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ACTINOMYCES NAESLUNDII ISOLATED FROM BONE MARROW BIOPSIES OBTAINED FROM PATIENTS WITH MALIGNANT LYMPHORETICULAR DISEASES

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ABSTRACT

Different types of *Actinomyces spp.* isolated from the oral cavity of human in healthy as well as in pathological state. Internally it is only found in association with pathological organs. Recently, *Actinomyces naeslundii* has been isolated from blood culture of a leukemic patient. Present studies indicate the association of this microorganism with cells obtained from bone marrow by punction in more than 40 percent of patients with malignant lymphoreticular disease.

INTRODUCTION

Investigations by Lord, Emons, Slak and others lead to conclusion that Actinomyces organisms, endogenous in man and animals leading a parasitic existence as non-pathogens in mucous membranes of the oral cavity in the caries and tartar of teeth in tonsillar crypts and alimentary tract (1)

Willard, 1974 (1) concluded three species of *A. israelii A. naes-*

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To inoculate the API 20-A strips gaspack anaerobic jars should be used and reaction completed after 48 and 72 hours of incubation. Before reading the reaction following procedures must be done.

1. Adding two drops of xylene then two drops of Ehrlich's reagent to indole tube. In case of positive reaction, red color develops in 5 minute.
2. Adding two drops of H_2O_2 3% into the microtubes including mannitol for catalase production.
3. Adding one drop of Bromcresol purple into all microtubes containing carbohydrates.

Yellow or light purple color of tubes containing carbohydrates is indicative of fermented reduction in this case one drop of 0.02% Bromcresol purple in aqueous solution must be added to each tube in positive fermented tubes yellow green color develps.

Positive gelatin liquefaction appears with black particles. Escolin hydrolysis was determined by the U.V. light.

RESULTS

Morphology. In thioglycollate broth, grows slowly below the surface, granular large or small or diffusing throughout the medium (Figure 1). Colonies on brain heart infusion agar (Difco), Actinomyces agar (Difco) and blood agar anaerobically appeared in 72 hours. Usually they were small, 1 to 2 mm in diameter, flat or convex with smooth surface and entire borders. In some colonies some degree of roughness was observed (Figure 2).

Microcolonies appeared with dense centers composed of a mass of diphtheroidal cells or filaments surrounded by long branched filaments propagating in all directions (Figure 3).

Gram positive, non-motile, non-acid fast and non-spore-forming organisms consisted of diphtherodial and branched filamentous forms less than 1 mm in diameter were observed. Branched forms frequently showed clubbed end and especially they were see in the lymphatic gland of infected rabbits (Figure 4).

Diphtheroidal forms had varying lengths with uneven staining, knobbed ends, frequently in v and y arrangements (Figure 5). Biochemical tests showed catalase negative, non-production urease or indole, or liquefied gelatin. In all instances the carbohydrates were fermented with the production of acid not gas. The results of the

carbohydrates fermentation was as the following. Positive tests were for glucose, saccharose, maltose, salicin, esculin, mannose, raffinose, trehalose. Negative tests were for manitol, xylose, arabinose, glycerol, cellobiose, melezitose, sorbitol, rhamnose, and it was variable for lactose.

All results from biochemical tests were identical to *A. naeslundii* according to Coleman et al 1969. (2)

As Table 1 indicated, *A. naeslundii* isolated from bone marrow samples in 25 cases and from blood samples in 5 cases, bone marrow biopsy was not available two of them. In two cases both blood and bone marrow cultivation was positive.

After necropsy of rabbits only, two were found infected, one in the liver and another in the mesenteric lymph gland. The mesenteric lymph gland was enlarged almost in the shape of a tumor. White necrotic areas were visible on liver surface. Gram positive organisms were observed in a piece of smashed liver on stained slide. Many sections were prepared from fixed lymph gland and stained with hematoxylin and eosin and periodic acid schiff (PAS) methods. Multiple colonies of PAS positive filamentous and branched mycelia were observed in different areas of lymph gland (Figure 6).

