	Conventional	Minimal disease	Germ-free/ dibiotic	Autopsy material	Total
Late foetal	2	-	-	-	2
Mature foetal	1	-	-	-	1
Neo-nate	-	4	-	-	4
1 w.o.	-	3	-	-	3
1 m.o.	5	-	1/3	-	9
2 m.o.	1	-	-	4	5
3 m.o.	1	-	-	1	2
4 m.o.	1	-	-	2	3
6 m.o.	-	6	-	-	6
10 m.o.	2	-	-	-	2
1 y.o.	1	-	-	-	1
2 y.o.	1	-	-	-	1
4 y.o.	-	1	-	-	1
Aged 10-12 y.o.	4	-	-	-	4

Table III.1: Sources of material for tonsillar ontogenetic study.

	Conventional	Minimal disease	Germ-free/ dibiotic	Autopsy material	Total
Late foetal	2	-	-	-	2
Mature foetal	1	-	-	-	1
Neo-nate	-	4	-	-	4
1 w.o.	-	3	-	-	3
1 m.o.	5	-	1/3	-	9
2 m.o.	1	-	-	4	5
3 m.o.	1	-	-	1	2
4 m.o.	1	-	-	2	3
6 m.o.	-	6	-	-	6
10 m.o.	2	-	-	-	2
1 y.o.	1	-	-	-	1
2 y.o.	1	-	-	-	1
4 y.o.	-	1	-	-	1
Aged 10-12 y.o.	4	-	-	-	4

Table IV.1: Afferent lymphatic drainage following deposition of dyes and India ink at various sites about the head.

	Right Ear	Right Nasal Mucosa	Left Nasal Cavity	Right Rostrum	Left Rostrum	Right Palate	Left Palate	Right Buccal Mucosa
Tonsil	-	-	-	-	-	-	-	-
Submandibular lymph node	-	+	-	-	-	+	+	+
Retropharyngeal lymph node	-	+	-	-	-	-	-	-
Parotid lymph node	+	+	-	+	+	+	+	-
Dorsal Superficial Cervical lymph node	+	-	-	-	-	-	-	-
Ventral Cervical Chain	+	-	-	-	-	-	-	-

Table V.1: Location of India ink following topical application to the oral surface of the palatine tonsils.

Hours post-application	Pig	Crypt Lumen	Crypt Epithelium	Sub-epithelium	Interfollicular tissue	Germinal centres	Trabeculae	Capsule
Control	654	-	-	-	-	-	-	-
1½	652	+	-	-	-	-	-	-
4	659	+	-	-	-	-	-	-
8	660	+	+	-	-	-	-	-
16	22	+	-	-	-	-	-	-
24	30	+	+	-	-	-	-	-
48	48	+	+	+	+	+	+	+
72	52	+	+	+	+	+	-	-
96	54	+	+	+	+	+	-	-
	55	+	+	+	+	+	+	+
120	74	+	+	+	+	+	+	+
	75	+	+	+	+	+	-	-
144	81	-	+	+	+	+	+	+
	82	+	+	+	+	+	+	-
168	62	-	+	+	+	+	+	+
	63	-	+	+	+	+	+	-

Table VI.1: Record of rectal temperatures (°F).

	PM34/1	PM34/2	PM34/3	PM34/4	PM34/5	PM34/6
Day 1	102.4 102.0	102.8 102.4	102.8 102.4	103.4 102.9	102.4 102.9	102.4 103.6
2	102.2 102.6	102.5 102.1	102.1 102.4	103.2 103.6	101.6 101.8	101.8 103.0
3	102.0 103.6	102.5 102.9	102.4 102.3	100.8 101.2	101.9 103.0	102.6 102.8
4	102.6 103.5	102.6 102.1	101.7 103.2	101.0 101.0	102.2 102.3	102.2 102.3
5	102.0 102.8	101.6 102.2	102.9 103.7	102.2 102.4	102.0 102.4	101.8 103.6
6	101.8	102.2	101.4	102.8	102.6	103.0
7	102.0	102.0	102.4	102.4	102.6	101.5
8	102.0	101.8	102.0	102.5	103.0	102.8
9	102.1	101.8	102.8	102.2	102.4	102.4
10	-	-	-	-	-	-
11	102.6	102.2	102.3	102.8	101.8	102.0
12	102.1	102.0	105.8	101.4	102.0	101.6
13	102.8	103.2	103.8	102.6	103.2	102.8
14	101.8	103.2	104.3	102.8	102.2	102.2
15	103.0	103.4	105.4	102.6	103.0	102.8
16	103.2	103.4	102.8	102.4	102.6	101.4
17	103.0	103.2	104.2	103.2	103.4	102.4
18	103.4	103.2	103.9	102.4	102.9	102.6
19	103.4	104.1	105.6	103.2	104.2	102.1
20	103.8	103.4	105.2	103.8	102.2	103.1
21	103.0	103.2	105.0	103.1	103.4	103.0
22	102.9	103.2	102.8	103.4	102.4	103.2
23	102.4	102.6	101.4	102.4	102.4	102.6
24	-	-	-	-	-	-

Table VI.2: Recovery of Strep. suis from daily blood samples and tonsillar swabs.

	PM34/1		PM34/2		PM34/3		PM34/4		PM34/5		PM34/6	
	T	B	T	B	T	B	T	B	T	B	T	B
Day 1	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-	-	-	-
4	-	-	-	-	+	-	+	-	-	-	-	-
5	-	-	-	-	-	-	+	-	-	-	-	-
6	NS	-	NS	-	NS	-	NS	-	NS	-	NS	-
7	-	-	-	-	+	-	-	-	-	-	-	-
8	-	-	-	-	+	-	-	-	-	-	-	-
9	-	-	-	-	+	-	+	-	-	-	-	-
10	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
11	-	-	-	-	+	+	+	-	-	-	-	-
12	+	-	+	-	-	+	-	-	-	-	-	-
13	-	-	-	-	-	+	-	-	-	-	-	-
14	+	-	-	-	+	+	-	-	-	-	-	-
15	+	-	-	-	-	+	-	-	-	-	-	-
16	-	-	-	-	-	+	-	-	-	-	-	-
17	NS	NS	NS	NS	NS	+	NS	NS	NS	NS	NS	NS
18	+	-	-	-	+	+	+	-	-	-	-	-
19	+	-	0	-	0	+	+	-	-	-	-	-
20	+	-	-	-	-	+	+	-	-	-	-	-
21	-	-	-	-	-	-	-	-	-	-	-	-
22	-	-	-	-	-	-	-	-	-	-	-	-
23	-	-	-	-	+	+	+	-	-	-	-	-
24	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

T = Tonsillar swab.
B = Blood sample.

\+ = Recovery of Strep. suis.
\- = No recovery of Strep. suis.
NS = Not sampled.
0 = Overgrown.

Table VI.3: Recovery of Streptococcus suis at post-mortem examination.

	PM34/1	PM34/2	PM34/3	PM34/4	PM34/5	PM34/6
Tonsil swab	-	+	+	-	-	-
Tonsil	+	0	+	+	-	0
Pharyngeal swab	-	-	-	-	-	-
Nasal swab	-	-	-	-	-	-
Submandibular lymph node	+	+	+	+	-	-
Retropharyngeal lymph node	-	+	-	-	-	-
Parotid lymph node	-	+	-	-	-	-
Spleen	-	-	+	-	-	-
Blood	-	-	-	-	-	-
CSF	-	-	+	-	-	-
Carpus	-	-	-	-	-	-
Tarsus	-	-	+	-	-	-
Abscess			+			

\+ = Recovery of Strep. suis.

\- = No recovery of Strep. suis.

0 = Overgrown.

Table VI.4: Localisation by immunofluorescent examination of *Strep. suis* antigen in the tonsils.

	PM34/1	PM34/2	PM34/3	PM34/4	PM34/5	PM34/6
Lumen	+	+	+	+	-	-
Epithelium	+	+	+	+	-	-
Subepithelium	+	+	+	+	-	-
Interfollicular lymphoid tissue	+	+	+	+	-	-
Germinal centres	(+)	(+)	(+)	(+)	-	-

\+ = Antigen present.

(+) = Antigen infrequently observed.

\- = Antigen absent.

Table VIII.1: Record of rectal temperatures (°F).

	PM34/21	PM34/22	PM34/23	PM34/24
Day 1	104.0	104.2	103.0	103.2
2	104.2	104.8	104.4	105.0
3	103.2	102.6	102.6	103.8
4	103.0	103.0	103.4	102.8
5	-	-	-	-
6	103.6	103.8	103.2	103.2 (†)
7	102.6	103.8	102.0	
8	102.0	102.6	102.0	
9	101.8	102.2	103.0	

\- = Not taken.

(†) = Pig died.

Table VIII.2: Recovery of Strep. suis from tonsillar swabs.

	PM34/21	PM34/22	PM34/23	PM34/24
Day 1	-	-	-	-
2	-	-	+	-
3	+	-	-	-
4	NS	NS	NS	NS
5	+	-	+	+
6	+	-	+	(0)
7	-	+	-	
8	+	-	-	
9	+	-	+	
10 - 20	NS	NS	NS	
21	+	-	+	
22	+	-	+	
23 - 38	NS	NS	NS	

+ = Recovery of Strep. suis.
- = No recovery of Strep. suis.
NS = Not sampled.
+
(0) = Pig died.

Table VIII.3: Recovery of Strep. suis at post-mortem examination.

	PM34/21	PM34/22	PM34/23
Tonsil	+	+	+
Submandibular lymph node	+	+	+
Retropharyngeal lymph node	-	+	-
Parotid lymph node	-	-	-
Spleen	-	-	-
Blood	-	-	-
CSF	-	-	-
Carpus	-	-	-
Tarsus	-	-	-

Table A.1: Record of rectal temperatures (^{o}F).

	PM34/10	PM34/11	PM34/12	PM34/13	PM34/14	PM34/15	PM34/16	PM34/17	PM34/18	PM34/19	PM34/20
Day 1	102.4	103.0	102.6	102.8	103.0	102.2	103.2	102.4	102.8	101.8	103.2
2	102.6	102.2	102.6	103.2	102.8	102.4	107.0	103.6	102.4	102.2	103.2
3	102.2	103.4	103.0	105.6	107.6 (†)	102.8	107.2	103.0	104.0	102.6	104.2
4	103.0	102.6	104.0	105.6		105.6	106.4	103.0	103.2	103.0	103.0

(†) = Pig died.

Table A.2: Recovery of Strep. suis from daily tonsillar swabs and blood samples.

	PM34/10		PM34/11		PM34/12		PM34/13		PM34/14		PM34/15		PM34/16		PM34/17		PM34/18		PM34/19		PM34/20	
	T	B	T	B	T	B	T	B	T	B	T	B	T	B	T	B	T	B	T	B	T	B
Day 1	+	-	NS	-	+	-	NS	-	+	-	NS	-	+	NS	NS	-	-	-	NS	-	-	-
2	NS	-	+	-	NS	+	+	+	NS	-	+	-	NS	+	x	-	NS	-	x	-	NS	-
3	NS	-	NS	+	NS	+	NS	+	NS	+ (†)	NS	+	NS	NS	NS	-	x	-	-	-	-	-
4	NS	+	NS	NS	NS	NS	NS	NS			NS	NS	NS	NS	NS	-	-	-	-	-	x	-

\+ = Recovery of Strep. suis.

\- = No recovery of Strep. suis.

x = Recovery of cross-reacting organism.

NS = Not sampled/examined.

(†) = Pig died.

Fig. II.1: Piglet accommodation. Individual heated straw bale pen.

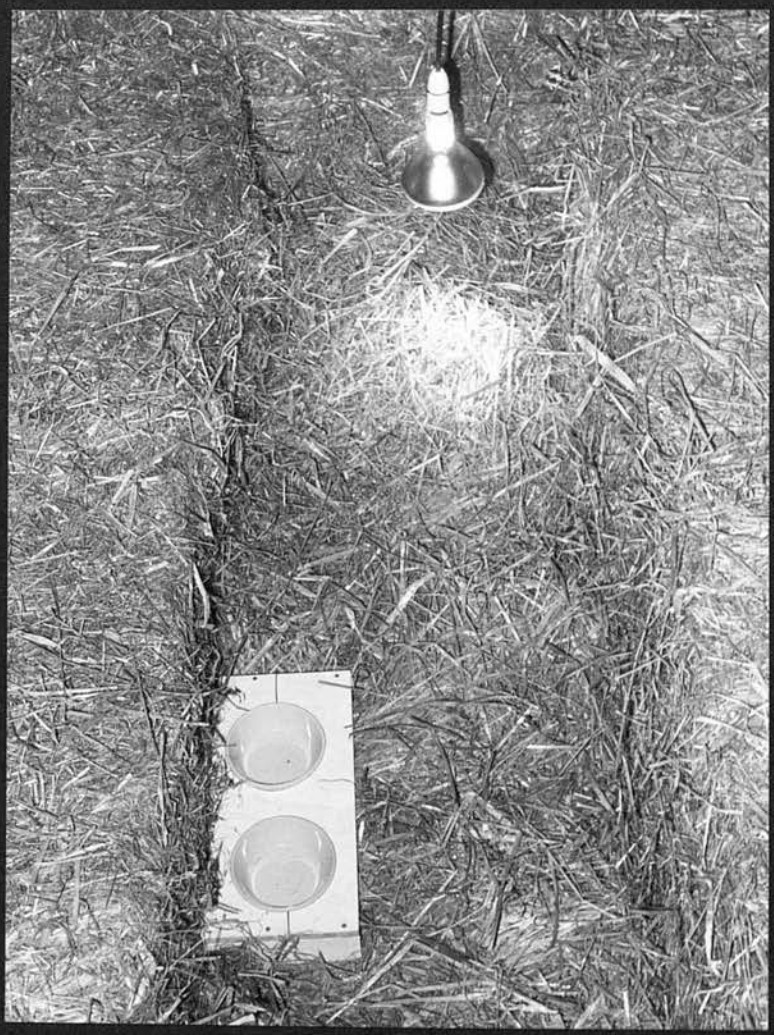


Fig. II.2: Ventral aspect of head and neck. Skin reflected to show submandibular lymph nodes (A).

Fig. II.3: Ventral aspect of head and neck. Skin and sterno-thyro-hyoideus muscle reflected to show submandibular (A) and retropharyngeal (B) lymph nodes.


A
A

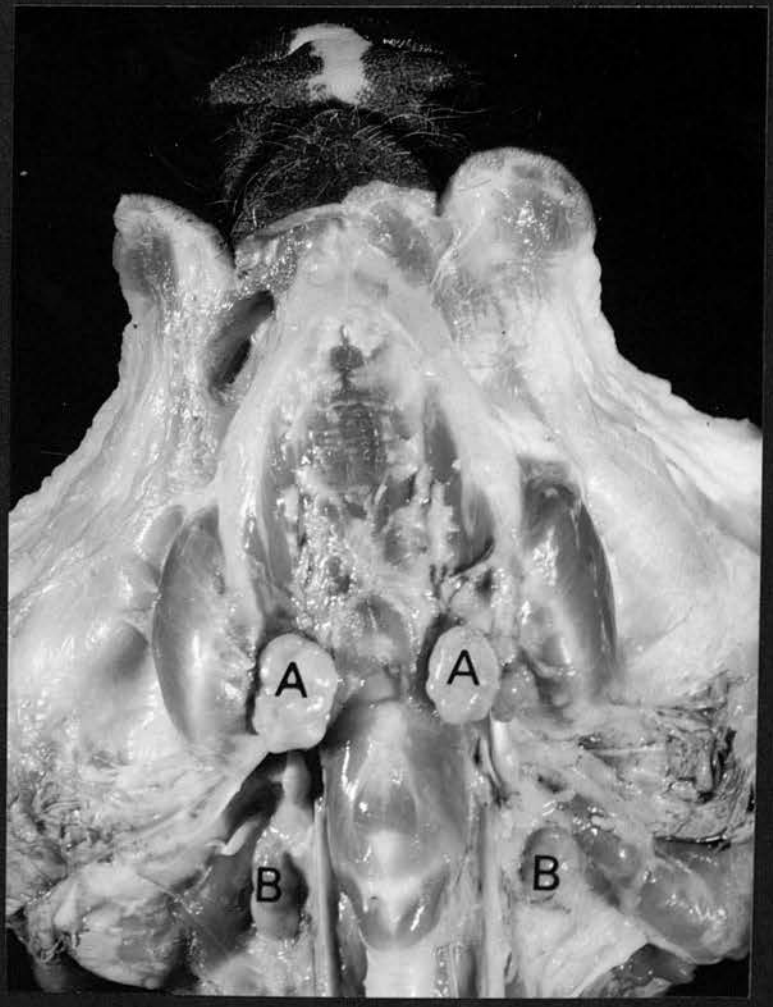

A
A
B
B

Fig. II.4: Ventral aspect of head and neck. Skin reflected, tongue, larynx and trachea excised to show submandibular (A) and retropharyngeal (B) lymph nodes and the palatine tonsils (C).

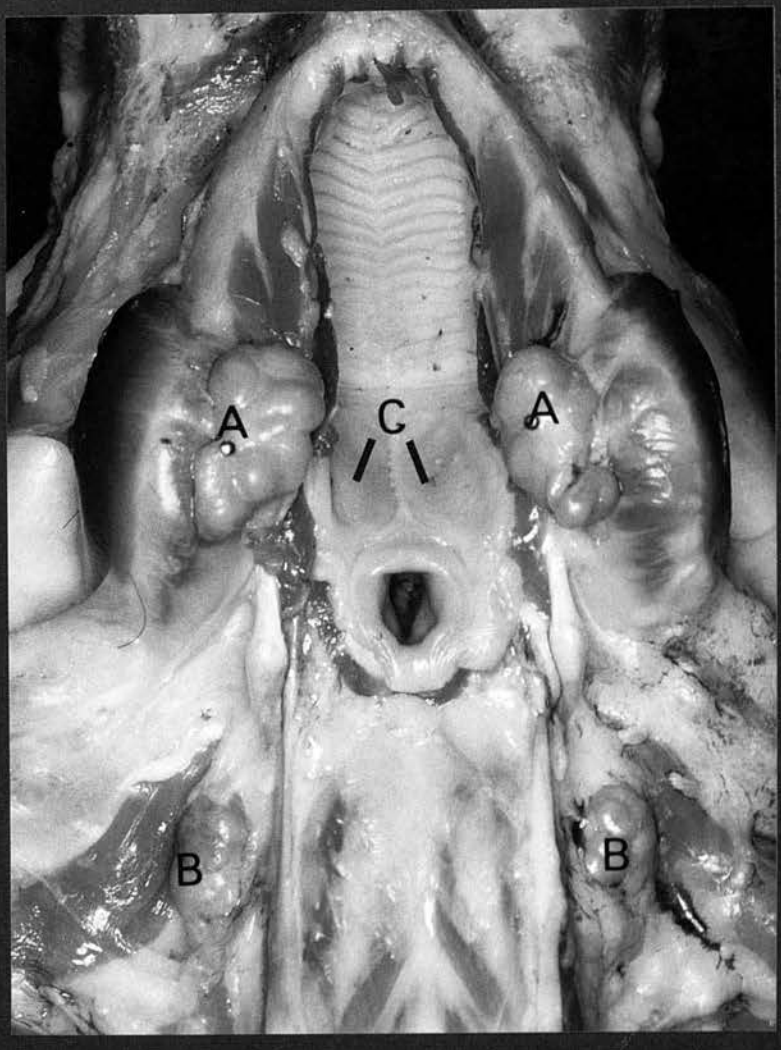

A
C
A
B
B

Fig. II.5: Capillary microprecipitin test. Specific reaction (right tube) at interface of PM34/A antiserum and *Streptococcus suis* antigen.

