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INCIDENCE OF LISTERIA IN RAW MILK

(With 2 Tables & 11 Figs.)

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مدى وجود ميكروب الليستريا في اللبن الخام

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أجريت هذه الدراسة على ٢٣٦ عينة لبن تم تجميعها على فترات زمنية من أقساط وتانكات مزرعة لبن واحدة في كل من محافظتي البحيرة والاسكندرية وذلك بغرض البحث عن ميكروب الليستريا واظهار نسبة تلوث اللبن بهذا الميكروب ذو الأهمية الصحية للمستهلك ـ تم عزل ميكروب الليستريا موسيتوجين والتعرف عليه بواسطة الاختبارات الكيمائية والاختبار المصلي التأكيدي حيث أظهرت نتائج الفحص أن نسبة التلوث بميكروبي الليستريا وحيدة الخلية (موتوسيتوجين) والليستريا أنكوا كانت ٣٪ ، ٥ر٨٪ على التوالي . تم حقن او مم والذي يحتوي على ٢ر٧ × $١٠^{٤}$ من ميكروب الليستريا موتوسيتوجين في الغشاء البريتوني للجرزان البيضاء وذلك لمتابعة التغيرات الباثولوجية التي تحدث خلال أسبوع من تاريخ الحقن وحتى حدوث الوفاة . حيث تم جمع عينات متتالية ابتداء من اليوم التالي للحقن وحتى اليوم الخامس من الأعضاء الداخلية للجرزان (الكبد ـ المخ) وفحصها باثولوجيا وتبين وجود احتقان وتهدد في الأوعية الدموية يتبعها وجود بقع دموية والتهاب منتشر في الخلايا مع انتشار في الخلايا الميتة حتى اليوم الخامس مما أدى إلى سكون في الحركة وذلك بعد ظهور الأعراض العصبية التي أدت إلى حدوث الوفاة . هذا وقد نوقشت الأهمية الصحية الاقتصادية للميكروبات المعزولة والاجراءات التي يمكن اتخاذها لمنع التلوث بهذا الميكروب .

SUMMARY

Two hundred and thirty six samples of raw milk were collected from bulk milk cans and tanks of individual dairy farms at El-Behera and Alexandria Governorates. Listeria was isolated from 27(11.4%) of 236 examined samples. The incidence of Listeria monocytogenes was 3% (7/236), while 8.5% (20/236) of raw milk samples were contaminated with L.innocua. The Listeria monocytogenes isolates were identified

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serologically by using Bacto Listeria "O" antisera Type 1 & 4.Experimental inoculation of L.monocytogenes into a white mice proved that the organism infect mice, replacing within the tissues and organs and lead to death. Histopathological examination for the liver and brain of sacrified mice revealed that the early macroscopic lesion appeared in the form of haemorrhagic foci and late lesion showed necrosis which started after the fourth day of inoculation and became prominant after the fifth day until death. The public health importance of the organism as well as the suggested measures for improving the quality of milk are discussed.

INTRODUCTION

Listeriosis constitutes a very important serious disease in several countries, the caustive organism is widespread in nature. L.monocytogenes has been reported as a cause of mastitis in dairy cow, a condition which can cause a significant rise in the number of causative organisms excreted in milk (GITTER, et al. 1980). High excretion rates of L.monocytogenes in milk from asymptomatic cows and goats have frequently been reported(FLEMING, et al. 1985).

Originally it was assumed that human aquired Listeriosis due to direct contact with infected animals. Recently a number of outbreaks have confirmed that this disease can be indirectly transmitted from infected animals to human through consumption of contaminated dairy products (FLEMING, et al. 1985; HAYES, et al. 1986 and FENLON and WILSON, 1989).

Chracteristically the syndrome in man including abortion in pregnant women, also menengitis or meningeoencephalitis, immunocompromised men and women have been reported (BARZA, 1985). The serious consequence of human Listeriosis makes it of utmost importance for public health significance and dairy industries (AL-ASHMAWY, 1990).