Clubs were better obsvered in staining slides were hematoxylin and eosin method (Figure 7-8). Colonies were seen surrounded by giant cells, eosinophils and macrophages, reactive but no malignant cells were observed. Recultivation from both organs showed positive.

DISCUSSION

Because of neutropenia and immunodeficiency, viral, bacterial, fungal, and protozoal infections have been reported in patients with acute granulocytic leukemia and in later stages of chronic lymphocytic leukemia and lymphocytic lymphoma. (6)

Actinomycosis of the larynx has been found in the postmortem of a patient with acute monoblastic leukemia. (7) Pneumonia and recurrent infection of the upper respiratory tract due to Pneumococcus has been reported among patients with plasma cell myeloma because of gamma globulin deficiency and neutropenia. (8) Usually, associated infection in such patients occurs in later stages of the disease when there is immunosuppression because of progression of the disease or type of treatment. Associated infection with septicemia cannot be

applied to our selected patients while they were among newly diagnosed and no treatment had been used before obtaining samples. From the other side in most of them while cells from bone marrow were positive, peripheral blood was found negative (except in few according to Table 1).

Surface Ig has been reported in normal B type lymphocytes by Piessens, 1973. (9) Whether *A. naeslundii* is the causative agent of malignancy in the cell, or not, it probably alters the cell surface Ig. The altered cell with different cell surface antigenicity will appear non-self to the host's immunodeficient system and blocking antibody will induce against it. Similar blocking serum has been reported among patients with malignant melanoma. (10)

In 1951, Harrington demonstrated an anti-platelet factor in the serum of patients with idiotypic thrombocytopenia. Later, this factor was proven to be an Ig antibody of the IgG class. (11)

Specific idiotypic Ig was reported in some circulating B cells of patients with multiple myeloma. (12) It has been shown that anti-idiotypic cell surface Ig was quite distinguishable from anti-commerical human Ig. Indeed, Actinomycete of normal flora of alimentary canal might be responsible in stimulating and sensitizing of both serum Ig and cell surface Ig. This must be studied in the future. Probably the degree of alteration of cell surface Ig Manifests the degree of malignancy of the disease. Antigen shared between normal lymphoid cell line and leukemic blast cells (acute lymphoid and myeloid leukemia) and also between histologically similar types of carcinomas has been reported respectively. (13) Relative improvement in clinical conditions of some leukemic patients has been reported by BCG therapy. (6)

Since tubercle bacillus belongs to Actinomycetales phylogenetically relative of Actinomyces and antigenetically close to this group might help in neutralizing blocking antibody in serum of patients.

Actinomyces naeslundii was isolated with no difficulty from patients with Hodgkin's disease, thrombocytopenia, and aplastic anemia. Etiologically these three types of disease classify between infectious and tremalignant lymphoreticular diseases.

Kinds of thrombocytopenia similar to idiopathic thrombocytopenia has been reported as the earliest manifestation of some autoimmune disease, tuberculosis, lymphoma and carcinomatosis. It is also occasionally an earlier manifestation of leukemia in the peripheral blood. (14)

Manifestations like aplastic anemia occur in miliary tuberculosis, disseminated fugnal disease, acute leukemia, multiple myeloma and Hodgkin's disease. (15)

In Hodgkin's disease many of the signs and symptoms suggested

the presence of infection, however to show any etiological relationship of Hodgkin's disease to human and avian tuberculosis, various diphtheroid bacteria, brucella, and anaerobic bacteria, have labored with little success. (16)

Difficult to interpret why from biopsies of some groups of patients *Actinomyces naeslundii* was isolated with such difficulty while in some other groups it was not isolated at all even after frequent passage.

In the former group microorganisms must be intracytoplasmic and released into the medium after rupturing of the cell and grows. While the latter group either bone marrow was not the site of infected cells or microorganism (if it was the causative agent) integrated into the DNA of nucleus thus the isolation was impossible. This subject is under investigation.