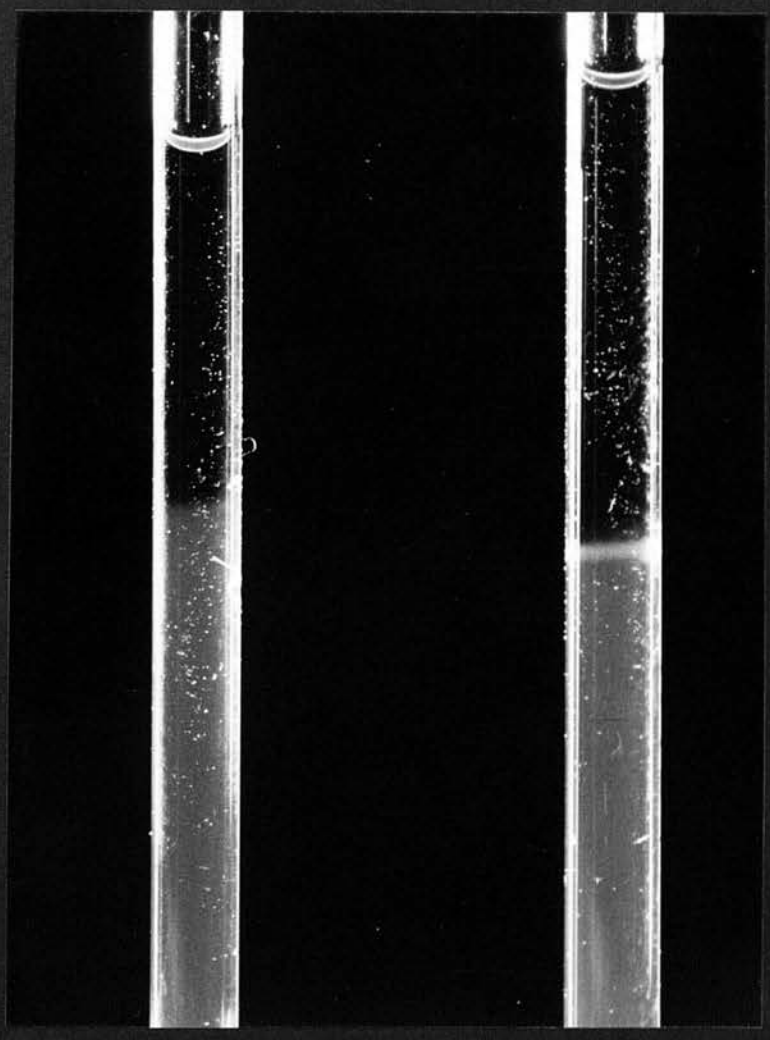
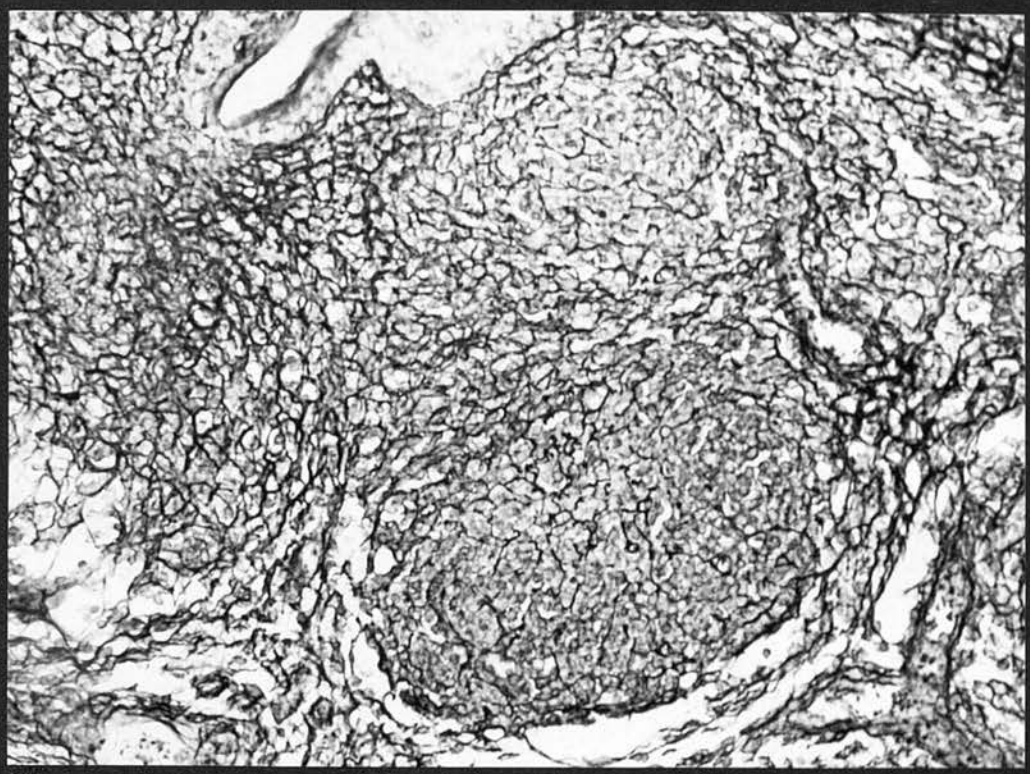


Fig. III.1: Tonsil, late foetus. Reticulin network illustrating follicular development.
Slidders' reticulin silver impregnation. x 250.

Fig. III.2: Tonsil, late foetus. Follicular lymphocytic cups related to crypts.
Haematoxylin and Eosin. x 200.



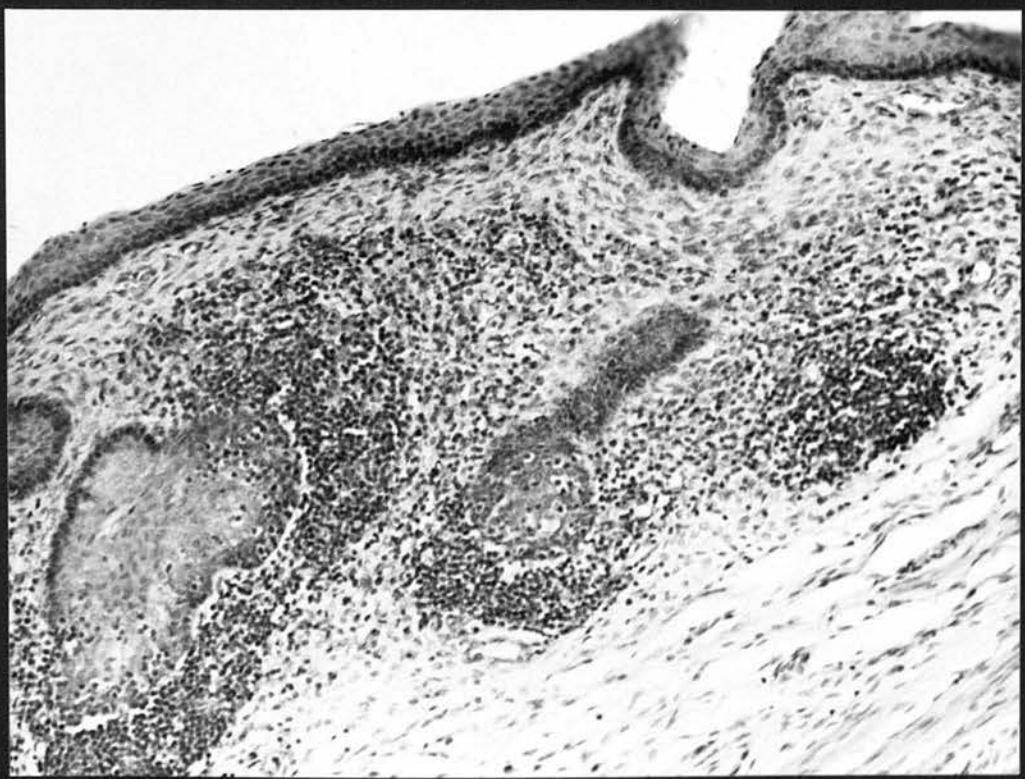


Fig. III.3: Tonsil, late foetus. Interfollicular tissue lightly populated by lymphocytic and large, pale-nucleated cells.

H & E. x 200.

Fig. III.4: Tonsil, full-term foetus. Crypts showing rudimentary branching.

H & E. x 125.

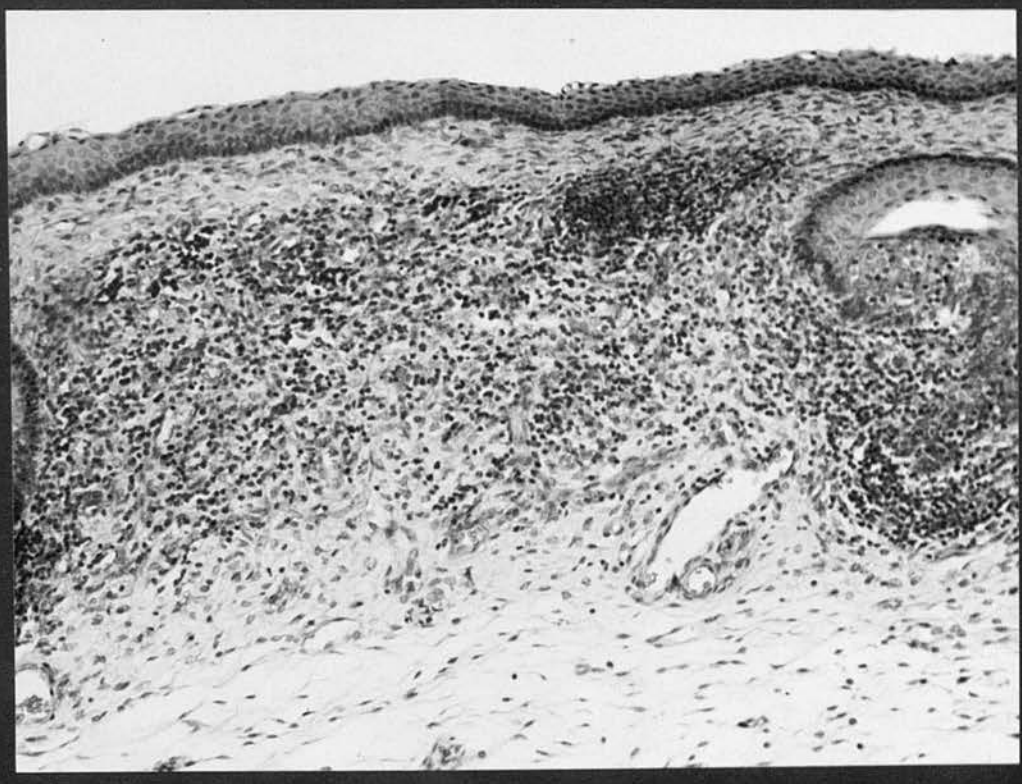
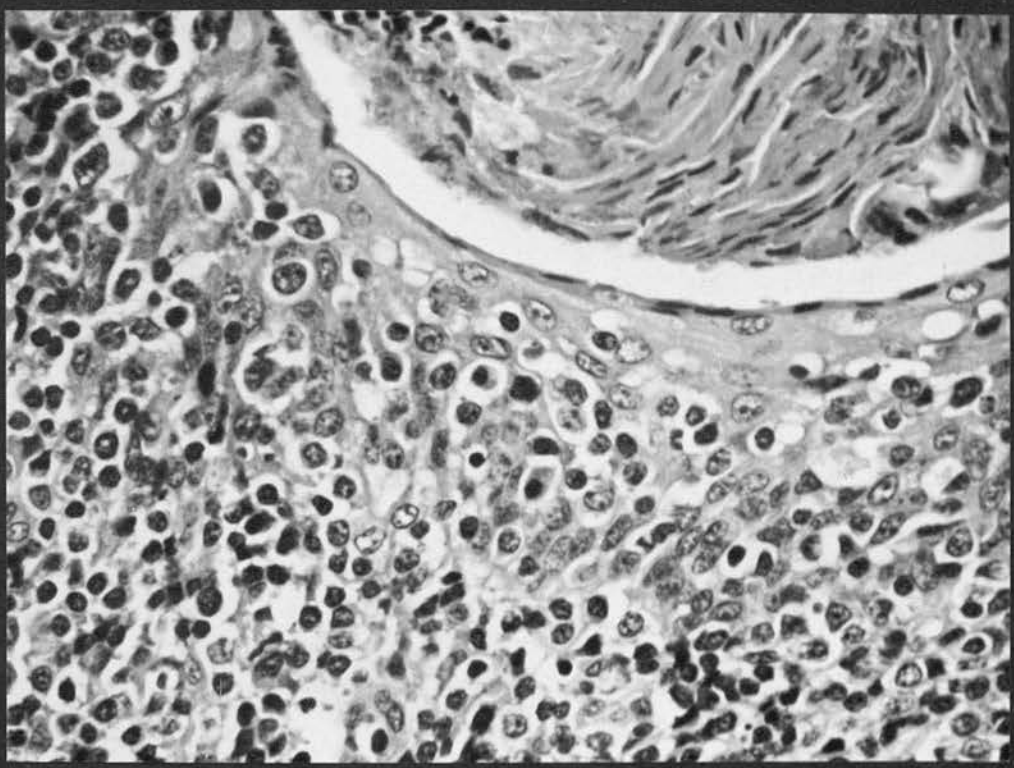


Fig. III.5: Tonsil, neo-natal piglet. Interfollicular tissue showing increased cellular population. H & E. x 125.

Fig. III.6: Tonsil, 1 w.o. piglet. Tonsillar depth substantially increased but density of interfollicular cellular population similar to Fig. III.5.
H & E. x 125.

Fig. III.7: Tonsil, 1 m.o. pig. Crypt epithelium infiltrated with plasma cells.
H & E. x 625.

Fig. III.8: Tonsil, 1 m.o. pig. Reticulin network delineating numerous germinal centres.
Retic. x 100.



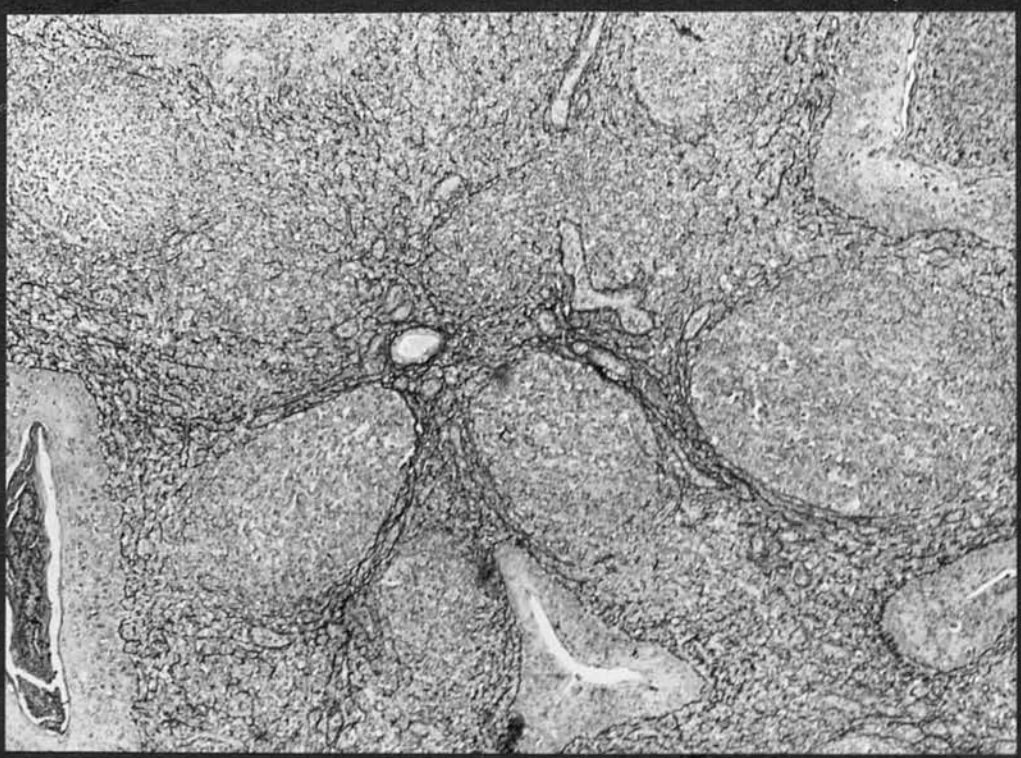
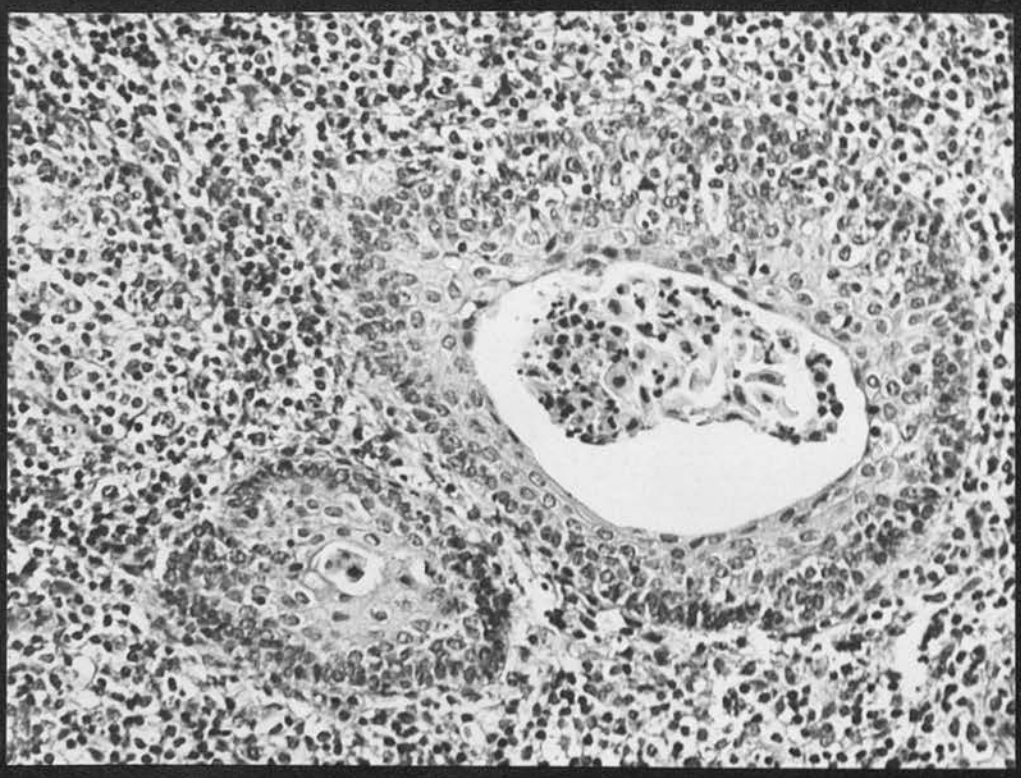


Fig. III.9: Tonsil, 1 m.o. pig. Germinal centre cells showing pyroninophilia.