The present investigation was undertaken to throw light on the incidence of Listeria in raw milk.

MATERIAL and METHODS

Sampling:

Two hundred and thirty six raw milk samples (200 ml each) were obtained from

bulk milk cans and tanks of individual dairy farms in El-Behera and Alexandria Governorates.

Samples were collected aseptically in sterile screw capped sampling bottles and transported on ice to the laboratory and refrigerated, until examined.

Isolation and identification:

The techniques recommended for recovery of Listeria in examined milk samples were assayed by food and Drug adminstration "FDA" (LOVETT, et al. 1985 & DONNELLY and BAIGNENT, 1986). Twenty five ml of each milk sample were added to 225 ml of Listeria enrichment broth (modified FDA broth) supplemented by thiamin dichloride 5 mg/L and tryptoflavin 10 gm/L and incubated at 30°C and examined after 2, 7 and 10 days by direct plating of 0.1 ml of diluted broth onto the surface of duplicate plates of Listeria selective agar which supplemented by thiamin dichloride 5 mg/L, tryptoflavin 10 gm/L and nalidixic acid 40 mg/L (FEINDET, 1976). The plates were incubated at 37°C for 48 hrs. Listeria like colonies were picked up and streaked onto tryptose soya agar (TSA) slants, incubated at 35°C for 24 hrs. and examined microscopically for characteristic morphology and identified according to SEELIGER and JONES (1986).

Serological examination:

The serological examination confirmed the isolates identified as Listeria monocytogenes according to DIFCO MANUAL (1984) with Listeria "O" antiserum type 1, 4 which obtained from Statens serum institute, Division of Microbiology, Denmark. The Listeria organisms were cultured twice on tryptose broth and incubated at 35°C for 24 h. The broth suspension was heated at 80°C for 1 hour in a water bath, then centrifuged directly after cooling. The bulk of the supernatant fluid was removed and the organisms were suspended in the remaining portion of the liquid. On a clean, dry glass slide one drop of heated organism was added to one drop of the antiserum diluted 1:20 in saline solution and rocked back and forth for 1-2 minutes. The presence of agglutination indicated positive results.

Mice pathogenicity and histopathological examination:

One of the cultural isolates which was confirmed as Listeria monocytogenes was grown two times in Tryptose soya broth with 0.6% yeast extract at 35°C for 24 h. The broth was centrifuged and the sediment was washed by 0.01 phosphate buffer saline at pH 4.7. The bacterial culture was diluted to give 10^4-10^5 colony forming unit (CFU)/1 ml of broth. 0.1 ml containing 2.7×10^4 cells were inoculated I.P into white mice (18-20 gm). Mice were observed for one week and deaths were recorded. The postmortem examination was performed on all infected and control mice immediately after death. At the same time one mice was sacrified after one

day of inoculation, two days, three days, four days and five days. The liver and brain of sacrified mice were grossly examined and the Listeria organisms were isolated from both organs (THOMAS, et al. 1971) by smearing these specimens over an selective Listeria agar surface and incubated at 37°C for 24-48 h. The organisms were identified biochemically and serologically, then tissue samples from both organs were fixed in 10% buffered formalin and 5 μ paraffin sections, stained with haematoxylin and eosin, were microscopically examined according to RODGERS (1980).

RESULTS

The results obtained were recorded in tables. 1 & 2 and in the described 11 figures.

DISCUSSION

The data presented in Tables (1 & 2) show that 27 (11.4%) of 236 raw milk samples were contaminated with listeria. Most of the positive samples (21/236) were obtained after 2 days of enrichment at 30°C and some positives (6/236) were obtained after 7 days of enrichment. No isolates were obtained after 10 days enrichment. The incidence of Listeria detected in this research nearly similar to that reported by other workers (FARBER, et al. 1988; LIEWEN and PLAUTZ, 1988).

The frequency distribution of isolates (Table 2) revealed isolation of L.monocytogenes and L.innocua spps. No other species of Listeria were isolated.

