularly or intracerebrally showed polioencephalomyelitis clinically and histopathologically. So we suggested that this enterovirus be pathogenic to some extents.

The occurrence of enterovirus infections in weanlings was at a time when colostral immunity presumably was low.[14,22] Further, the enterovirus infections seemed to be associated with concurrent bacterial infections and certain stress factors occurring in or immediately following the weanling period.[22] In experiment Ⅲ, pigs inoculated with enterovirus and hog cholera vaccine known as a stress factor were recumbent within 14th days post inoculation following neural disorders similar to the experiment Ⅱ, moreover CNS disturbance was rapidly progressed than the pigs inoculated with enterovirus only. It was also confirmed in this paper stress factors including recent hog cholera vaccine, weaning, castration, deworming and movements connected with sales and purchases could collaborate the outbreak of porcine polioencephalomyelitis.[22]

All the pigs inoculated with enterovirus revealed the pathological changes of nonsuppurative polioencephalomyelitis in the central nervous system, which consisted of degeneration of nerve cells, neuronophagia, diffuse and focal gliosis, glial nodules and perivascular lymphocytic infiltration in both grey and white matter. These changes agree quite well with the lesions of polioencephalomyelitis induced in specific pathogen free pigs by pathogenic enterovirus.[1,5,6,14,15,26,24] On the basis of the characteristics of pathological changes induced and distribution in the central nervous system and other organs, the infectious disease reproduced in this experiment was similar to Talfan disease (milder form of classical Teschen disease),[6] because the lesions of Teschen disease[11,15] were practically confined to the grey matter, while the lesions of Talfan disease[6] were to be found on both grey and white matter, though usually more severe in the grey.

Concerning the distribution of prominent histopathological changes in CNS of the pigs infected with enterovirus, Harding *et al.*[6] described that the histopathological changes of the cerebellum were particularly striking, rather than the rest of the CNS. Long *et al.*[14] said that the lesions were present in the gray matter at all levels of spinal cord, brain stem, and cerebellum, but usually not in the cerebrum. Morimoto *et al.*[16] described the pathological changes were distributed most remarkably in the medulla oblongata and extended to the pons and the cerebral cortex. Manuelidis *et al.*[15] said that meningitis was one of the early manifestations of Teschen disease; the inflammation of the meninges wsa very marked in the cerebellum, moderate in the cerebrum, and slight in the spinal cord.

In this paper, the lesions similar to the previous reports[1,6,14] were found in all the levels of CNS. Especially cerebral meningitis was severe in the experiment I, and glial nodules were easily found in the gray matter of the cerebrum in the experiment Ⅰ and Ⅱ. Focal gliosis in the molecular and the Purkinje's cell layer of cerebellum in the experiment Ⅰ and Ⅱ. In experiment Ⅲ, diffuse gliosis was marked at all levels of CNS.

On the basis of these results, it was possible to draw decisive conclusions on the pathogenicity of the

enterovirus isolated in Korea.

Summary

A total of 10 colostrum-deprived pigs(1 or 2-day-old) and 6 pigs (35-day-old), which had been raised by natural maternal nursing, were used to study the pathogenicity of the porcine enteroviruses by the intracerebral and intramuscular routes of inoculation, which the enterovirus were isolated from the diseased pigs in Korea.

The porcine enteroviruses produced an identical polioencephalomyelitis in colostrum-deprived pigs and 35-day-old pigs, which manifested clinical signs and histopathological changes. Clinically it was characterized by incoordination, rise in rectal temperature, ataxia, flaccid paralysis in all the experimental groups. Histopathologically, the lesions were present in both grey and white matter at all levels of central nervous system, though usually more severe in the grey matter. These changes were characterized by meningeal infiltration, degeneration of nerve cells, neuronophagia, diffuse and focal gliosis, glial nodules and perivascular lymphocytic infiltrations. Ganglionitis of the dorsal root ganglia was frequently observed. On the basis of the clinical and histopathological changes mentioned above, it was concluded that porcine enteroviruses isolated in Korea were pathogenic strains which could produce polioencephalomyelitis in pigs.

The most severe disease was produced by the inoculation of both enterovirus and hog cholera vaccine in the 35-day-old pigs at a time when colostral immunity presumably was low. The porcine enterovirus infections seemed to be associated with certain stress factor such as hog cholera vaccine in or immediately following the weanling period.

Acknowledgment: The authors wish to express their appreciation to Dr. H. J. Kwon for his generous advices.