Methyl-green/pyronin. x 220.

Fig. III.10: Tonsil, 1 m.o. germ-free pig. Crypt epithelium showing reticulation and cellular infiltration. H & E. x 250.

Fig. III.11: Tonsil, 1 m.o. germ-free pig. Crypt epithelium showing infiltrated plasma cells (arrows). H & E. x 625.



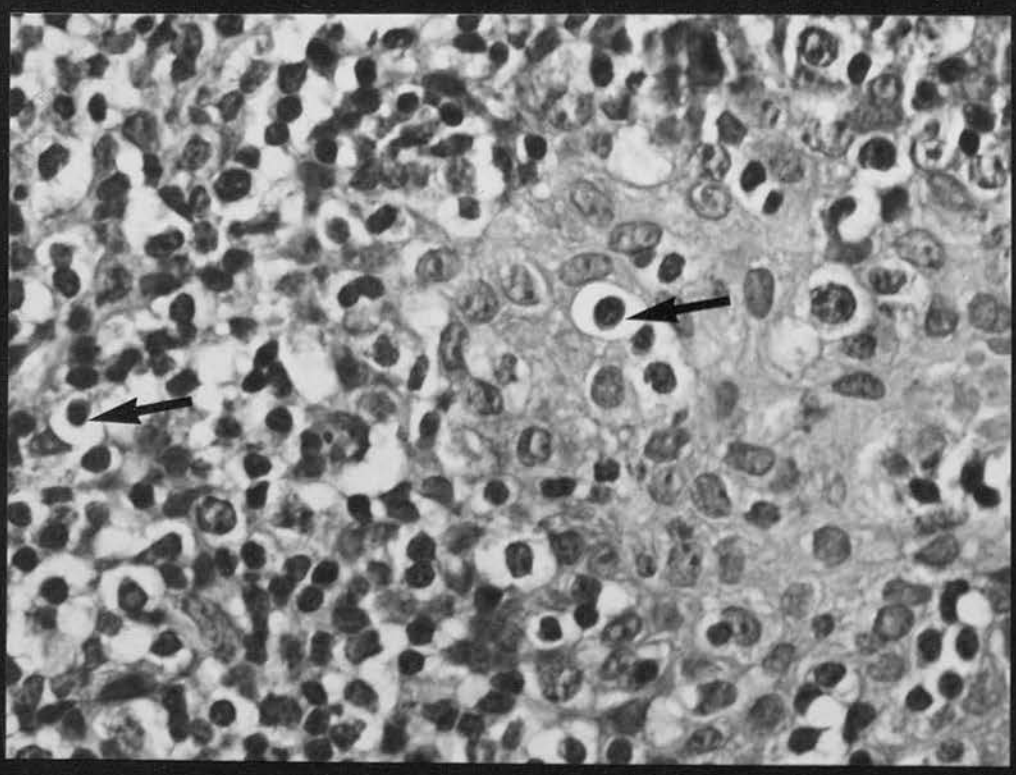


Fig. III.12: Tonsil, 1 m.o. germ-free pig. Reticulin network depicting numerous germinal centres.
Retic. x 80.

Fig. III.13: Tonsil, 1 m.o. germ-free. Germinal centre containing macrophages, lymphocytes, lymphoblasts and cellular fragments.
H & E. x 500.

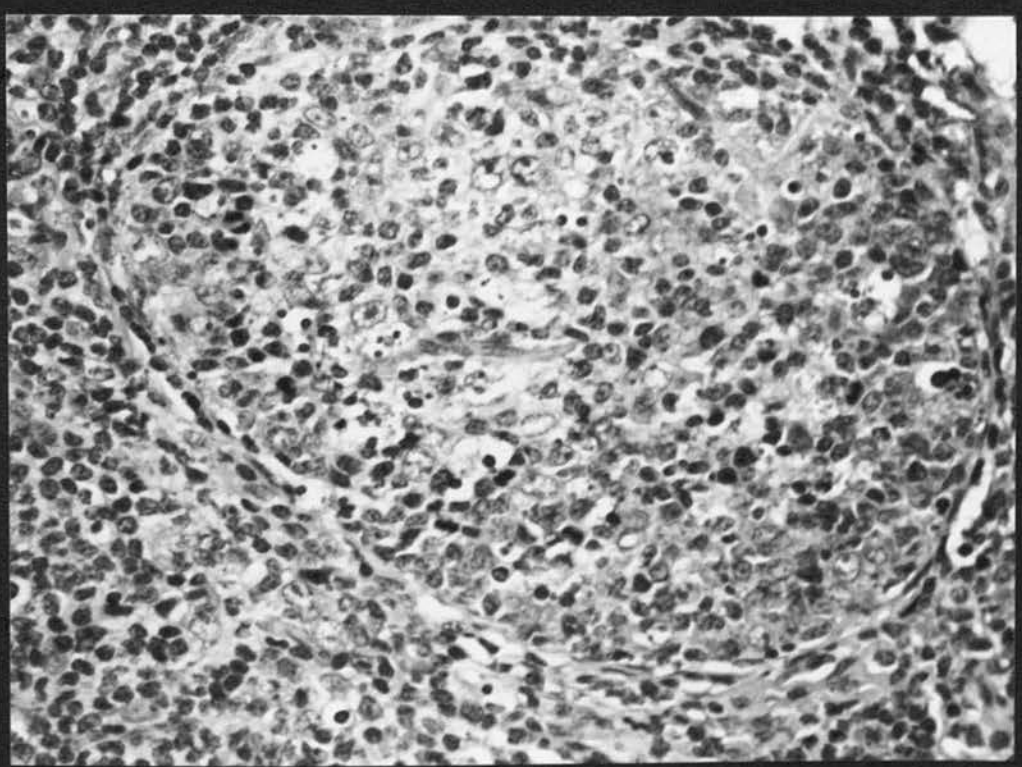
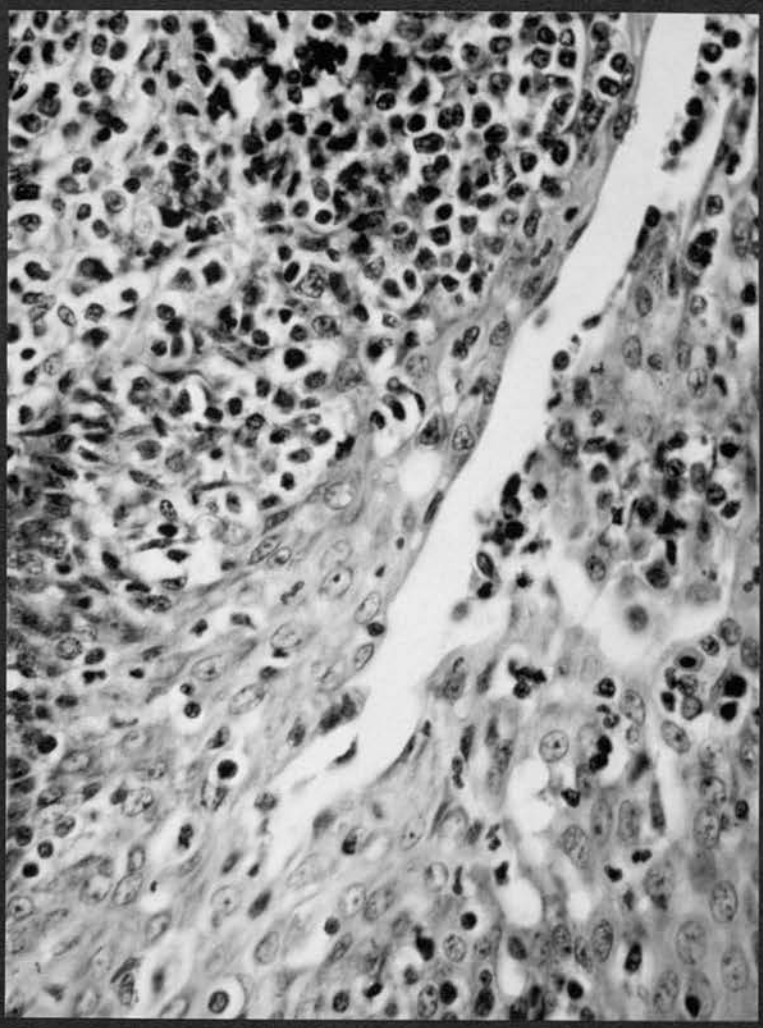


Fig. III.14: Tonsil, 2 m.o. pig. Reticulation of crypt epithelium and infiltration with lymphocytes and plasma cells.
H & E. x 500.

Fig. III.15: Tonsil, 2 m.o. pig. Germinal centre showing mitotic figures.
H & E. x 500.



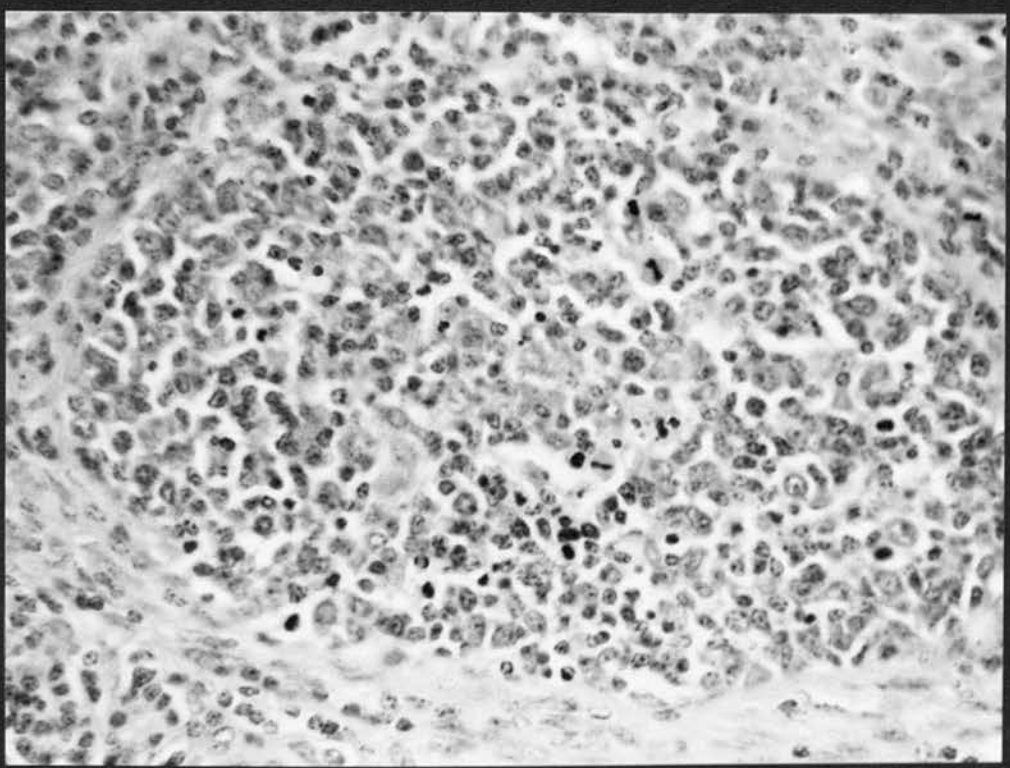


Fig. III.16: Tonsil, 2 m.o. pig. Reticulin meshwork clearly defining germinal centres.

Retic. x 100.

Fig. III.17: Tonsil, 2 m.o. pig. Crypt epithelium and subepithelial zone containing plasma cells (arrows).

Methyl-green/pyronin. x 440.

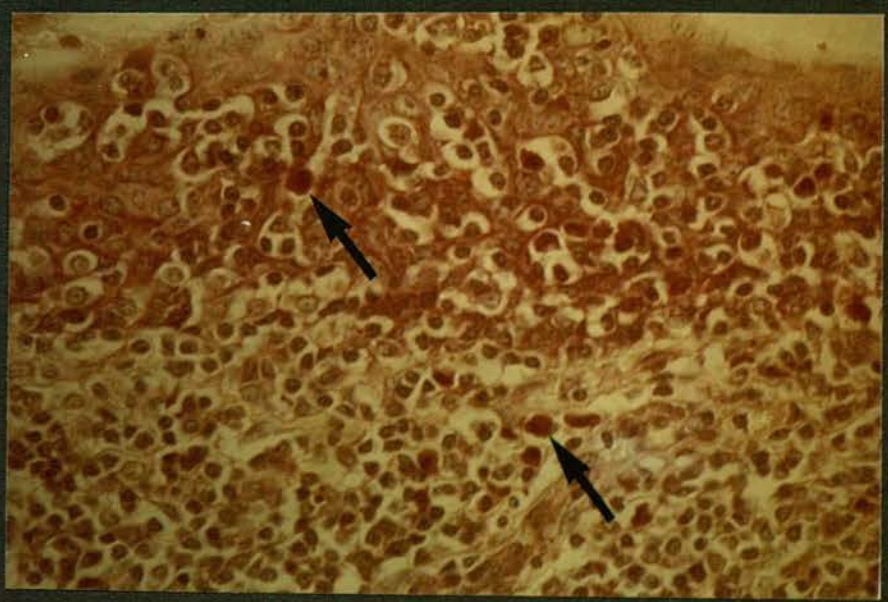


Fig. III.18: Tonsil, 1 y.o. pig. Interfollicular tissue extensive compared with germinal centres.
Retic. x 100.

Fig. III.19: Tonsil, 4 y.o. pig. Germinal centres showing overall decrease in size.
Retic. x 100.

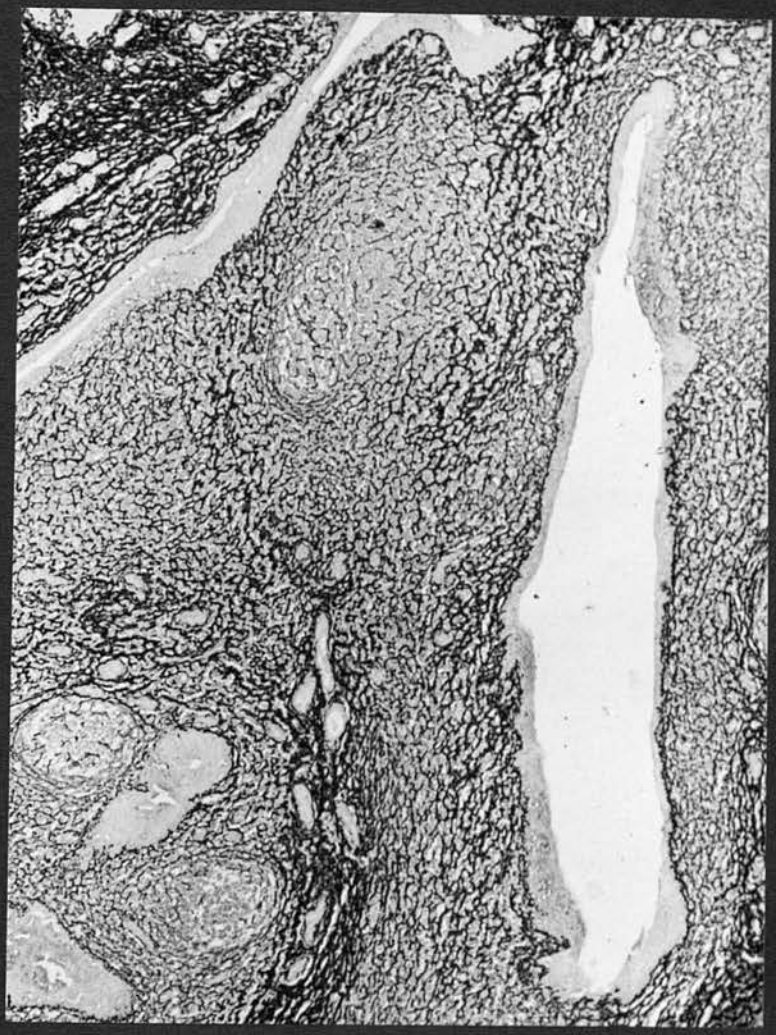
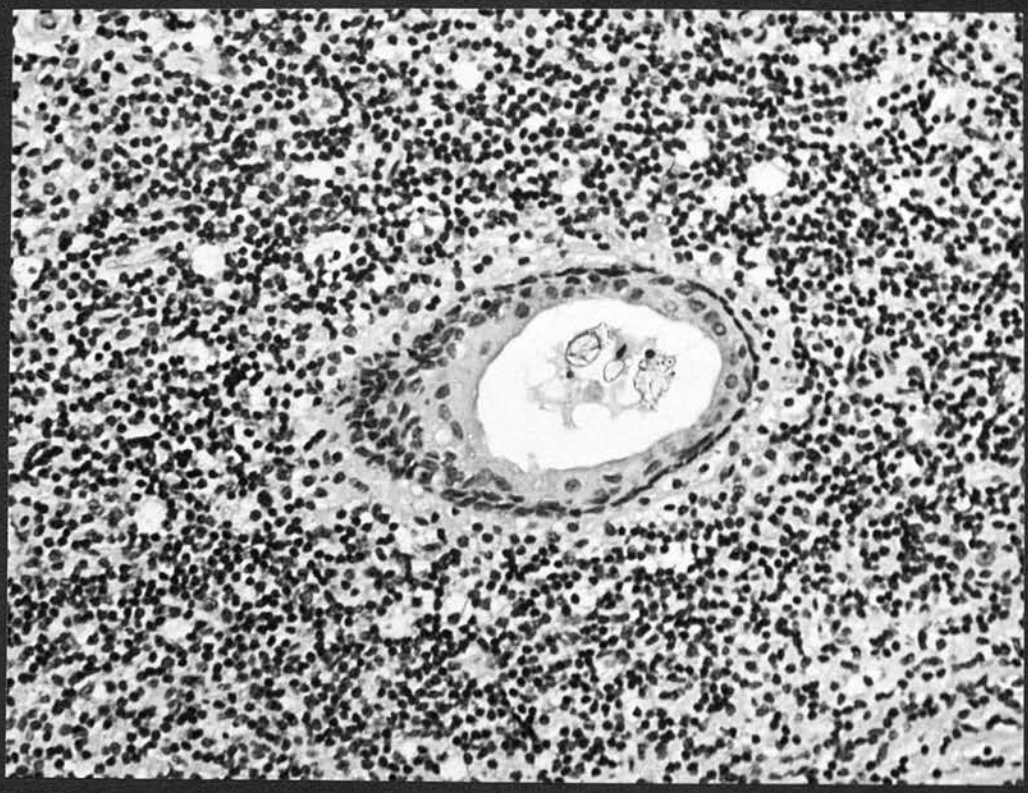


Fig. III.20: Tonsil, aged pig. "Pavement" cells in basal germinal layer, and reduction in thickness of crypt epithelium.
H & E. x 250.