RESUME

Diverses souches d'Actinomycetes furent isoles de la cavite buccale du sujets humains malades ou en bonne sante. Ces organismes furent trouves en association seulement avec des organes internes atteints de lesions. Actinomyces naeslundii fut isole recemment du sang des malades atteints de leucemie.

Nous avons trouve ce microorganisme associe aux cellules lors de la ponction de la moelle osseuse en plus de 40 pour cent des cas de la maladie maligne du systeme lymphoreticulaire.

				Results of cultivation and frequency of passage			
				Bone marrow		Blood	
No.	Sex	Age	Type of Disease	Result	No Passage	Result	No Passage
1	M	40	CML	+	2	—	5
2	M	4	ALL	—	—	+	4
3	M	30	CML	—	—	+	4
4	M	18	ALL	+	4	—	4
5	M	37	CML	+	4	—	4
6	F	40	CML	—	4	—	4
7	F	29	ALL	+	2	—	4
8	M	27	ALL	+	2	—	4
9	M	16	AA	—	4	—	4
10	F	45	AML	—	4	—	4
11	M	14	AML	—	4	—	4
12	M	20	HD	+	1	—	4
13	M	24	AA	+	1	—	4
14	F	25	AID	—	4	—	4
15	F	50	CML	—	4	—	4
16	M	75	LL	—	4	—	4
17	F	50	CML	+	2	—	4
18	M	22	AA	—	4	—	4
19	M	24	AA	—	4	—	4
20	M	30	CML	+	4	—	4
21	M	22	HD	+	1	—	4
22	F	40	AID	—	4	—	4
23	F	15	ALL	+	2	—	4
24	M	60	L	—	4	—	4
25	M	22	CML	—	4	—	4
26	F	40	CML	+	4	—	4
27	F	21	AML	—	4	—	4

28	M	70	CML	+	4	—	4
29	M	32	L	+	3	+	2
30	F	35	L	—	4	—	4
31	M	22	AA	+	2	—	4
32	M	18	AID	—	4	—	4
33	M	21	ALL	—	4	—	4
34	F	35	MM	—	4	—	4
35	F	20	L	—	4	—	4
36	M	41	L	—	4	—	4
37	F	40	MM	—	4	—	4
38	M	30	T	+	2	+	2
39	F	45	CLL	—	4	—	4
40	M	20	CLL	—	4	—	4
41	M	58	MM	+	1	—	4
42	M	28	AML	—	4	—	4
43	M	38	ALL	—	4	—	4
44	M	18	AA	—	4	—	4
45	M	35	CML	+	3	—	4
46	F	45	CML	+	4	—	4
47	M	28	L	+	2	—	4
48	F	25	T	+	1	—	4
49	M	21	ALL	—	4	—	4
50	M	35	ALL	1	4	—	4
51	M	52	MM	+	1	—	4
52	M	39	L	—	4	—	4
53	F	18	AID	—	4	—	4
54	F	50	CML	—	4	+	4
55	F	14	T	+	1	—	4
56	M	45	L	+	4	—	4
57	M	23	CML	+	3	—	4

Table 1. Actinomyces isolated from bone marrow and blood of patients with lymphoreticular disease.

CML: Chronic Myeloid Leukemia
ALL: Acute Lymphocytic Leukemia
AA: Aplastic Anemia
AML: Acute Myeloid Leukemia
HD: Hodgkin's Disease
AID: Anemia of Iron Deficiency
LL: Lymphoid Leukemia
L: Lymphoma
MM: Multiple Myeloma
T: Thrombocytopenia
CLL: Chronic Lymphoid Leukemia