L.innocua was the species most frequently isolated from raw milk samples, being found in 8.5% (20/236) of the samples analyzed. This compares favorably with results reported by LOVETT, et al. (1985) who analyzed raw milk samples in the united states in the Tri- State, Massachusetts, and California areas and found isolation rates for L.innocua of 7.7; 9.5 and 4.0%, respectively.

FARBER, et al. (1988) inferred L.innocua has most frequently isolated and was found in 9.7% (43/445) of the raw milk samples, while LIEWEN and PLAUTZ (1988) could isolate the organism from 5% (10/200) of raw milk samples obtained from bulk storage tanks of dairy farms in Eastern Nebraska.

L.monocytogenes was found in only 7 of 236 (3%) raw milk samples analyzed. Our results are similar to those reported by LOVETT, et al. (1985); HAYES, et al. (1986); FARBER, et al. (1988); LIEWEN and PLAUTZ (1988) and FENLON and WILSON (1989). AL-MEDHAGI (1991) could isolate Listeria monocytogenes from 2 (5%) of 40 raw milk samples and 1 (5%) of 20 Domietta cheese samples.

The role of milk and dairy products in transmission of Listeria monocytogenes from infected dairy cattle to human through consumption of contaminated milk and dairy products make it unreleastic to expect that they may be excluded completely from dairy factories. Especially Listeria was able to attach to stainless steel equipments at different pH, 5-8 and temp., 10-35ºC (LOVETT, et al. 1987).

Survey in Germany and France strongly suggested that cheese made from pasteurized milk are frequently contaminated with L.monocytogenes (FLEMING, et al. 1985).

The excretion of L.monocytogenes in cow's milk is well recognized. Cows with Listeric mastitis may produce normal appearing milk containing large numbers of bacteria moreover, the presence of L.monocytogenes in milk may be caused by exogenous contamination from the farm, feed or faecal contamination (FLEMING, et al. 1985). Some of the organisms may survive pasteurization and may then grow better than competting species at refrigerator temperatures (BARZA, 1985).

Pathological lesions:

In our material it was found that if the inoculum size was sufficiently large, death occured without the initial development of specific lesions. The same results have been reported by PATERSON (1940); JAGER and MYERS (1954) and DONTENWELL and KNOTHE (1956).

It was evident that L.monocytogenes as a facultative interacellular pathogen, infect mice, replicating within the tissues and organs and lead to death. In the present work it has been also noticed macroscopically that early lesions were in the form of haemorrhagic foci and late lesions showed necrosis, which is compatible with that of DONTENWELL and KNOTHE (1956) and BASHER, et al. (1984).

In this study three morphologically distinct types of lesion were seen as early lesions in the form of vascular dilation and congestion after the first two days, followed by massive mononuclear inflammatory cellular infiltrate after the third day, then necrosis started after the fourth day and became prominant after the fifth day. These results coincide with that of DONTENWELL and KNOTHE (1956); BASHER, et al. (1983) and BASHER, et al. (1984) in their work on newly hatched chicks.

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LEGENDS

Fig. 1: Section of normal mouse liver showing hepatic lobule with central vein and regular raws of hepatocytes separated from each other by sinusoids. H & E (a x125, b x500).

Fig. 2: Section of normal mouse brain showing multipolar neyrone cell-bodies scattered in a mass of axons and dendritis of other numerous supporting cells and small blood vessels. (a x125, b x500).

Fig. 3: Liver section after the first day, showing mild vascular dilation and congestion (central vein & sinusoids) H & E (a x125, b x500, c x1250).

Fig. 4: Brain section after the first day showing the some mild vascular dilation and congestion, H & E (a x125, b x1250).

Fig. 5: Liver section after the second day showing severe vascular dilatation and congestion with mild lymphocytic infiltration H & E (a x500, b x1250).

Fig. 6: Brain section after the second day showing the same severe vascular dilatation and congestion. H & E (a x 500, b x1250).

Fig. 7: **(a & b):** Liver and brain sections after the third day showing massive infiltration with mononuclear inflammatory cellular infiltrate. H & E (a x125, b x125).