Fig. III.21: Tonsil, aged pig. Crypt epithelium showing reticulation and cellular infiltration.
H & E. x 250.



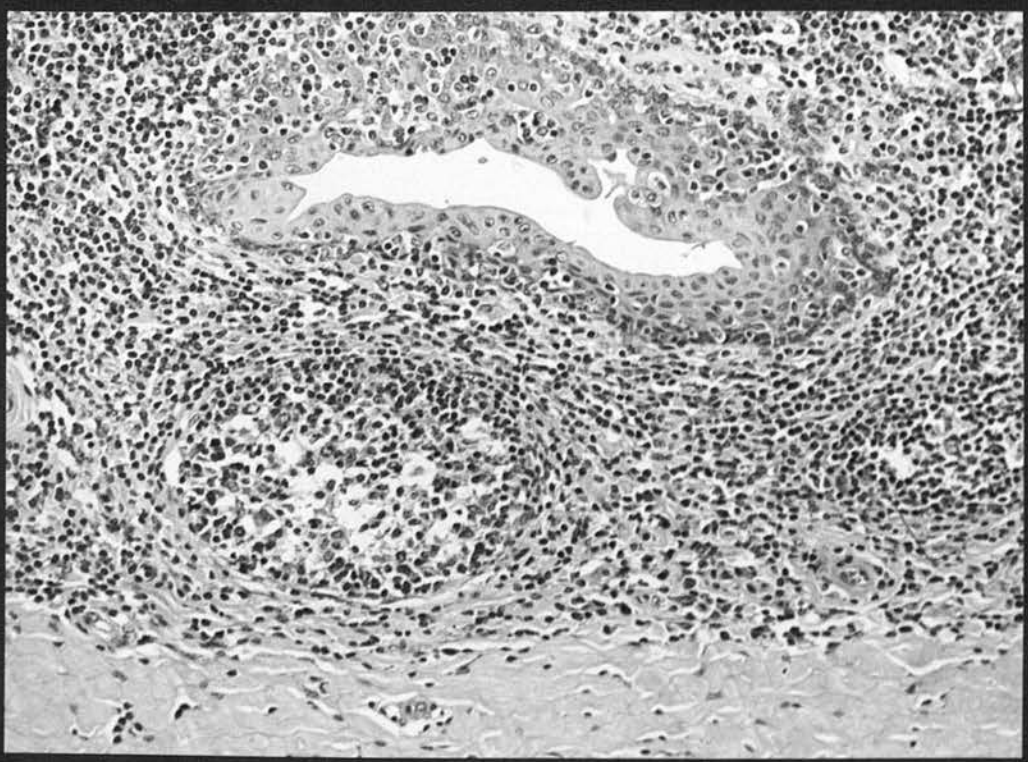


Fig. III.22: Tonsil, aged pig. Connective tissue stroma markedly increased.

H & E. x 80.

Fig. IV.1: Latero-ventral aspect of head. Reflection of skin to show afferent lymphatic vessel (A) leading to parotid lymph node from dye injection site (B) in rostrum.

Fig. IV.2: Palatine tonsils and submandibular lymph nodes. Tonsils unstained but submandibular lymph nodes containing India ink and dye following injection of these into the buccal mucosa and anterior hard palate.

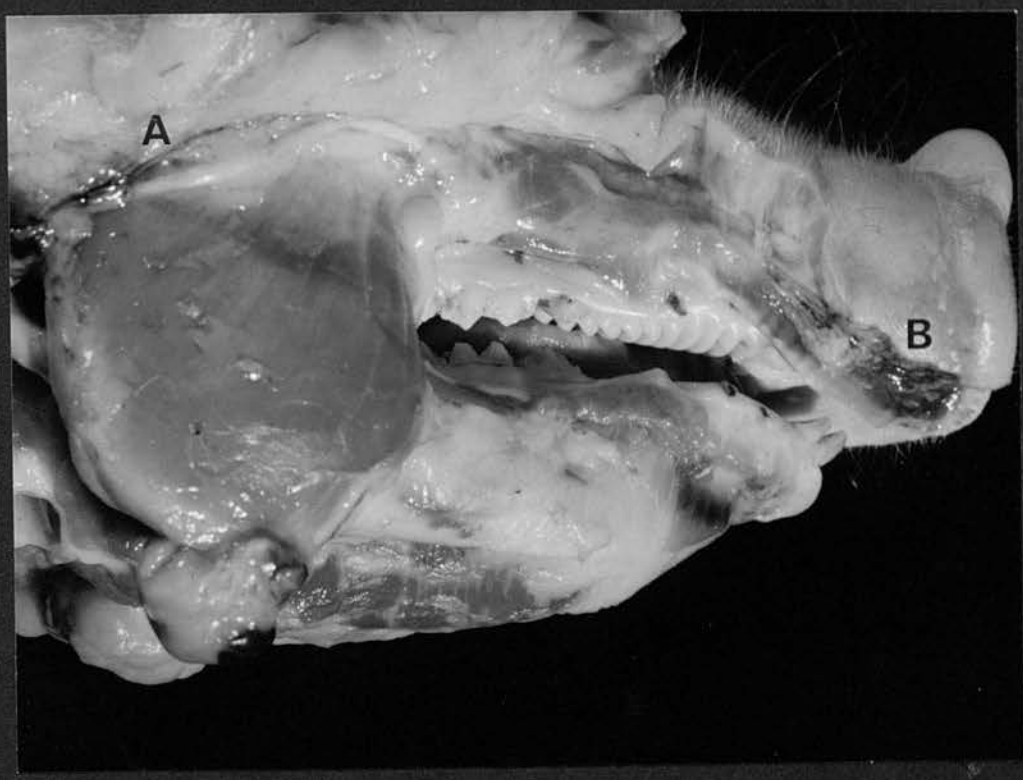

A
B

Fig. IV.3: Ventral aspect of head and neck. Tongue and upper respiratory tract excised to show site of intra-tonsillar injection and drainage of dye to submandibular, retropharyngeal and parotid lymph nodes.

Fig. IV.4: Tonsil. Vascular bed perfused with India ink. H & E. x 25.

Fig. IV.5: Tonsil. Vascular bed perfused with India ink, illustrating blood supply to interfollicular tissue and germinal centres.

H & E. x 125.

Fig. IV.6: Tonsil. Vascular bed perfused with India ink, showing only blood vessels in interfollicular tissue.

Retic. x 80.

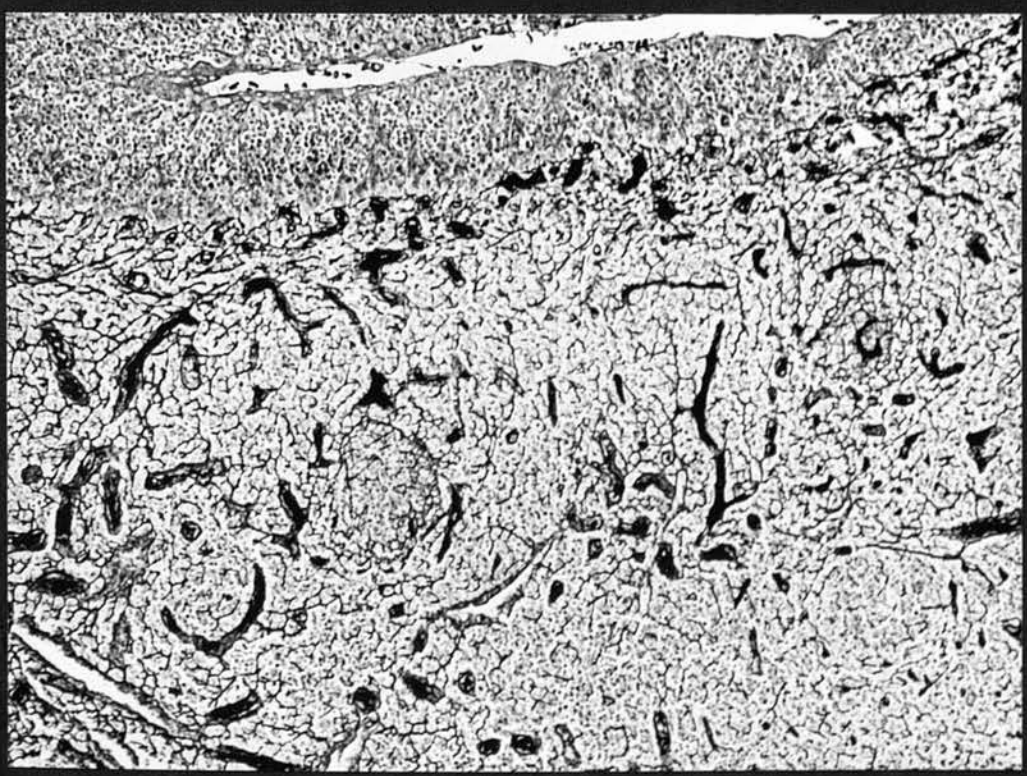
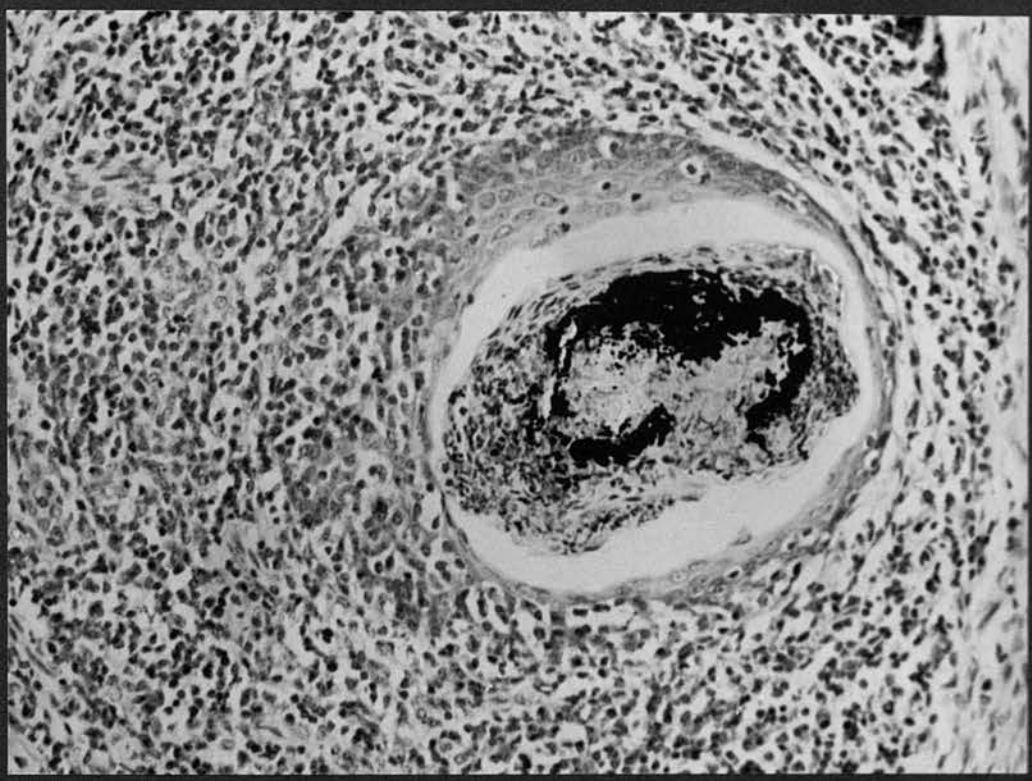


Fig. V.1: Tonsil. India ink in crypt lumen following topical application.

Haematoxylin & Tartrazine. x 125.

Fig. V.2: Tonsil. Cells of crypt epithelium containing India ink particles.

H & Tart. x 625.



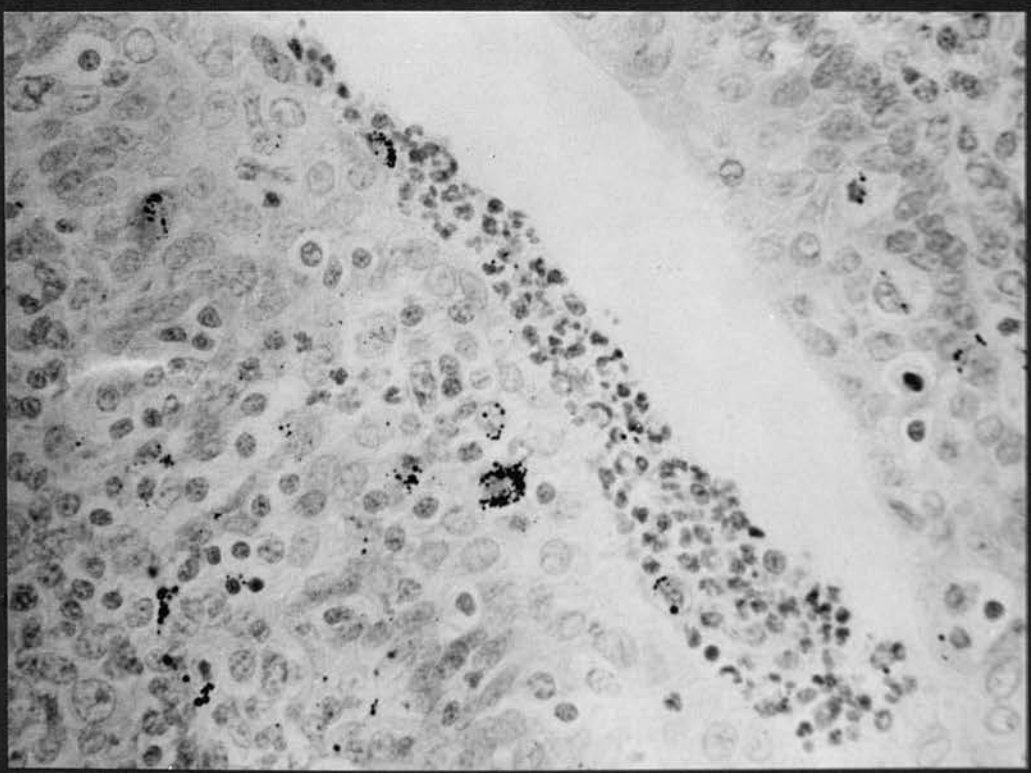


Fig. V.3: Tonsil. India ink in crypt lumen, epithelium and extending into interfollicular tissue. H & Tart. x 140.

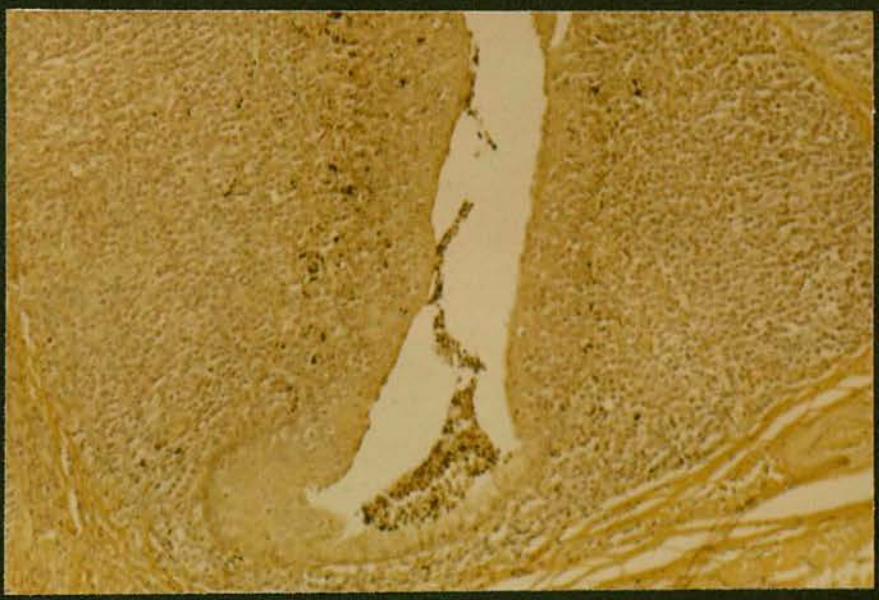
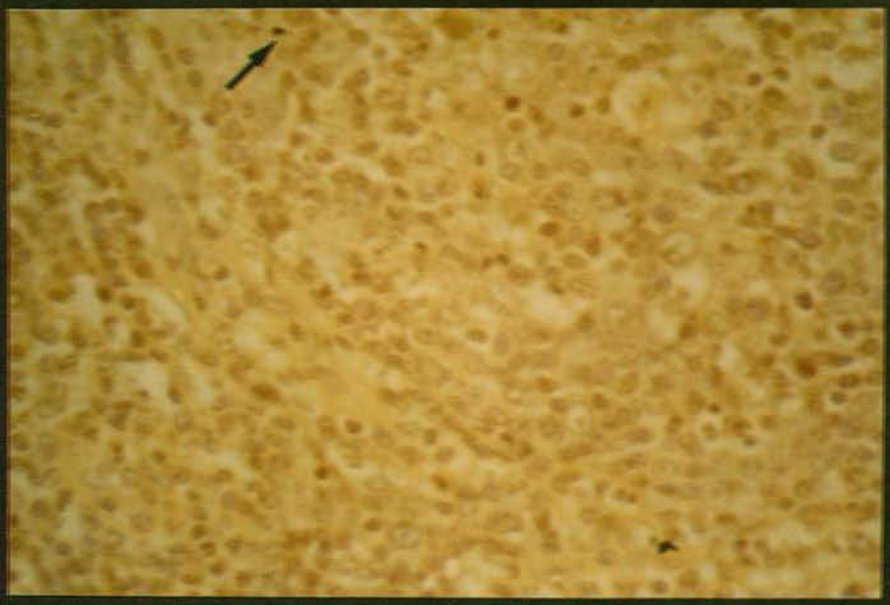


Fig. V.4: Tonsil. Germinal centres with intracellular India ink particles (arrows).