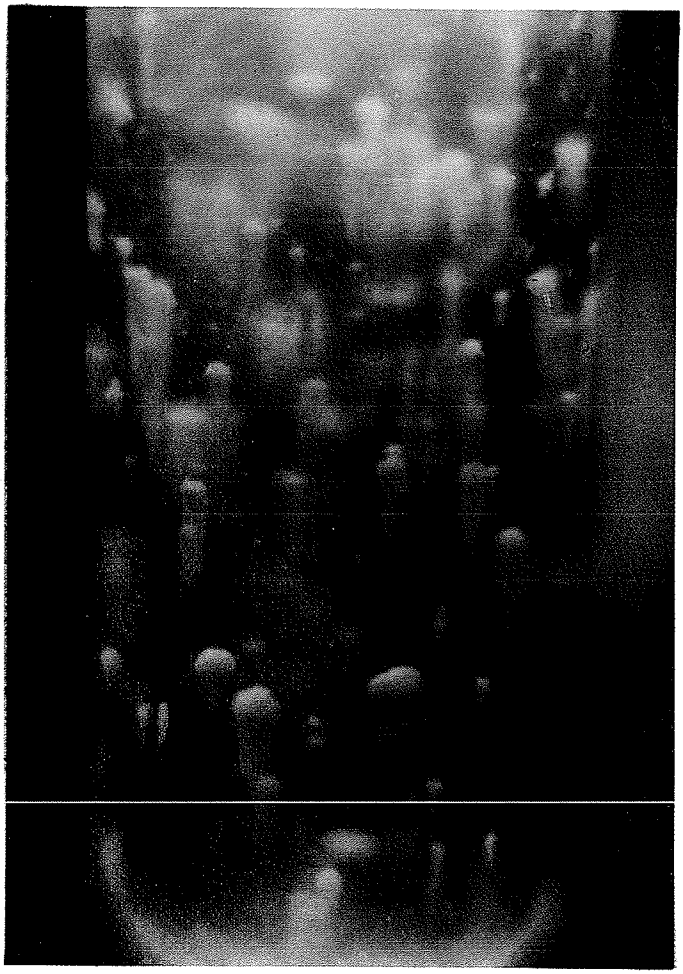


Figure 1: Colonies of *Actinomyces naeslundii* in thioglycollate agar. x4.5.

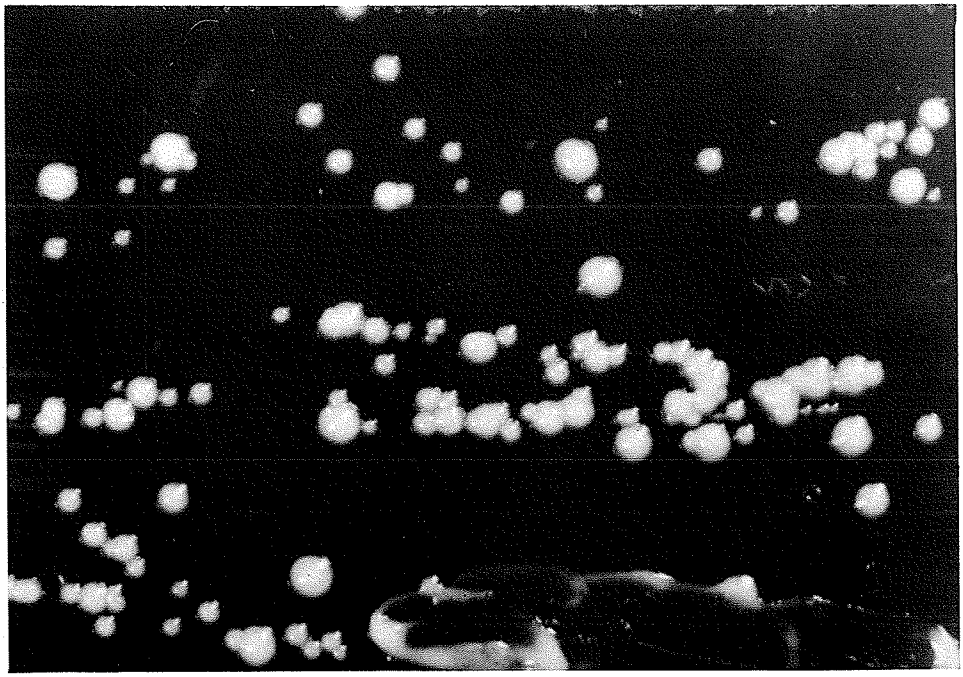


Figure 2: Colonies of *A. naeslundii* x45.