Explanations of Figures

Fig. 2. Pig intramuscularly inoculated with enterovirus shows ataxia and severe emaciation(Experiment Ⅱ).

Fig. 3. Microscopical appearance of cerebrum of piglet intracerebrally inoculated with enterovirus (Exp. Ⅰ). Severe meningitis is seen. H-E. ×67.

Fig. 4. Cerebrum of piglet intracerebrally inoculated with enterovirus(Exp. Ⅰ). Formation of microglial nodule in the gray matter is seen. H-E. ×33.

Fig. 5. Cerebrum of pig intramuscularly inoculated with enterovirus(Exp. Ⅱ). Formation of microglial nodule is seen in the gray matter. H-E. ×67.

Fig. 6. Higher power view of microglial nodule in Fig. 5. Formation of microglial nodule with degenerated nerve cells is seen. H-E. ×200.

Fig. 7. Cerebrum of pig intramuscularly inoculated with enterovirus(Exp. Ⅱ). Severe perivascular cuffing and necrosis of vascular walls are seen. H-E. ×67.

Fig. 8. Cerebrum of pig simultaneously inoculated with both enterovirus and hog cholera vaccine. (Exp. Ⅲ) Perivascular cuffing in gray matter and meningitis are seen. H-E. ×33.

Fig. 9. Cerebellum of pig intramuscularly inoculated with enterovirus(Exp. Ⅱ). Meningitis is seen. H-E. ×33.

Fig. 10. Cerebellum of pig intramuscularly inoculated with enterovirus(Exp. Ⅱ). Diffuse and focal gliosis are seen in the Purkinje's cell layer. H-E. ×50.

Fig. 11. Cerebellum of pig intramuscularly inoculated with enterovirus(Exp. Ⅱ). Degeneration of Purkinje's cell is conspicuous. Thionin. ×50.

Fig. 12. Cerebellum of pig inoculated with both enterovirus and hog cholera vaccine(Exp. Ⅲ). Perivascular cuffing in gray matter and degeneration of Purkinje's cells are observed. H-E. ×132.

Fig. 13. Medulla oblongata of pig intramuscularly inoculated with enterovirus(Exp. Ⅱ). Severe infiltration of mononuclear cells beneath meninges and diffuse gliosis are seen. H-E. ×33.

Fig. 14. Medulla oblongata of pig intramuscularly inoculated with enterovirus(Exp. Ⅱ). Two nerve cells showed degeneration being compared with normal cell. Thionin. ×132.

Fig. 15. Medulla oblongata of pig inoculated with enterovirus and hog cholera vaccine(Exp. Ⅲ). Perivascular cuffing, degeneration of nerve cells and diffuse gliosis are seen. H-E. ×33.

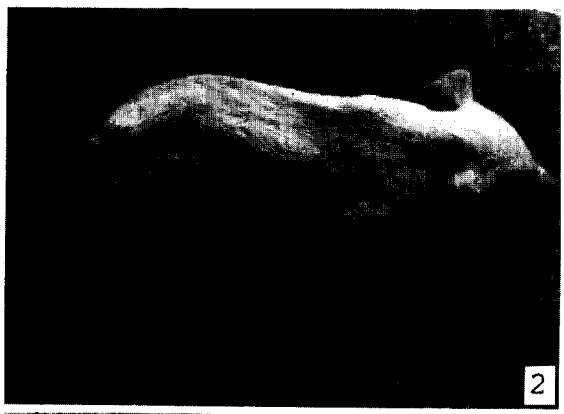
Fig. 16. Spinal cord of pig intracerebrally inoculated with enterovirus(Exp. Ⅰ). Diffuse gliosis and degenerative nerve cells are seen in the gray matter. H-E. ×33.

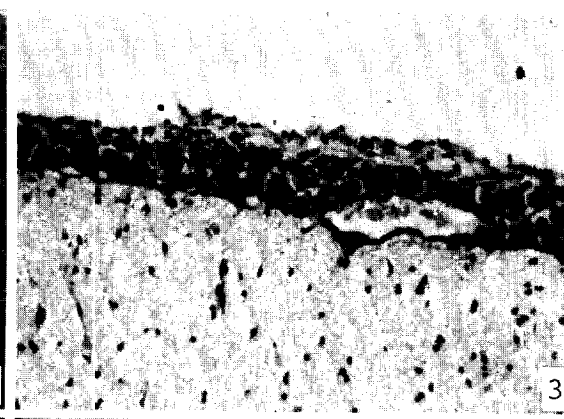
Fig. 17. Dorsal root ganglion of piglet intracerebrally inoculated with enterovirus(Exp. Ⅰ). Heavy mononuclear cell infiltration and degenerative ganglion cells are observed. H-E. X33.