H & Tart. Top x 440.

Bottom x 600.



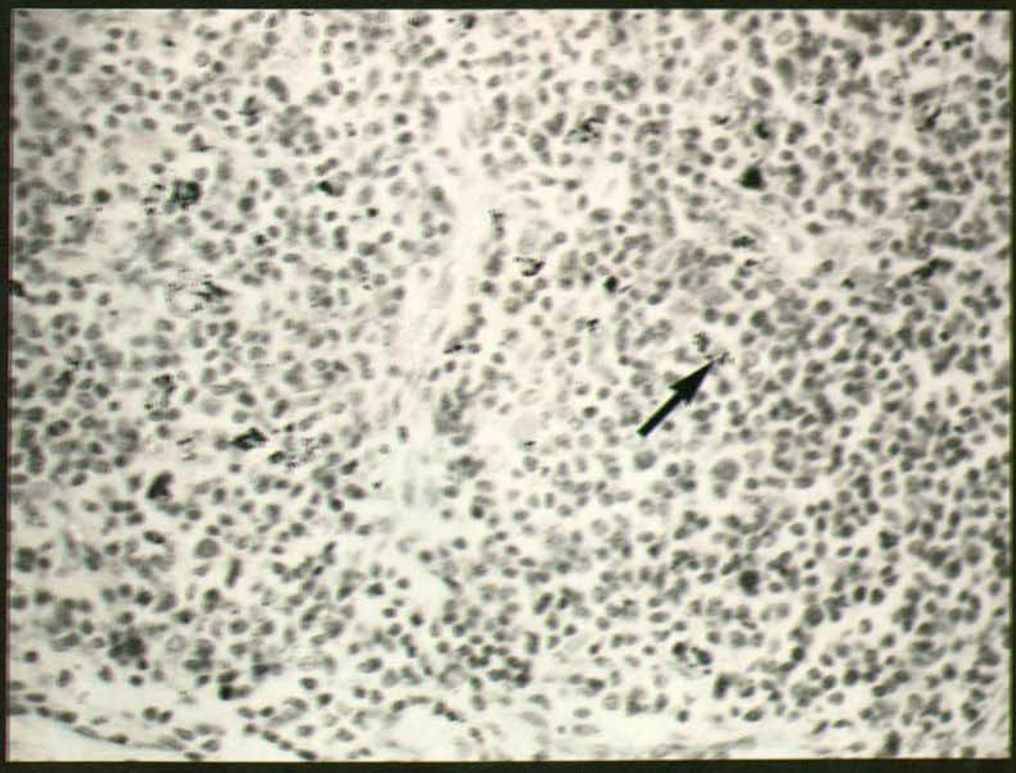


Fig. V.5: Tonsil. India ink in trabeculae and aboral capsule adjacent to ink-positive interfollicular tissue.

H & Tart. x 800.

Fig. V.6: India ink particles.

Unstained. x 35,000.

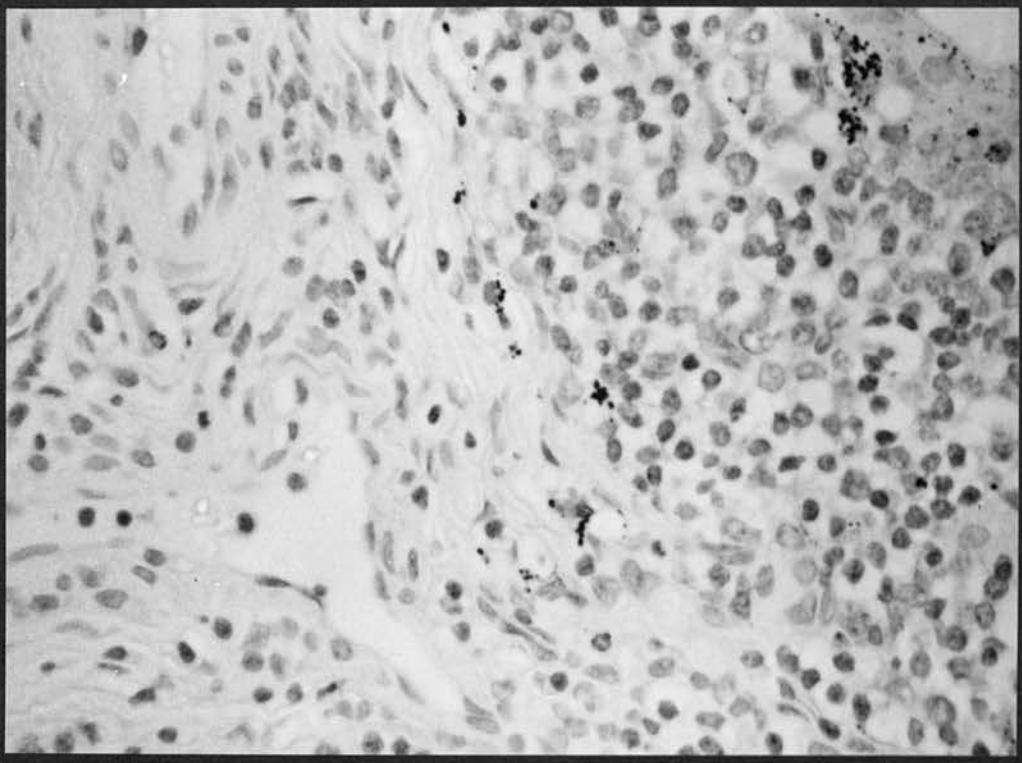
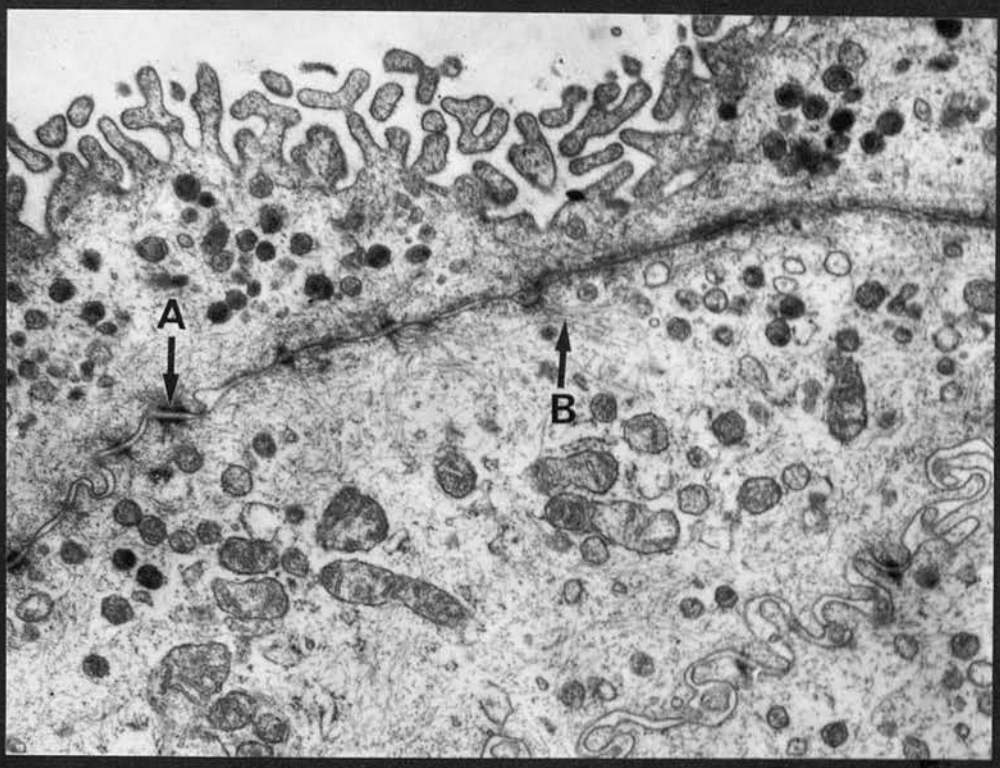


Fig. V.7: Tonsil. Microvilli on free luminal surface of crypt epithelium. Desmosomes (A) and tonofilaments (B).
Uranyl acetate, lead citrate. x 23,000.

Fig. V.8: Tonsil. Crypt epithelial cell showing desmosomes and tonofilaments.
Uranyl acetate, lead citrate. x 18,000.


A
B

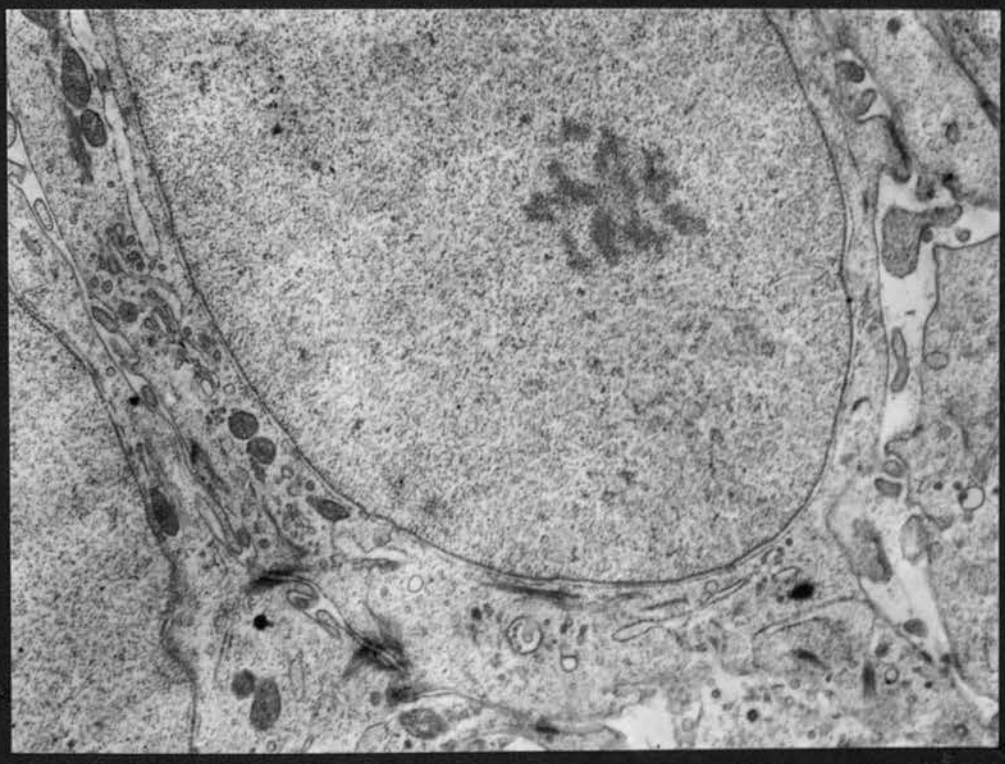
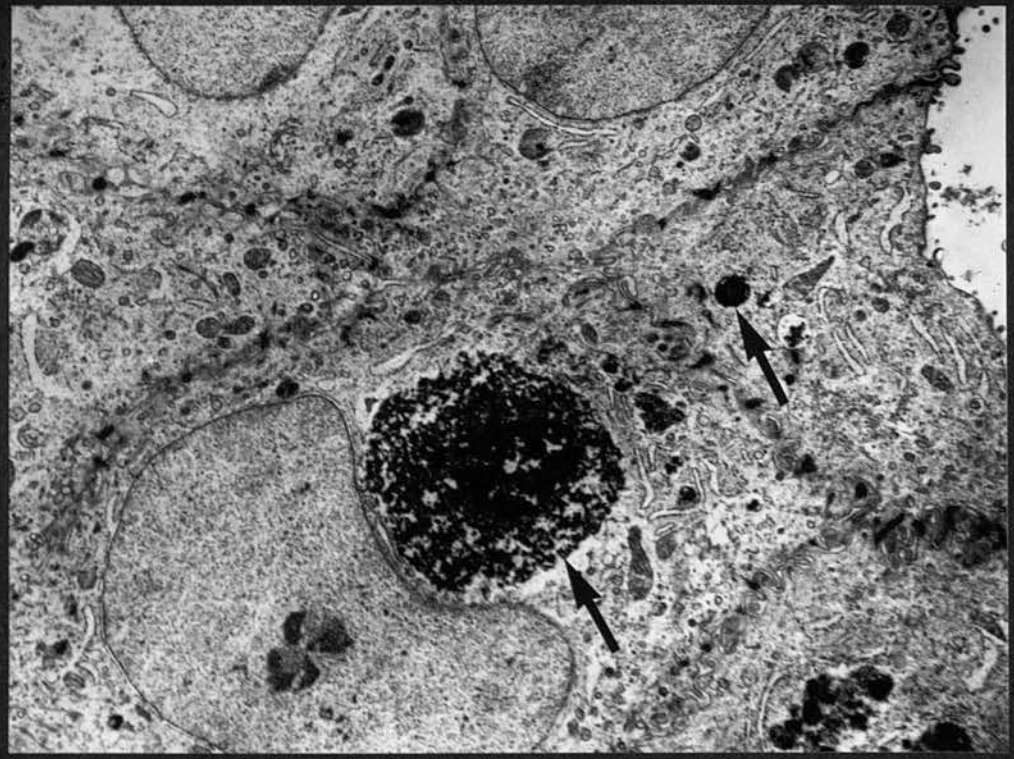


Fig. V.9: Tonsil. India ink particles free within crypt lumen.

Uranyl acetate, lead citrate. x 12,500.

Fig. V.10: Tonsil. India ink particles (arrows) in crypt epithelial cells.

Uranyl acetate, lead citrate. x 7,500.