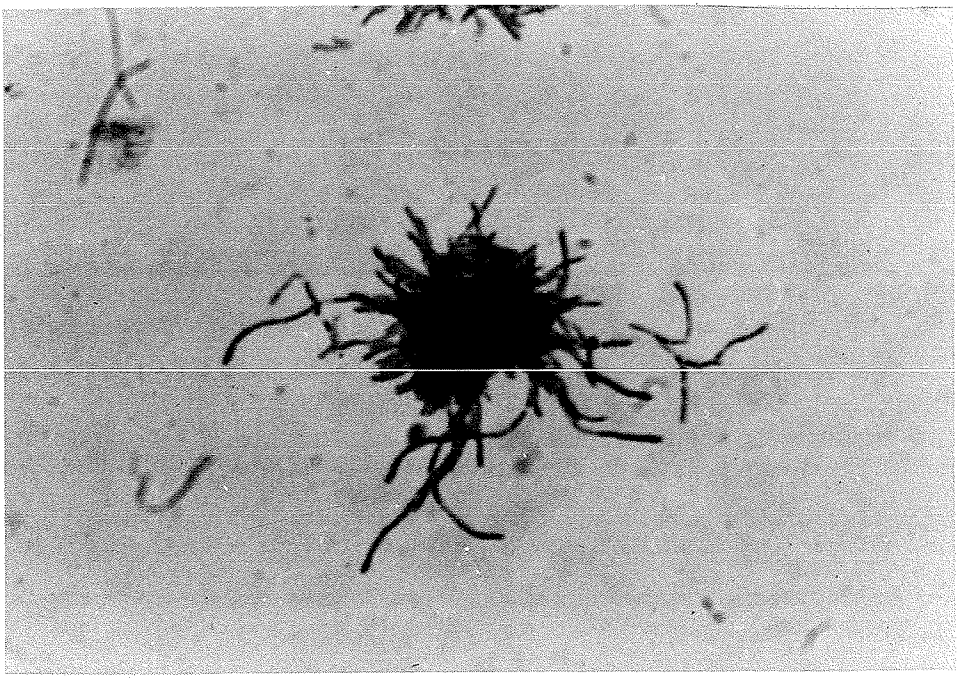


Figure 3: Microcolony of *A. naeslundii* gram stain x1000.

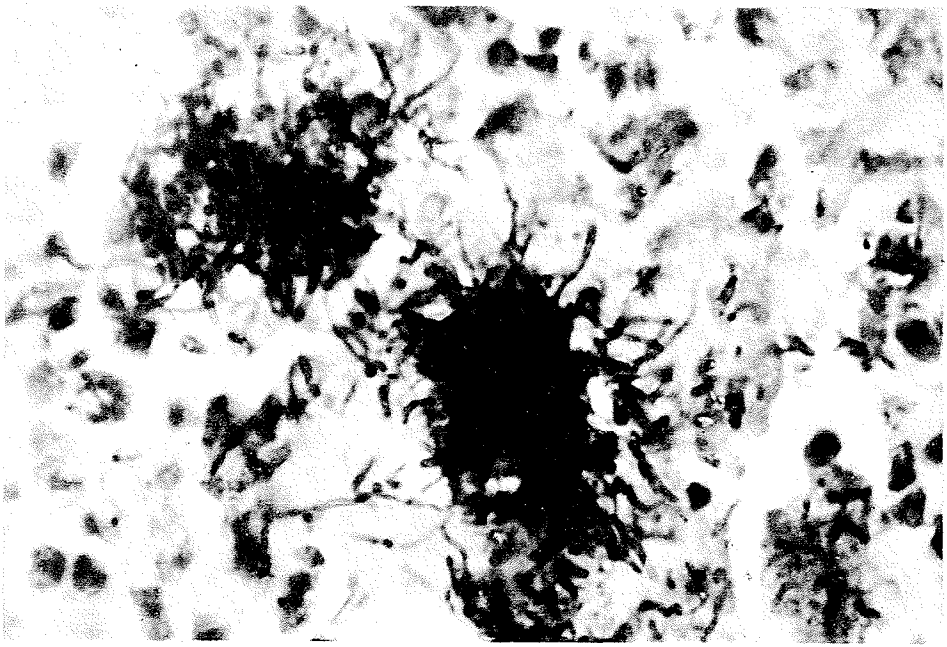


Figure 4: Infected lymphatic gland in rabbit PAS stain x1250.