Fig. 8: Liver section after the fourth day showing degenerative changes (cytoplasmic granularity, oedema & nuclear enlargement) H & E (a x125, b x500).

Fig. 9: Brain section after the fourth day showing degenerative changes (cytoplasmic granularity, oedema & nuclear enlargement H & E (a x125, b x500).

Fig. 10: Liver section after the fifth day showing marked necrotic changes. H & E (a x125, b x500, c x1250).

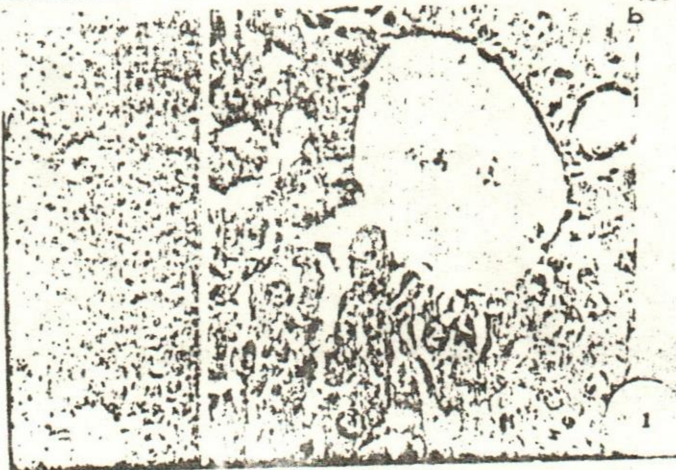
Fig. 11: Brain section after fifth day showing marked necrotic changes H & E (a x125, b x500, c x1250).

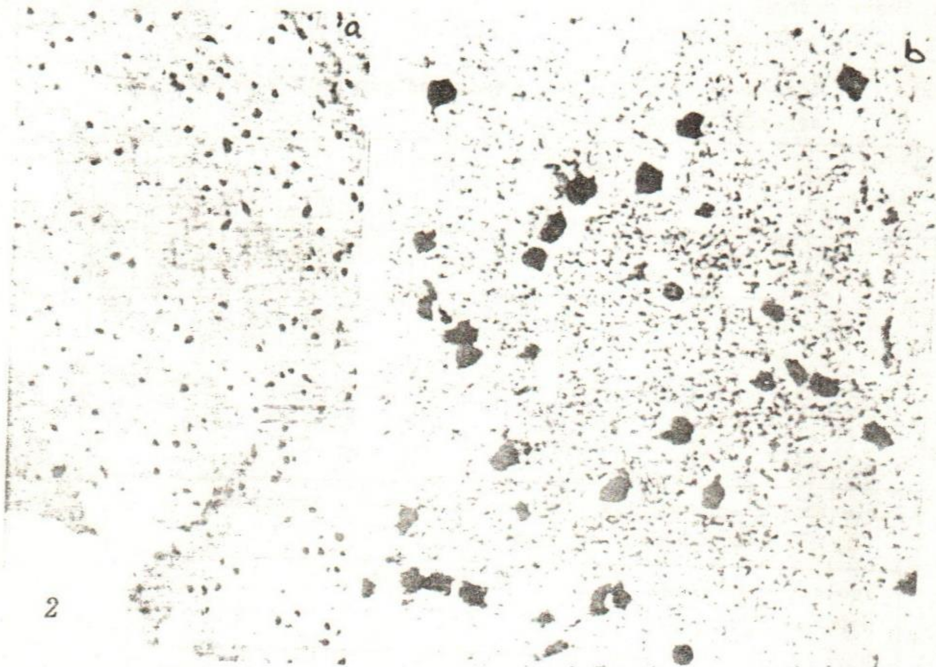
Table (1): Incidence of Listeria in examined raw milk samples after 2, 7 and 10 days

Total No. of samples	Period of incubation			Total
	2 days	7 days	10 days	
	No. of +ve samples (%)	No. of +ve samples (%)	No. of +ve samples (%)	
236	21 (8.9)	6 (2.5)	-	27 (11.4)