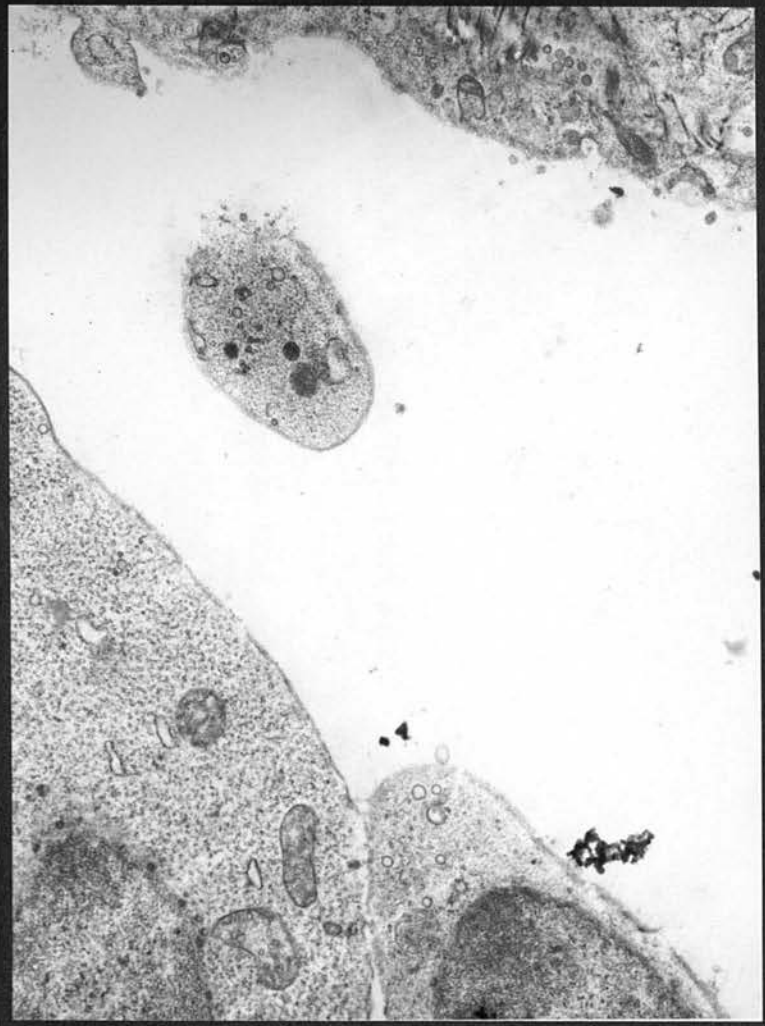
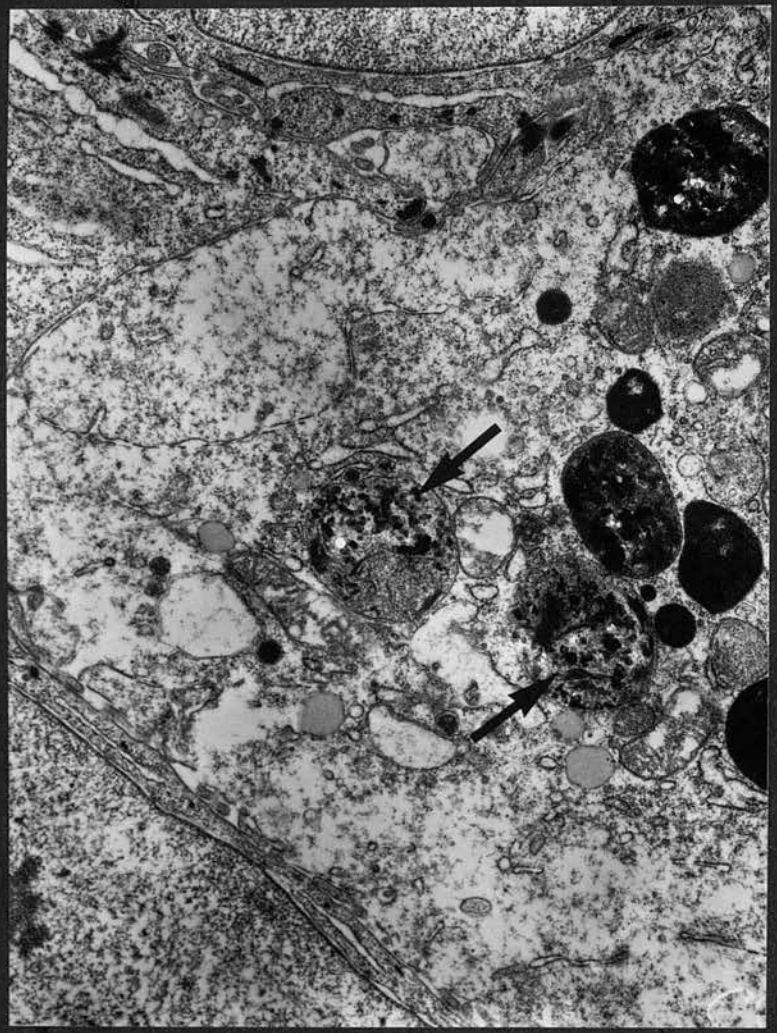
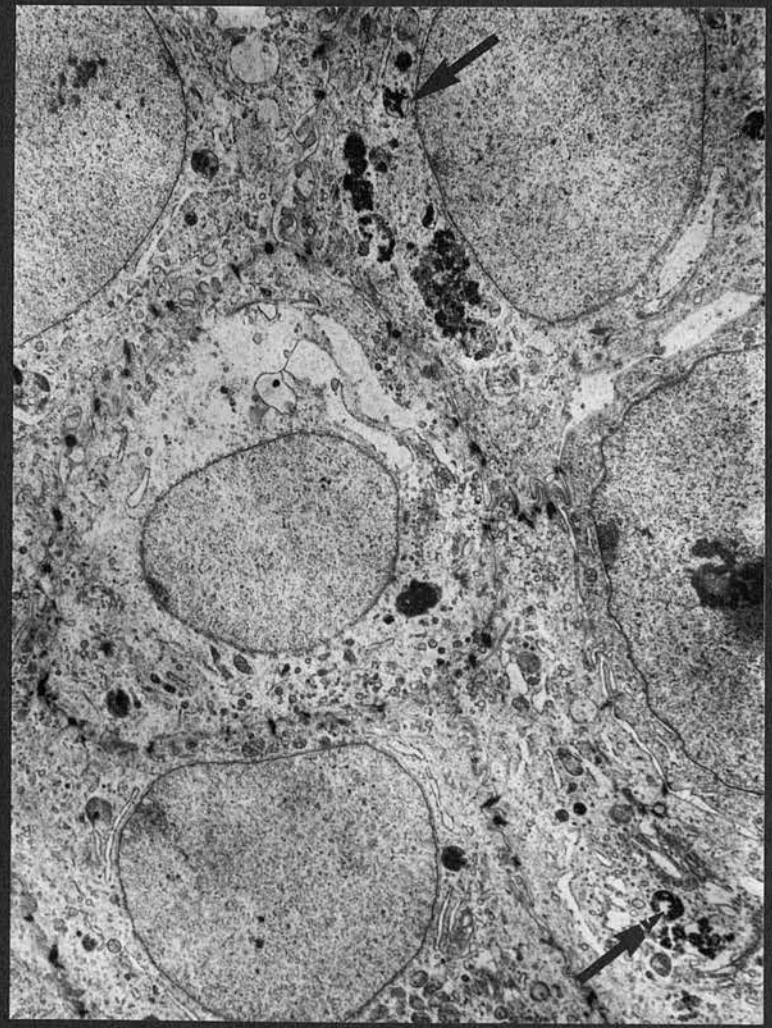


Fig. V.11: Tonsil. Crypt epithelial cells containing India ink particles (arrows).
Uranyl acetate, lead citrate. x 6,400.

Fig. V.12: India ink particles (arrows) contained in phagosomes within cells of crypt epithelium.
Uranyl acetate, lead citrate. x 28,500.





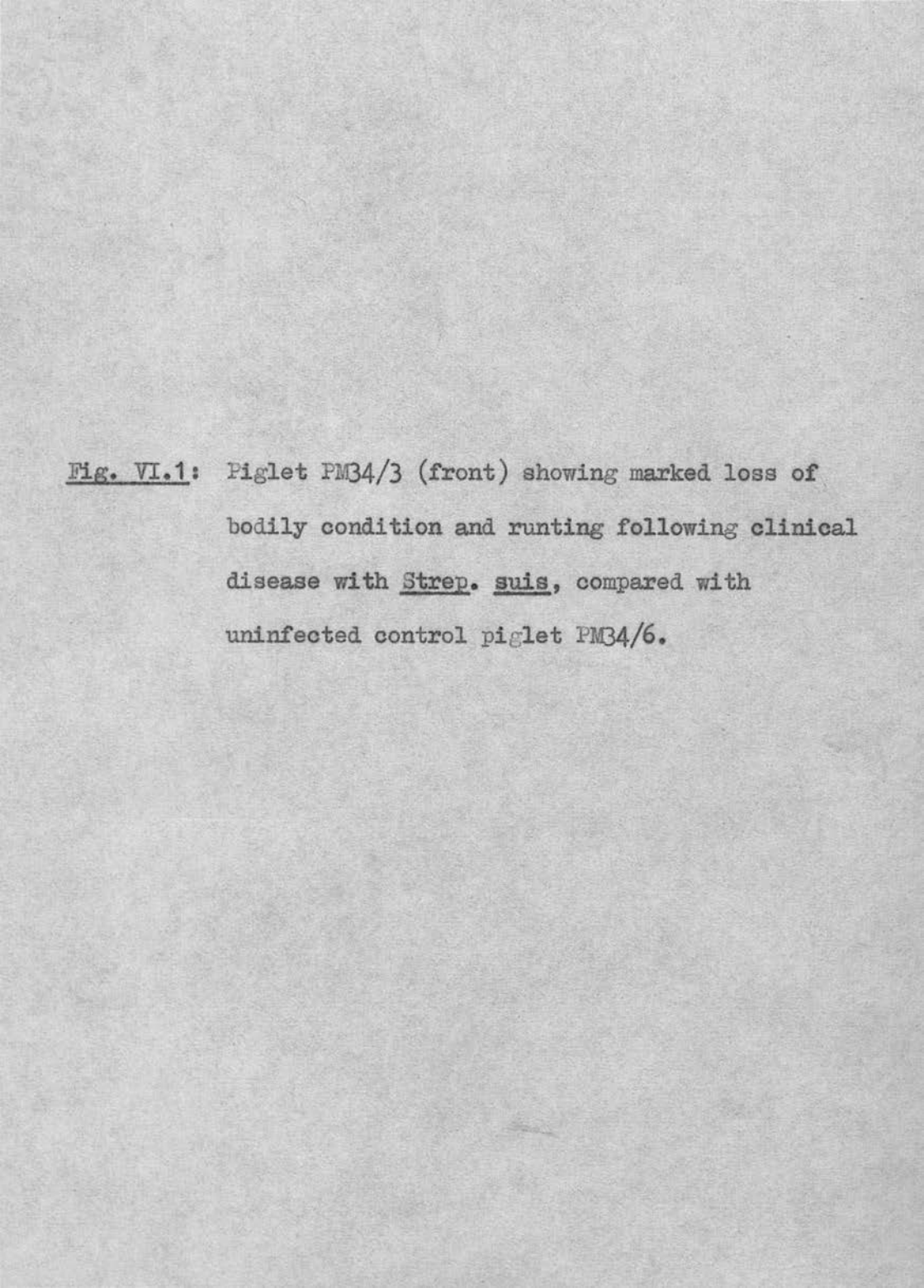


Fig. VI.1: Piglet PM34/3 (front) showing marked loss of bodily condition and runting following clinical disease with *Strep. suis*, compared with uninfected control piglet PM34/6.

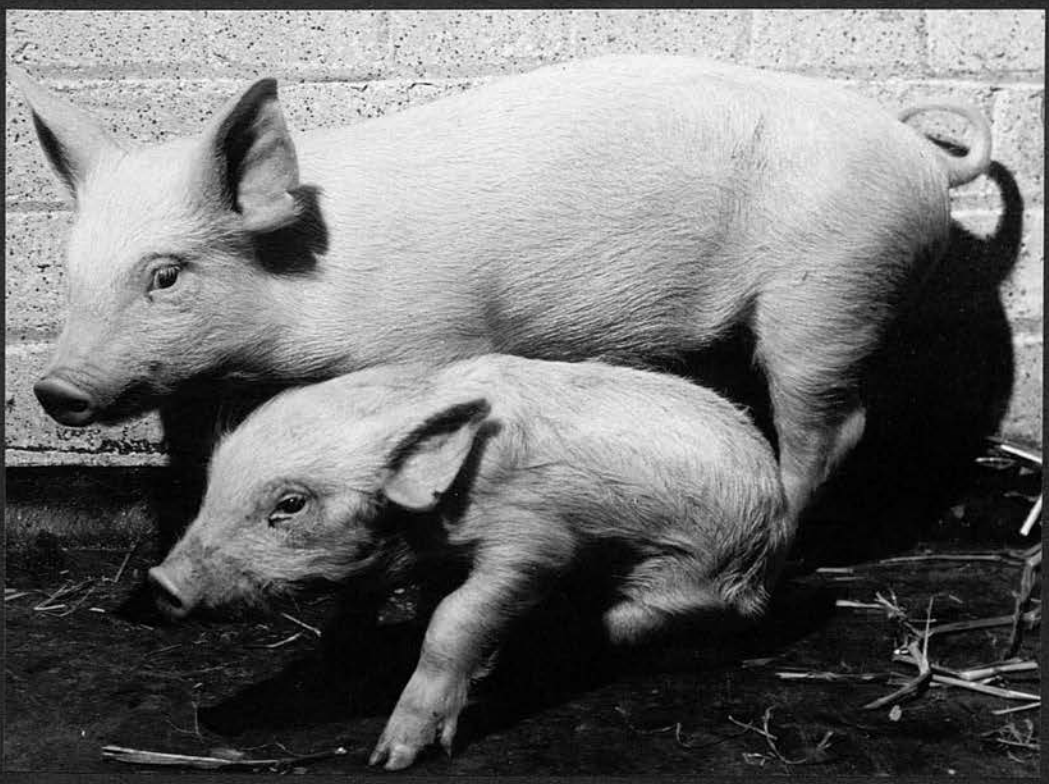


Fig. VI.2: Tonsil from infected but clinically healthy piglet. Lymphoid parenchyma showing normal pattern with numerous germinal centres. H & E. x 100.

Fig. VI.3: Tonsil. Crypt lumen and epithelium containing Gram-positive bacteria (arrows).
Gram's stain. x 220.

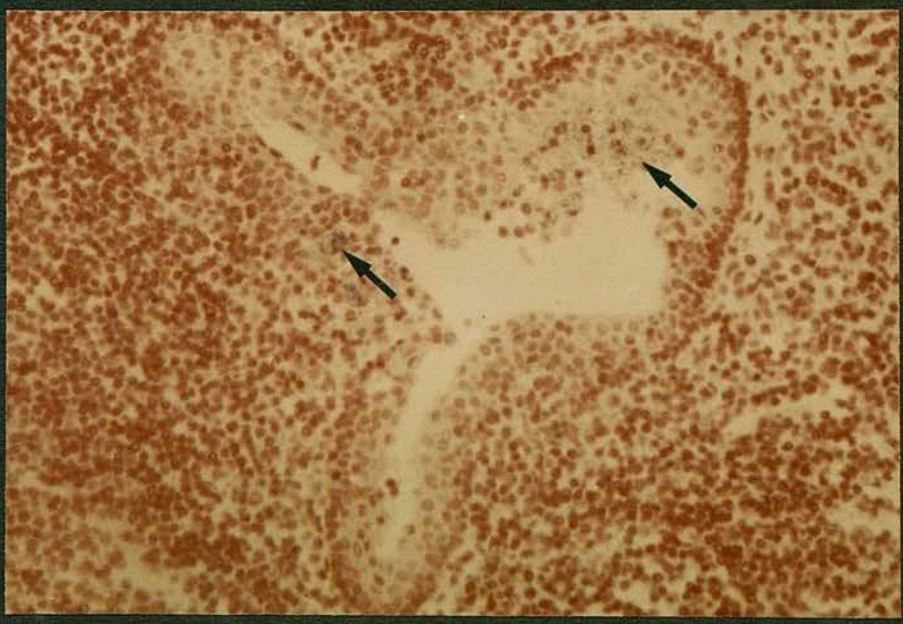
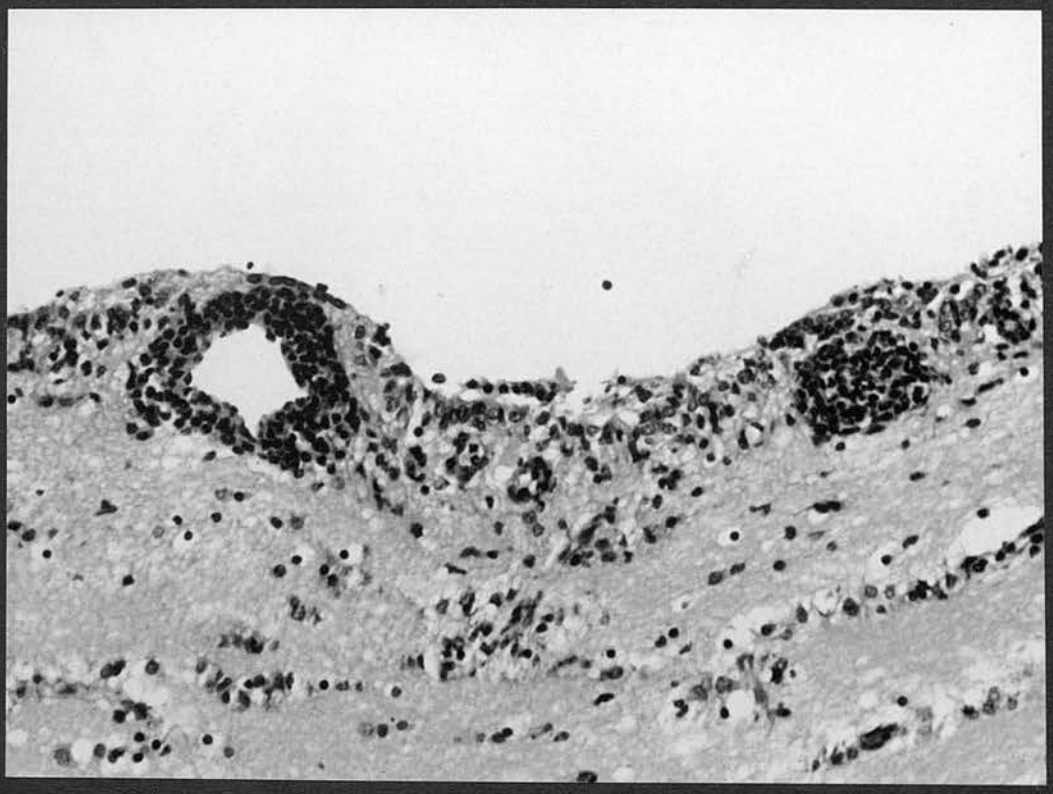


Fig. VI.4: Brain. Mononuclear perivascular cuffing adjacent to fourth ventricle.

H & E. x 300.

Fig. VI.5: Brain. Plasma cells in perivascular cuff.

H & E. x 600.



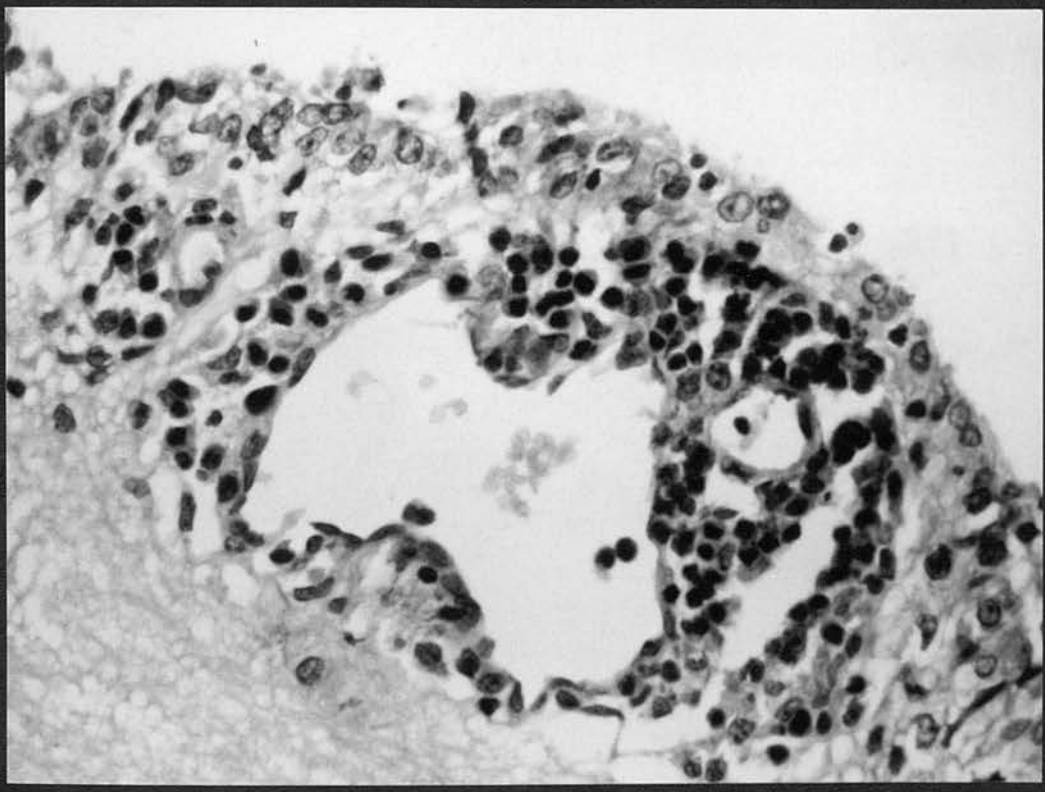
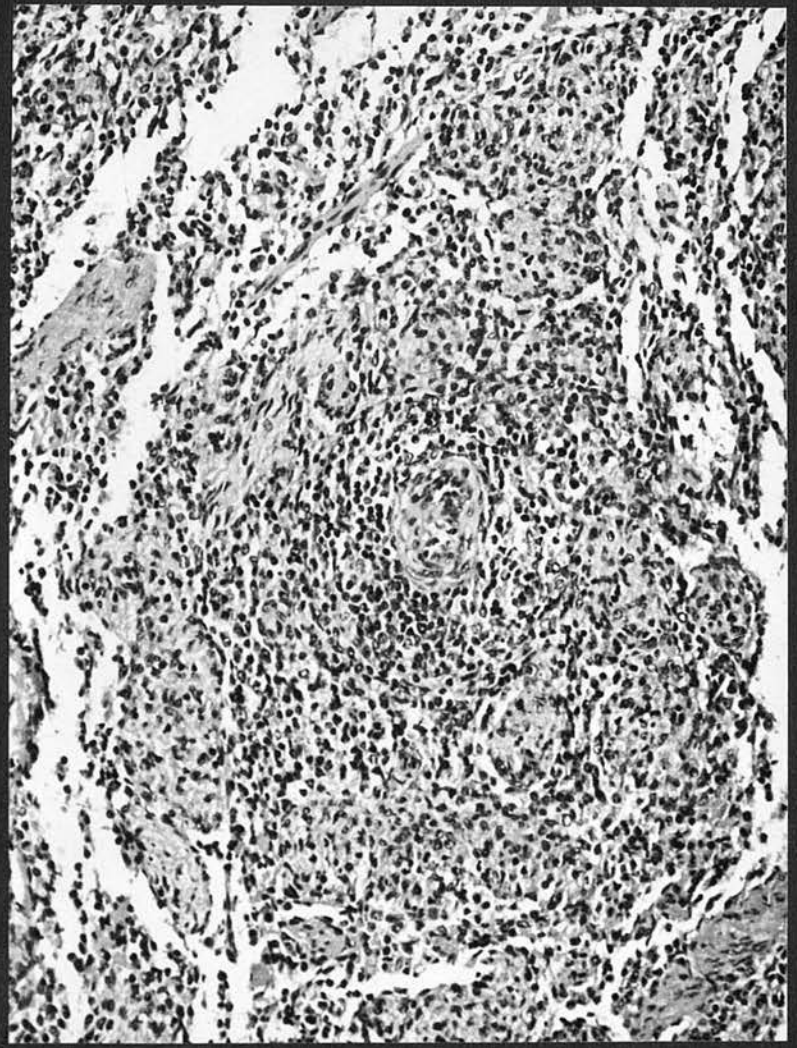


Fig. VI.6: Spleen. Plasma cells (arrows) in red pulp.
H & E. x 800.