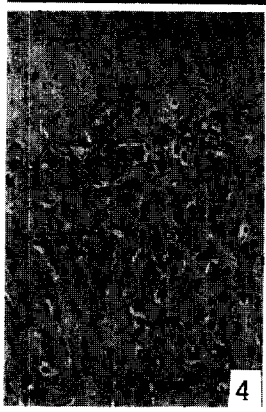
Fig. 18. Spinal cord of pig intramuscularly inoculated with enterovirus(Exp. Ⅱ). Degeneration of nerve cells, perivascular cuffing, and diffuse and focal gliosis are seen in the ventral horn of gray matter. H-E. ×33.

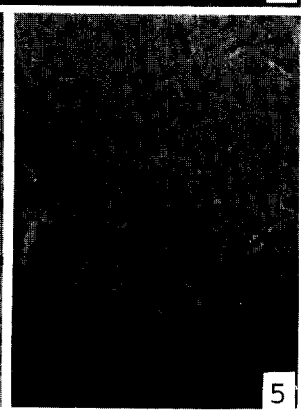
Fig. 19. Dorsal root ganglion of pig intramuscularly inoculated with enterovirus(Exp. Ⅱ). Severe ganglionitis is seen. H-E. ×50.

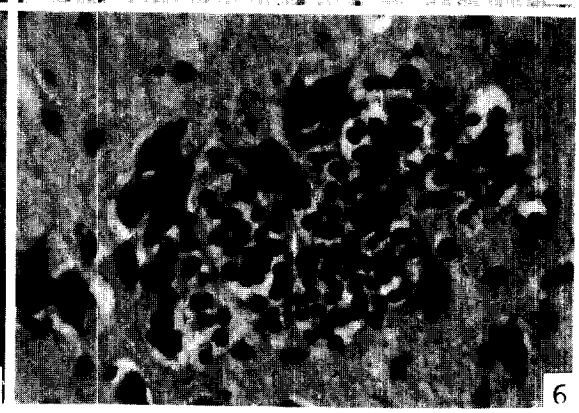
Fig. 20. Dorsal root ganglion of pig intramuscularly inoculated with both enterovirus and hog cholera vaccine (Exp. Ⅲ). Degenerations of ganglion cells are seen. Thionin. ×67.