Table (2): Frequency disrtibution of Listeria Spp. isolated from raw milk samples

Isolated Listeria Spp.	Frequancy			
	Period of incubation			Total
	2 days	7 days	10 days	
L. monocytogenes	3 (1.3)	4 (1.7)	- -	7 (3)
L. innocua	18 (7.6)	2 (0.9)	- -	20 (8.5)

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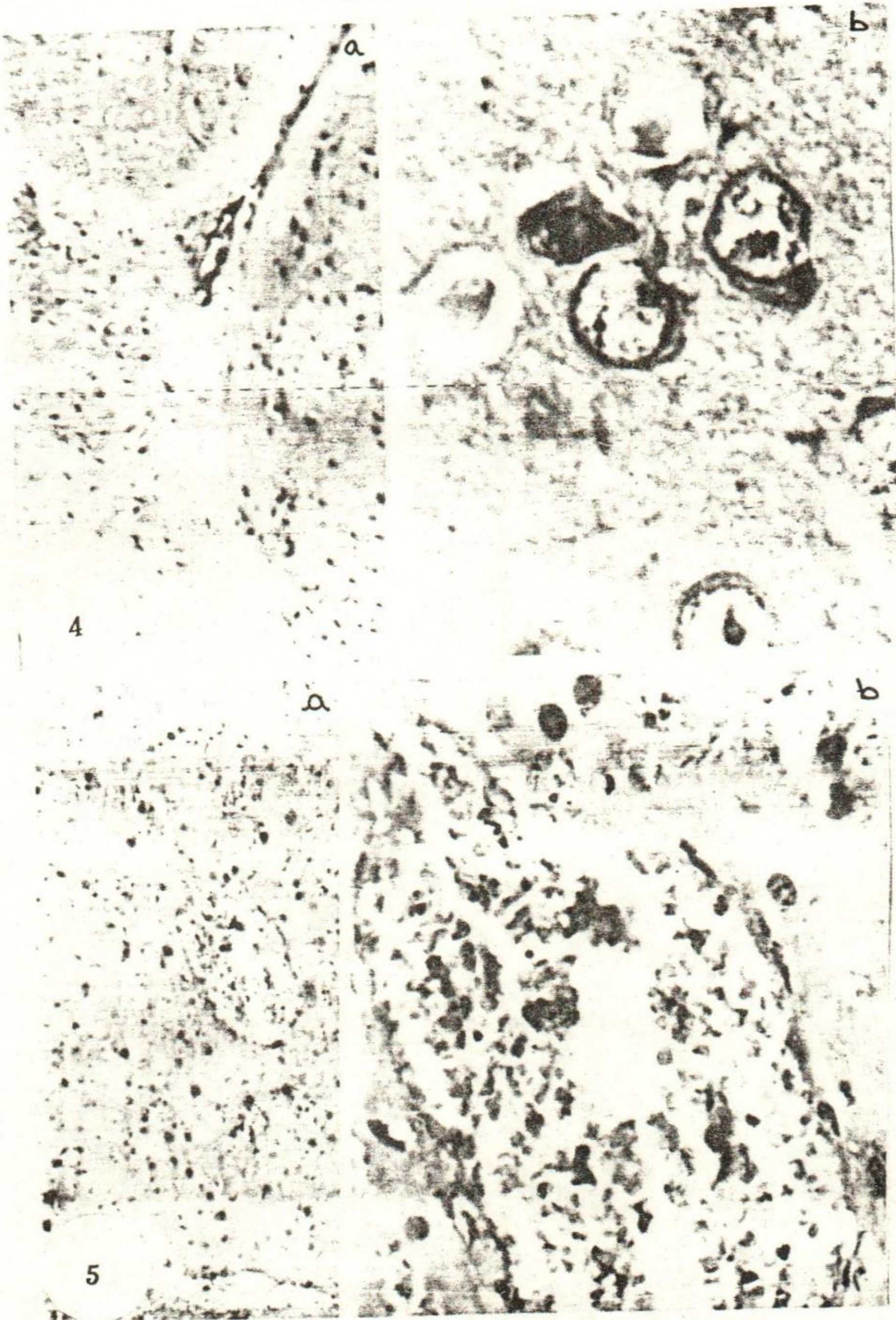
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