Fig. VI.7: Spleen. Inactive white pulp.
H & E. x 250.



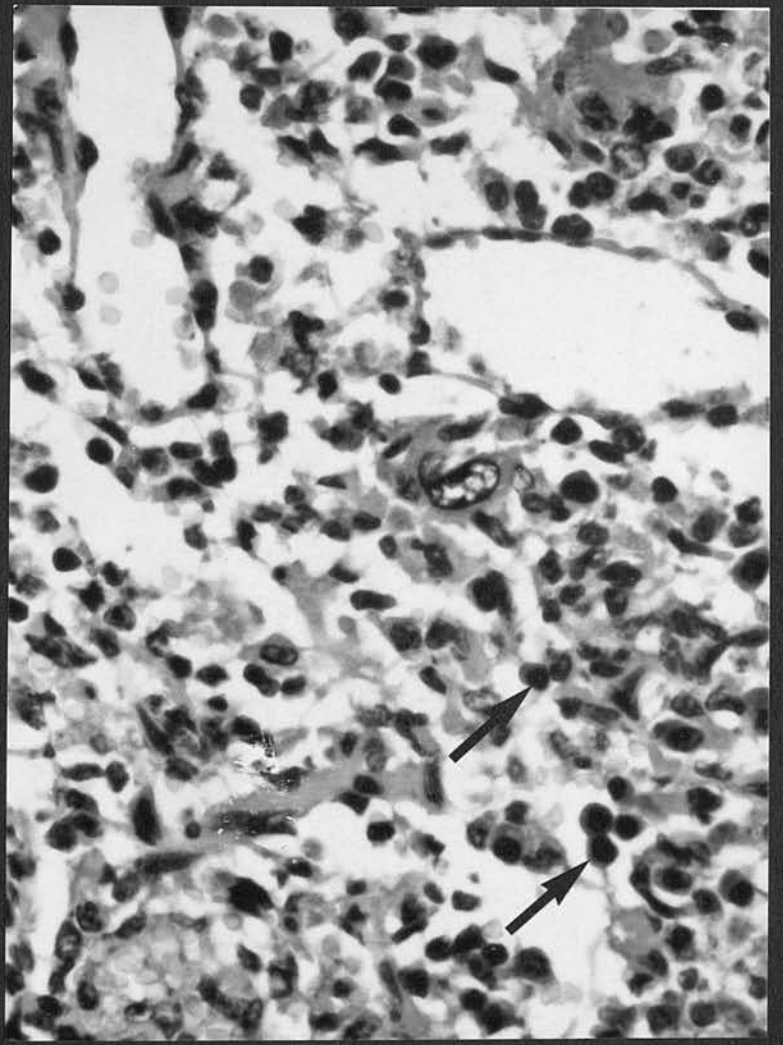


Fig. VI.8: Tonsil. Depleted germinal centre adjacent to crypt. Oedema of lymphoid parenchyma.
H & E. x 30.

Fig. VI.9: Tonsil. Epithelium and subepithelial zone infiltrated with plasma cells.
Methyl-green/pyronin. x 440.

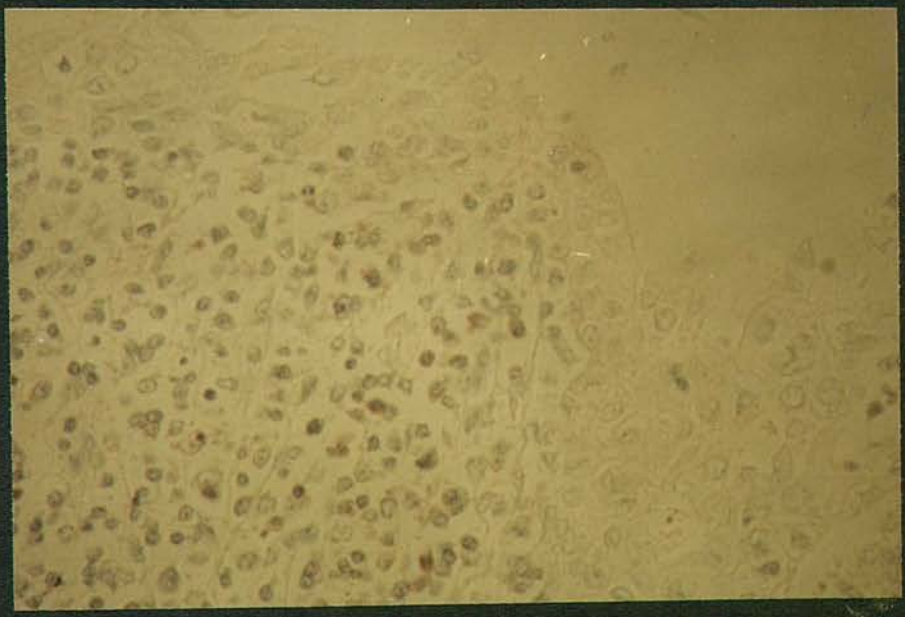
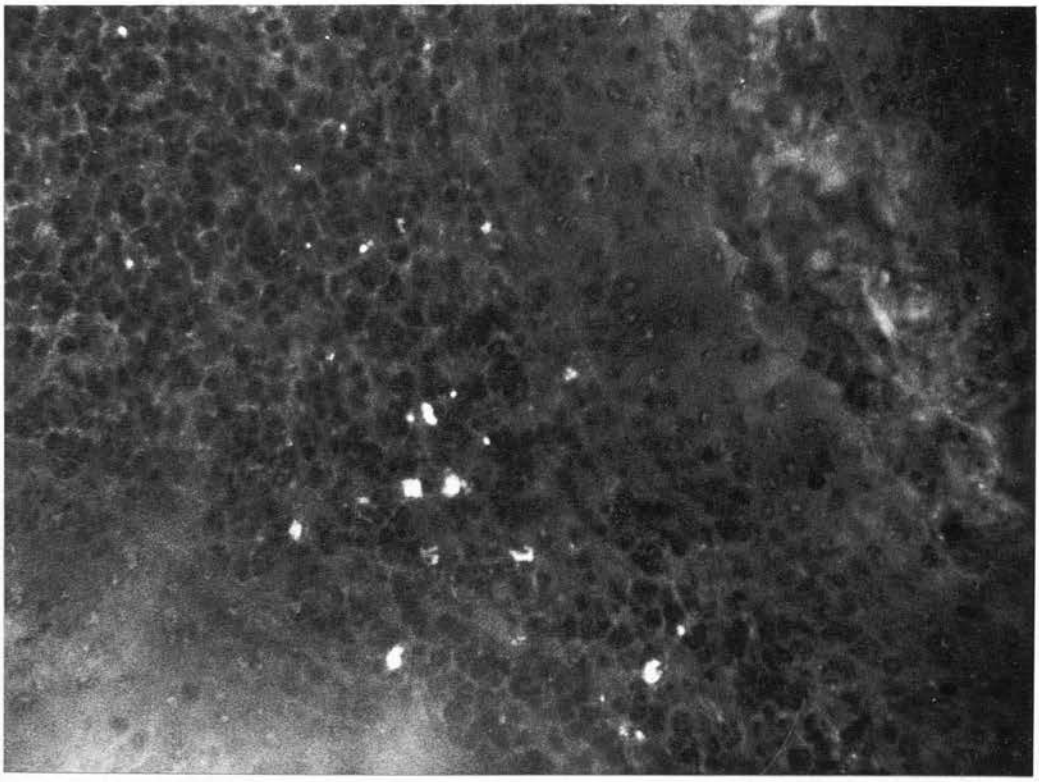


Fig. VI.10: Tonsil. Lymphoid parenchyma showing autofluorescent particles. Specific fluorescence absent.
Indirect stain. Immune rabbit globulin (PM34/B), goat anti-rabbit fluorescein isothiocyanate conjugated serum (FITC-GAR).
x 250.

Fig. VI.11: Tonsil. Crypt lumen containing numerous *Strep. suis* bacteria.
Indirect stain. PM34/B, FITC-GAR. x 500.



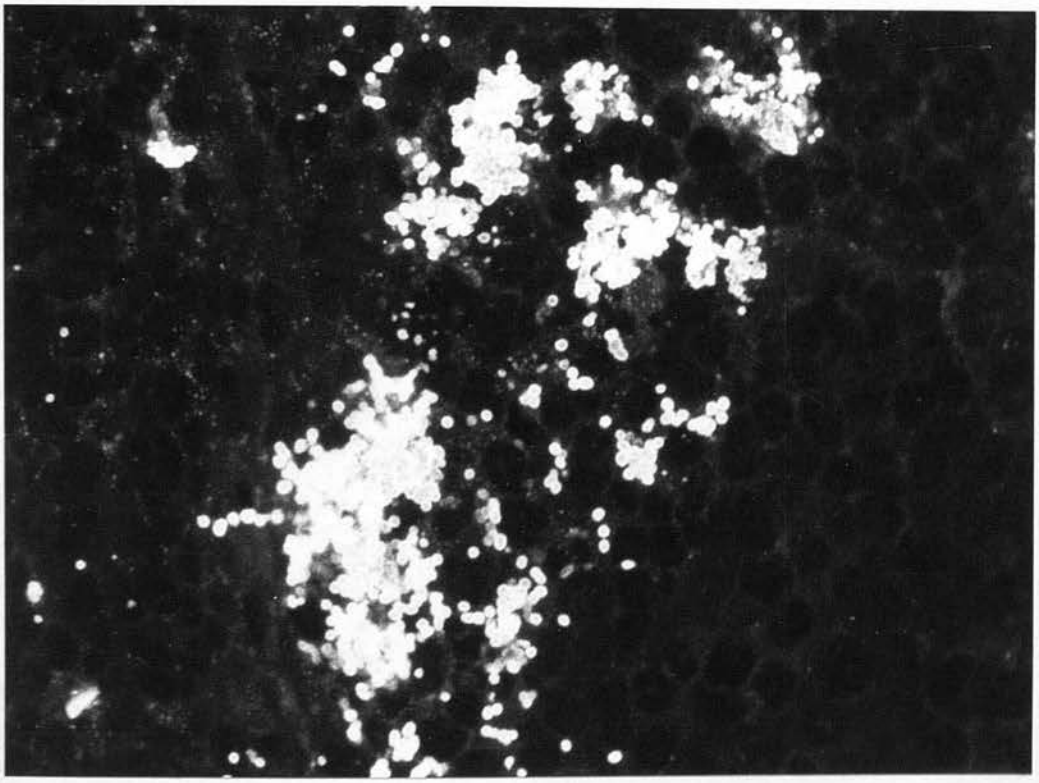
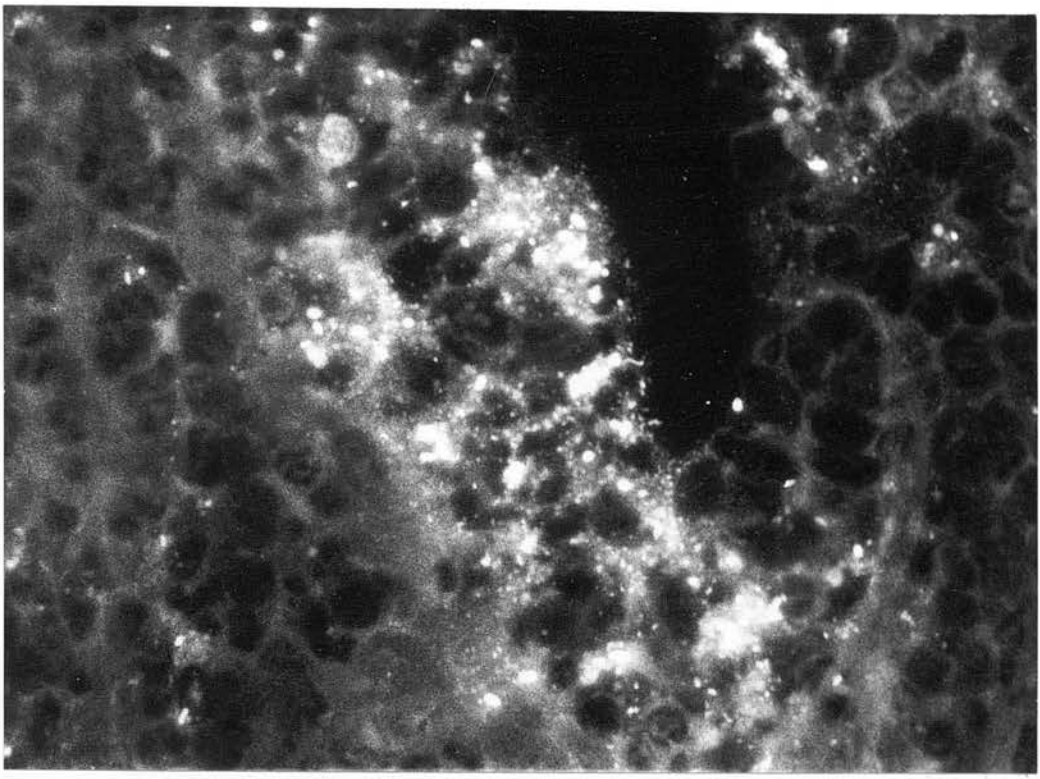


Fig. VI.12: Tonsil. Crypt lumen and epithelium with particulate and amorphous Strep. suis antigen.

Indirect stain. PM34/B, FITC-GAR. x 500.

Fig. VI.13: Tonsil. Germinal centre containing Strep. suis antigen.

Indirect stain. PM34/B, FITC-GAR. x 500.



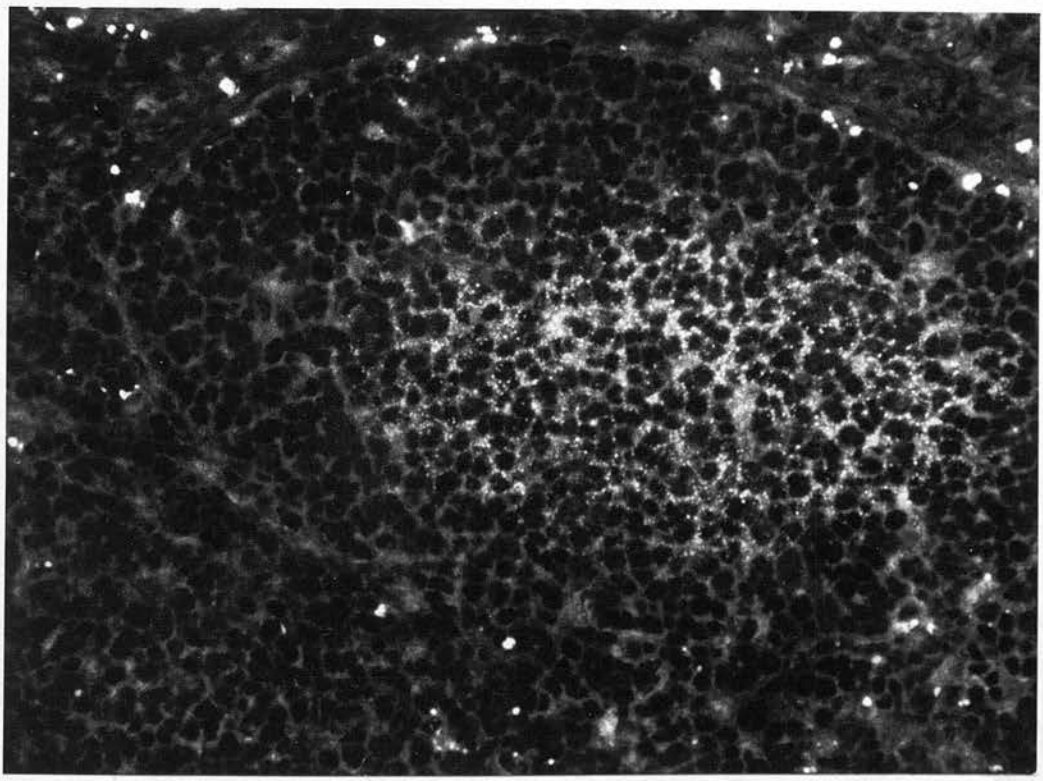


Fig. VI.14: Tonsil. Absence of specific fluorescence in control sections of infected material. Indirect stain. Non-immune rabbit serum, FITC-GAR. x 250.