Figure 5: *Actinomyces naeslundii* diphtheroid type gram stain x1000.

lundii and *A. eriksonii* all are normal flora of the human buccal cavity and tonsillar crypts and are of limited invasive ability.

They probably can not elicit disease without the aid of trauma to the tissue and the presence of associated bacteria.

Coleman et al 1969 (2) isolated *A. naeslundii* from circulating blood and wound abscess and empyema of the gall bladder. Another species of Actinomyces *(A. bovis)* has been isolated by us from *Cysticercus bovis* (3) and abnormal growing (Pseudoalveolar hydatid cyst (4) in cow.

Observations indicate Actinomycets has wide range of population which varies biochemically and antigenetically according to their growing media or niche and associates.

Harmless microflora of normal oral cavity, in time become associated with pathological organs, abnormal budding cysts and malignant cells of the host. Present study indicate the association of microorganism with the tumor cell obtained from bone marrow of patients with malignant lymphoreticular diseases.

MATERIALS AND METHODS

Study continued from September 1978 to March 1980. Meanwhile 57 patients and controls were selected to study in blood transfusion centers of Tehran hospitals (Imam Khomeini and Amir Alam) and diffinitive diagnosis of disease were given by Dr. H. Fardin, Dr. Zamanian Pour, Dr. M. Keyhani.

Sternal biopsy (punction) and leukocytes collected from circulating blood from patients with malignant lymphoreticular disease were cultured separately in thioglycollate and were kept anerobically in anaerobic jar in 37^{0} centigrade for two weeks. Microorganisms from the culture growing in brain heart infusion agar (difco) were used to inject intravenously into five rabbits, three times, during 15 days according to Slak, 1942.(5)

Indentification was supplemented by API system. A system applied in Auburn University, Department of Microbiology, School of Veterinary Medicine.

Prior to inoculation of the *Actinomyces naeslundii* in basal medium (API) anaerobic culture obtained from lyophlyzed microorganisms. Typical colonies were selected and emulsified in the medium as quickly as possible. Then the tube and cupule of the gel tube filled with the emulsion and the cupule section of the indole tube filled with the mineral oil.