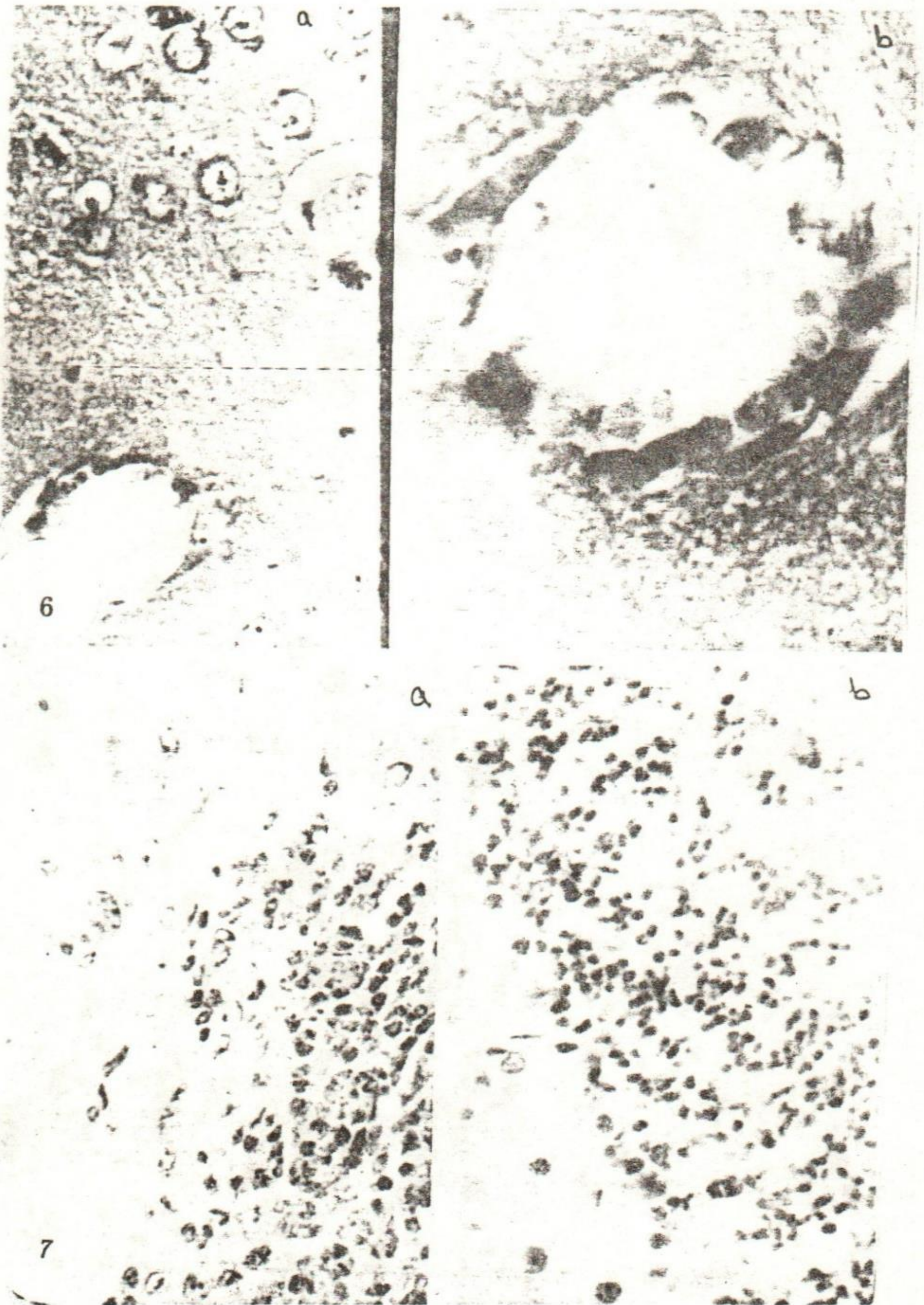
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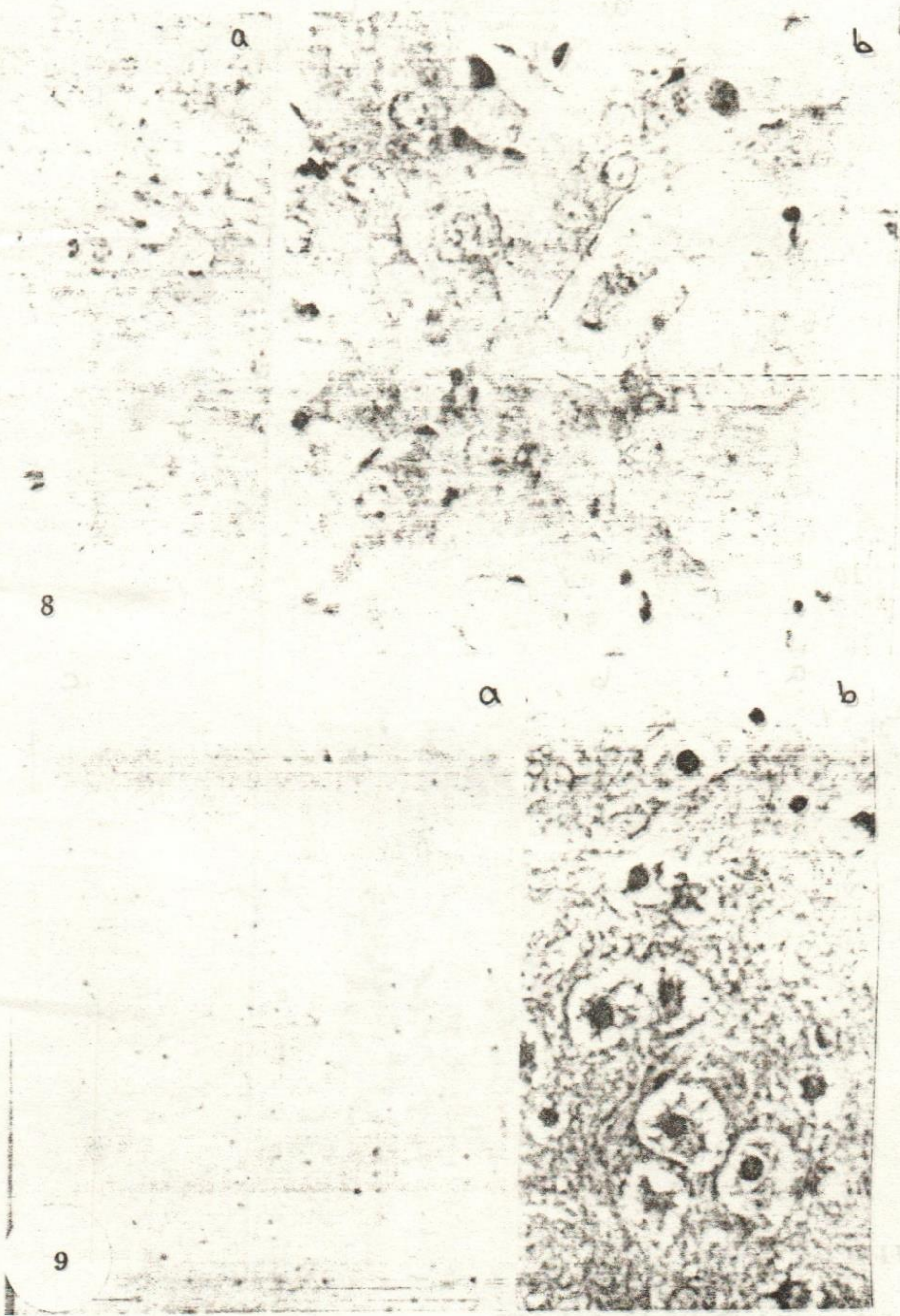
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