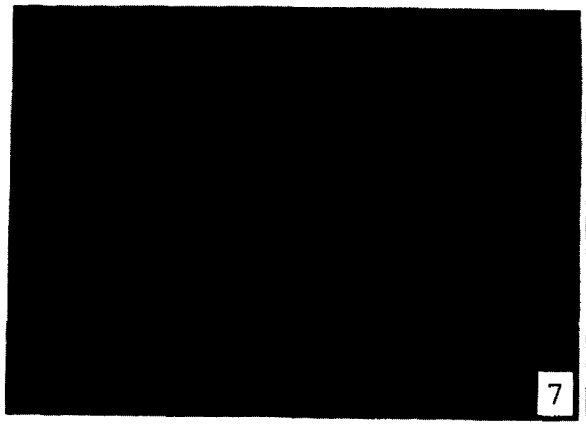








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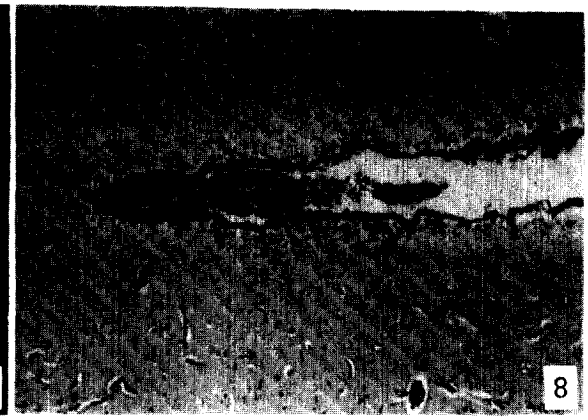
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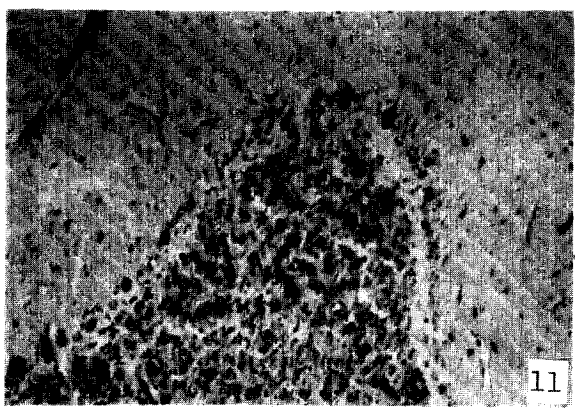
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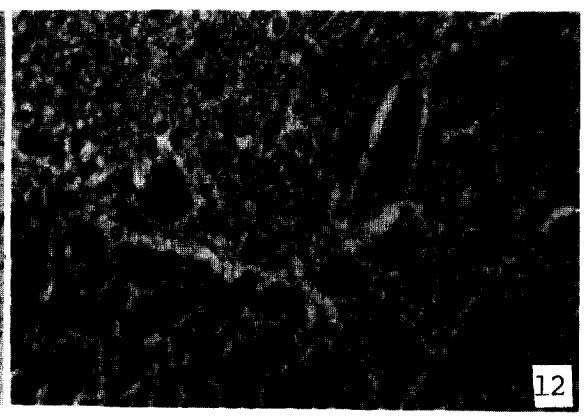
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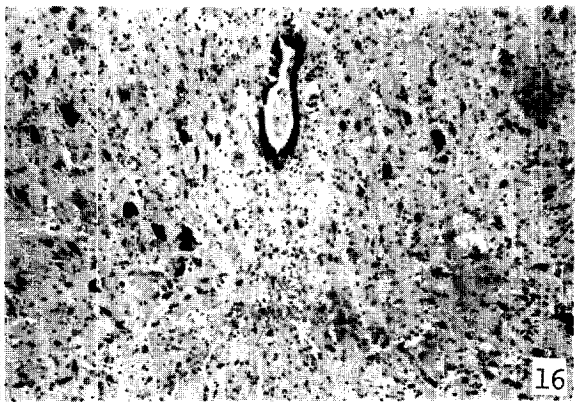
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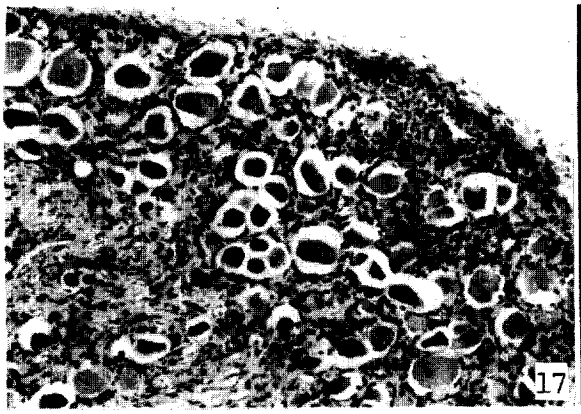
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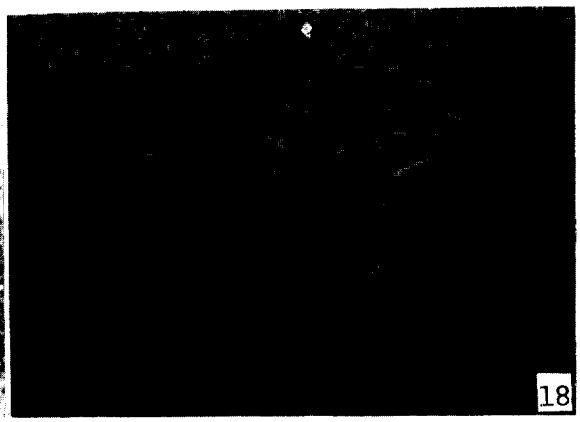
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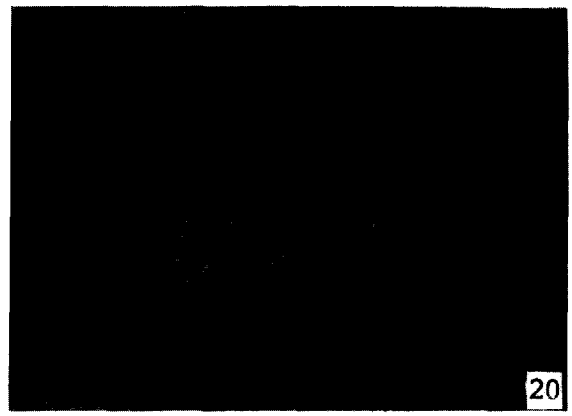

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