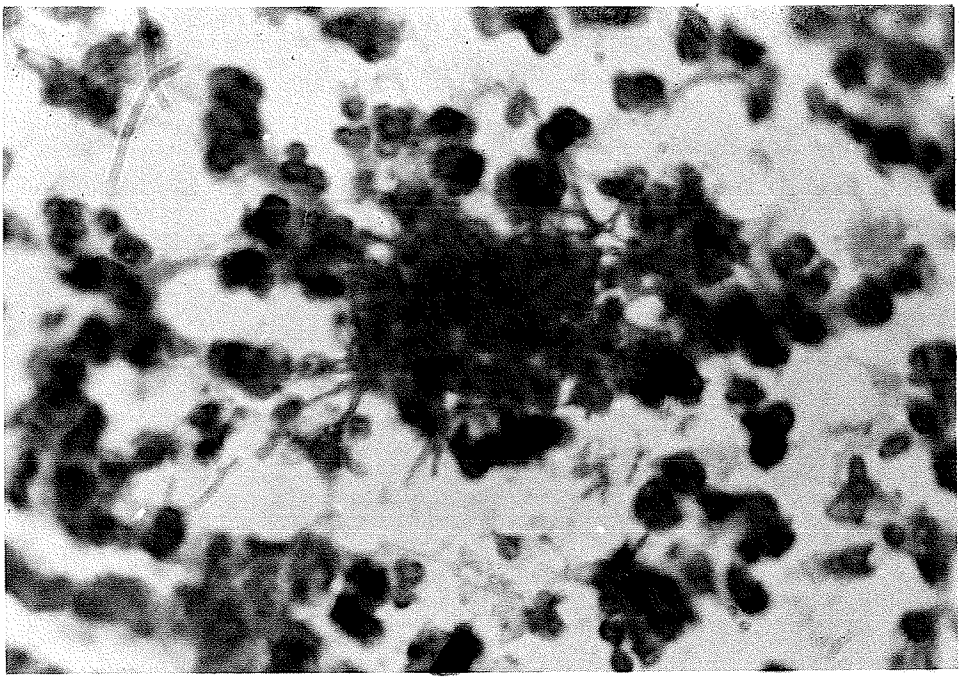


Figure 6: Infection in lymphatic gland of rabbit. PAS stain x1250.

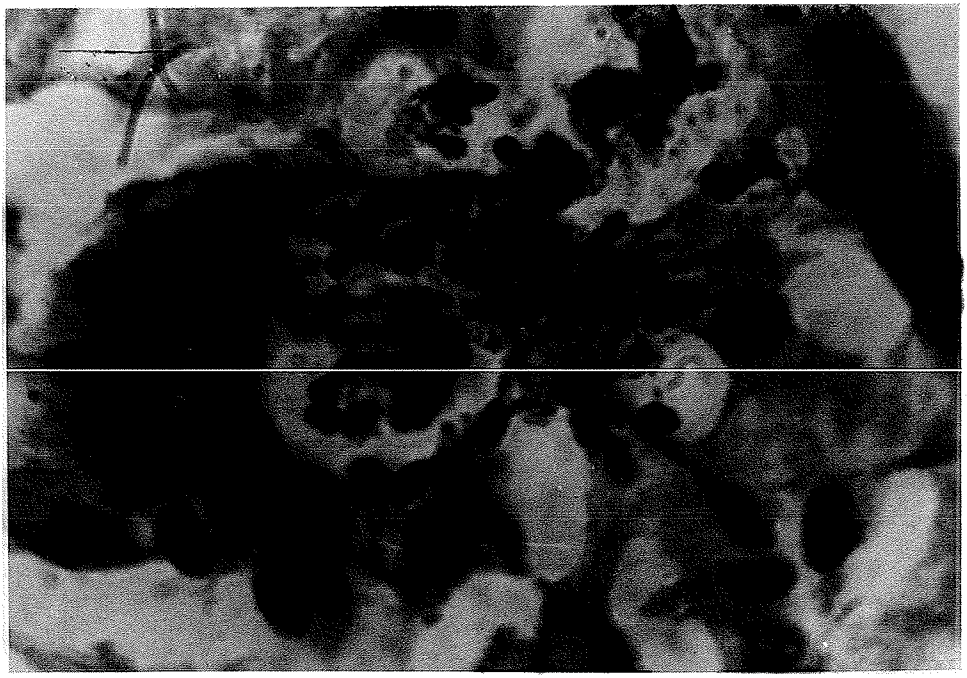
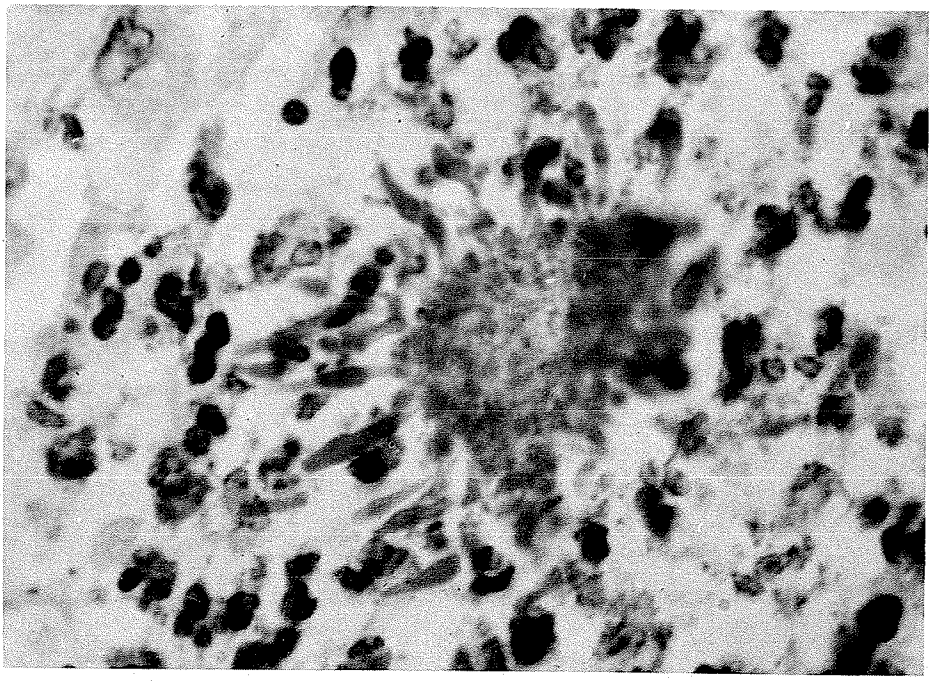