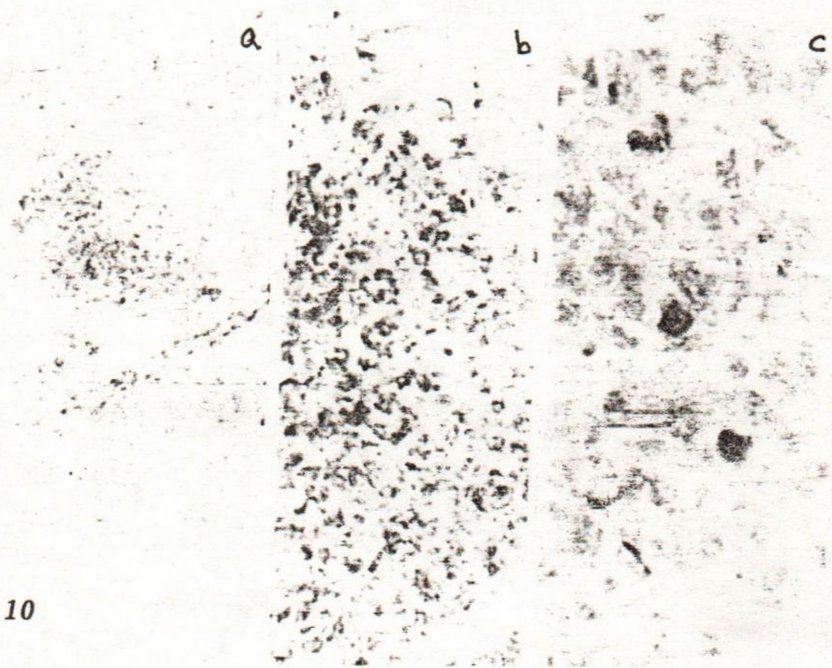
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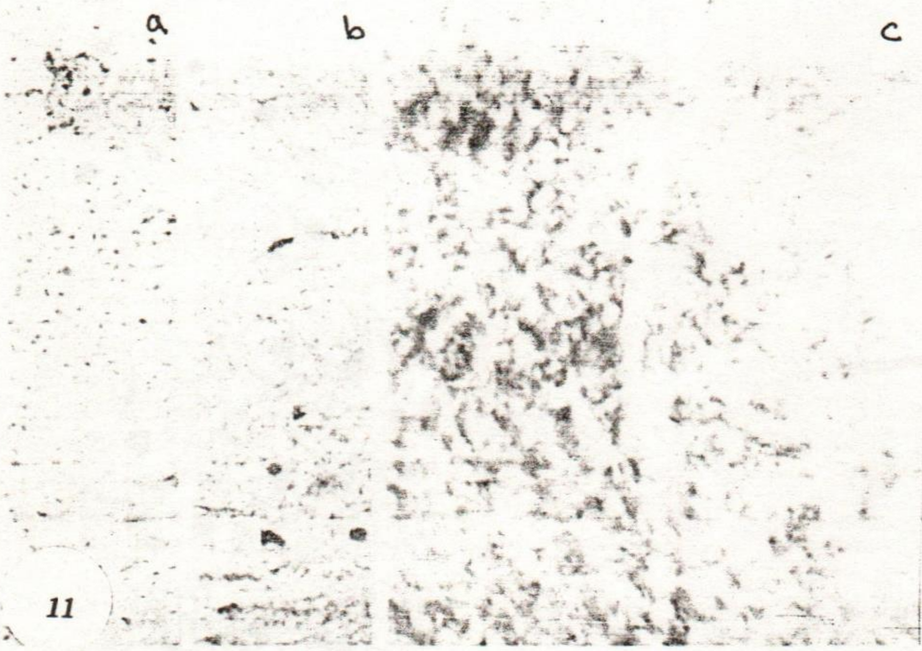
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