Figure 7-8: Infection in lymphatic gland in rabbit, H and E Stain x 2000.



REFERENCES

1. Rippon, John. Willard (1974) Medical Mycology (patho-genic Actinomycetes),Philadelphia, Sunders pp. 387.

2. Coleman, R.M., George, L.K., Rozzell, A.R. (1969). Appl. Microbiol. 18 (3), 420-426.

3. Mobedi-I.Vand-Youssefi, J., Esterabadi, A.J. (1978) *Actinomyces bovis*, a possible cause for calcification of the parasite in the host tissue, Arch. Inst. Razi, *30*, 117-128

4. Mobedi-I.Vand-Youssefi, J., Talebi-Nejad, A. (1979), *Actinomyces bovis* in pseudo-alveolar, hydatid cysts in cow. Arch. Inst. Razi. in Press.

5. Slack, J. 1942. The Source of Infection in Actinomycosis, J. Bact, *43*, 193-209.

6. Williams, W.J., Beutler, E. Erslev, A.J., Rundles, R.W. (1977). Hematology, Second edition, McGraw-Hill Book Company. A Blakiston Publication. New York, pp. 1755.

7. Billingham, R.P., Giner, P.F., (1972). Actinomycosis in acute leukemia. New York State J. Med. Nov. 15, pp. 2797-2800.

8. Bergsagel, O.E., (1977). Plasma cell myeloma in hematology of williams et al. Blakston Publication, New York.

9. Piessens, W.F., Schur, P.H., Moloney, W.B., Churchill, W.H. (1973): Lymphocyte surface immunoglobulin (distribution and frequency in lymphoproliferative diseases) New Engl. J. Med. 25, (4) 176-180.

10. Hellstrom, I., Warner, G.A., Hellstrom, K.E., Sjogren, H.O.: (1973) Sequential studies on cell-mediated Tumor Immunity and Blocking serum activity in ten patients with Malignant malanoma. Int. J. Cancer *11*, 280-292.

11. McClure, P.D. (1975). Idiopathic thrombocytopenic purpura in

children. Diagnosis and Management Pediatrics, *55*, 68-74.

12. Abdou, N.I., Abdou, N.L. (1975). The monoclonal nature of lymphocytes in multiple myeloma (effects of therapy). Ann. Intern. Med. Med. *84* 42-45.

13. Durantex, A., Zighelboim, J., Thieme, T., Fahey, J.L., (1975). Antigen shared by leukemic blast cell and lymphoblastoid cell lines detected by lymphocyte - dependent antibody, Cancer Research *35*, 2693-2698.

14. Aster, R.H., (1977). Disorders of hemostasis-quantitative platelet disorders, in Hematology of Williams et al. Blackston Publication, New York.

15. Ersleve, A., (1977). Aplastic anemia in hematology of Williams et al, Blackston Publication, New York.

16. Rundles, R.W., (1977). Hodgkin's disease in Hematology of Williams et al, Blackston Publication, New York.