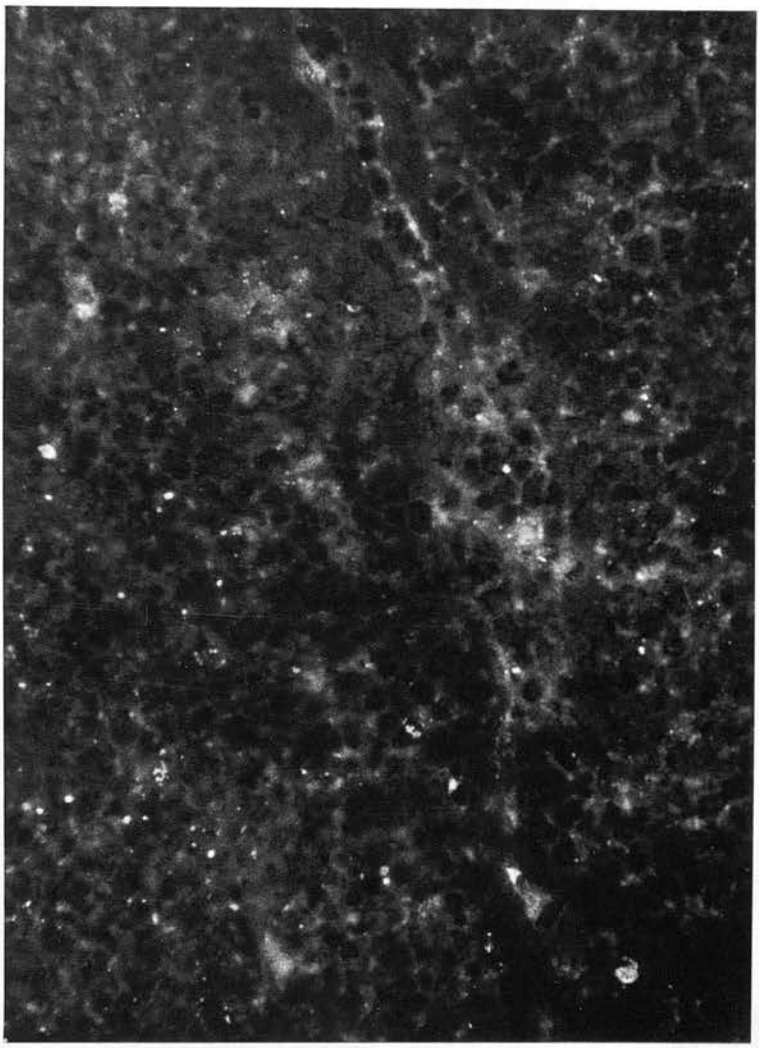
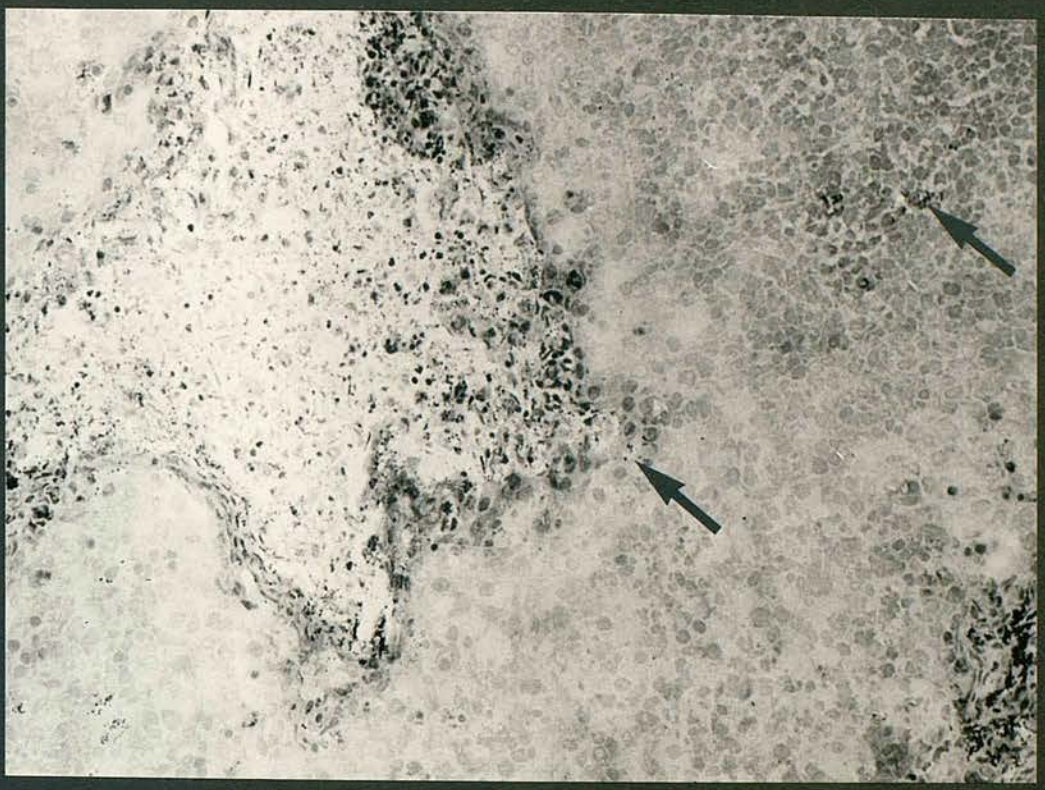


Fig. VI.15: Tonsil. Crypt lumen, epithelium and subepithelial zones containing Gram-positive bacteria (arrows). Gram's stain. Top x 250.
Bottom x 350.



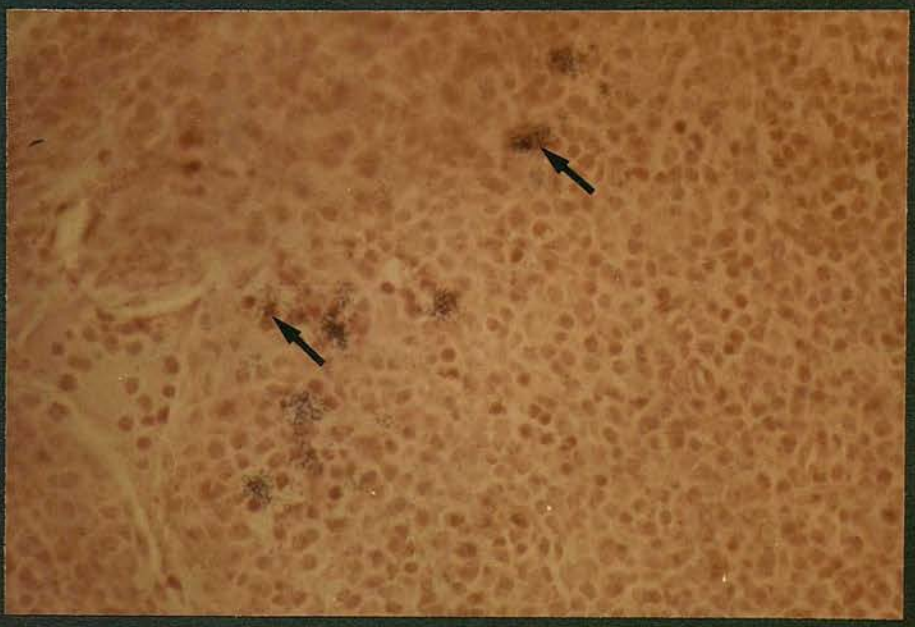


Fig. VII.1: Tonsil. Crypt epithelium and subepithelial zone showing infiltration with plasma cells. H & E. x 500.

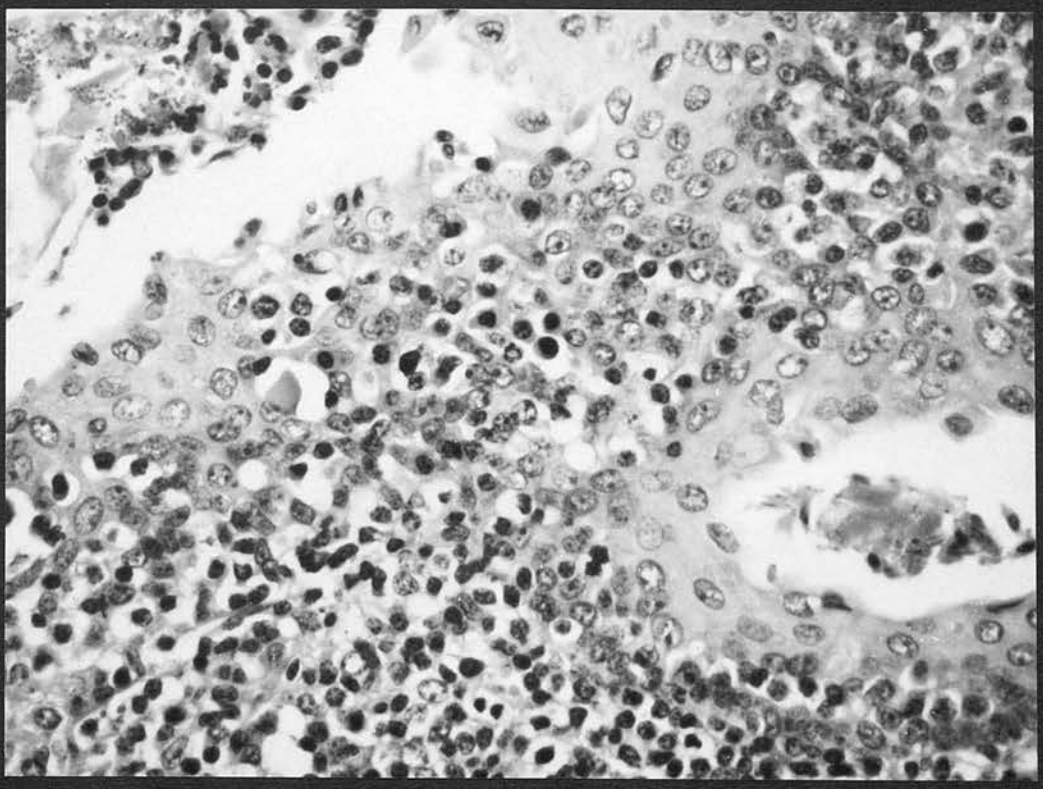
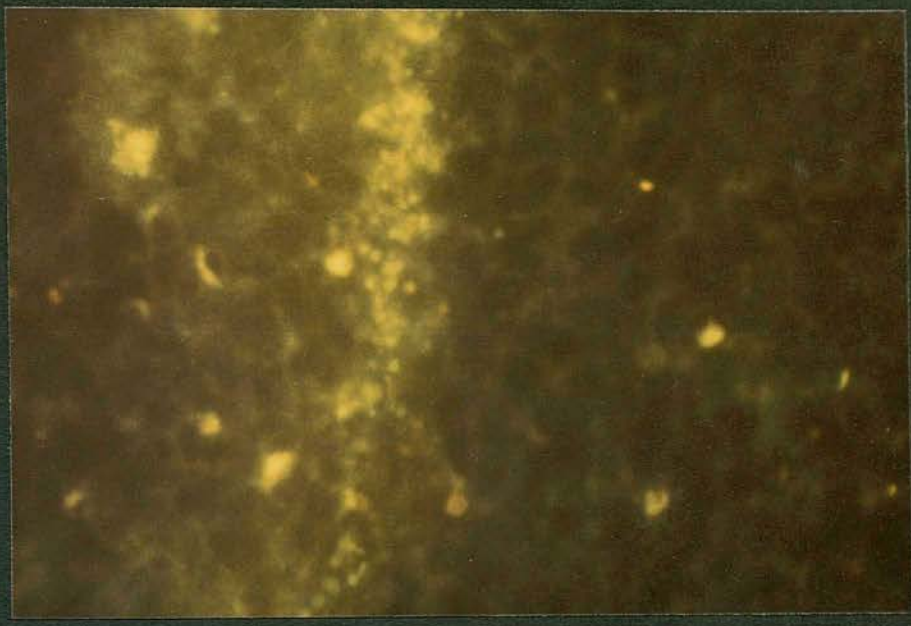


Fig. VII.2: Tonsil. Crypt lumen and epithelium containing *Strep. suis* bacteria.
Indirect stain. PM34/B, FITC-CAR. x 440.

Fig. VII.3: Tonsil. Gram-positive bacteria in crypt lumen and epithelium.
Gram's stain. x 500.



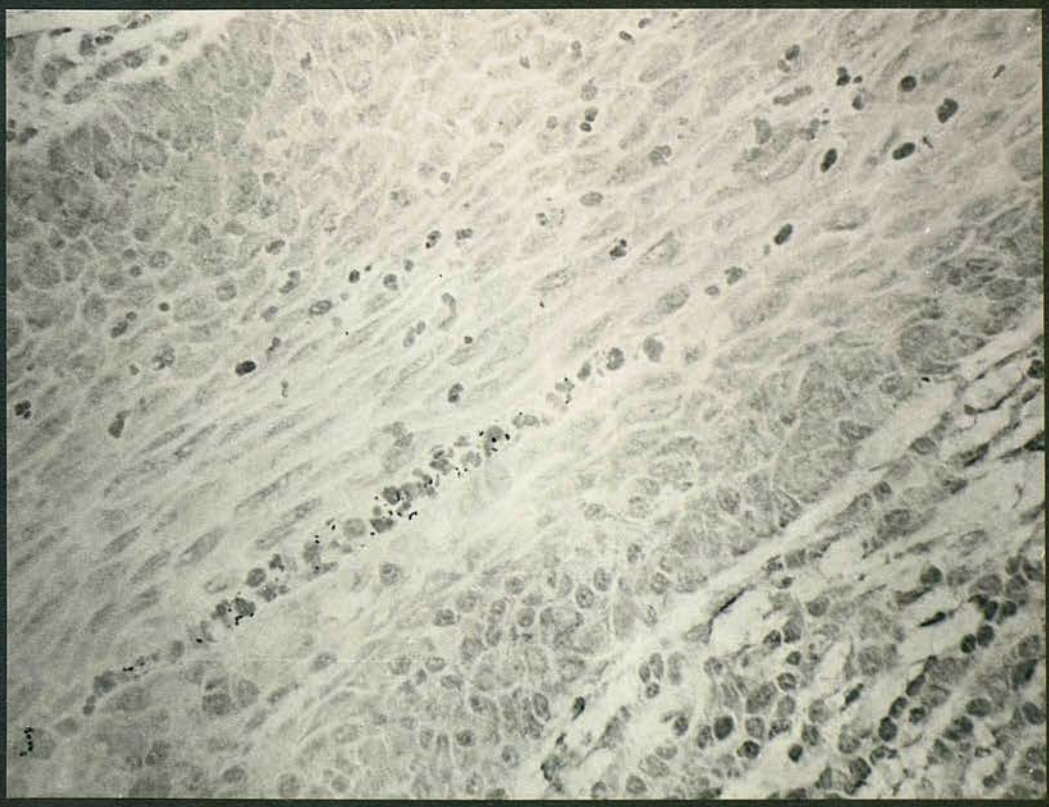


Fig. VII.4: Strep. suis. Bacteria in pairs, showing distinct partition zone.

Uranyl acetate. x 20,000.

Fig. VII.5: Strep. suis. Bacterial cell wall and capsule composed of double membrane with filamentous projections.

Uranyl acetate. x 40,000.

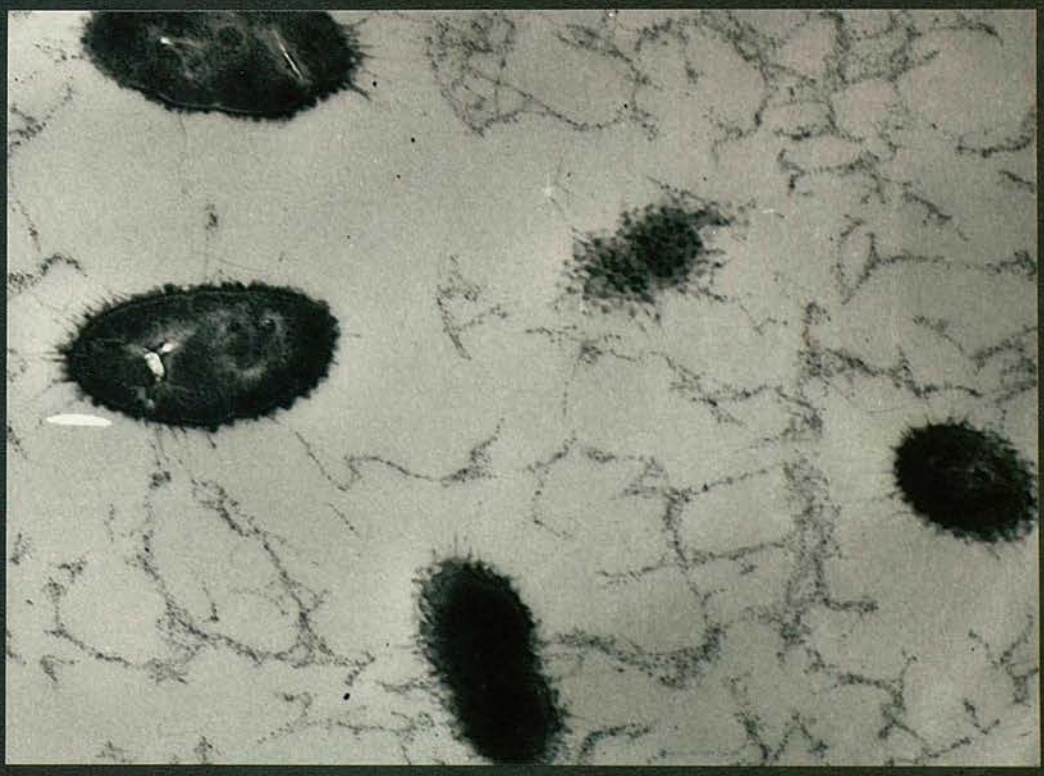


Fig. VII.6: Tonsil. Crypt epithelium typical of that selected for electron-microscopical examination. Thionine/toluidine blue. x 300.

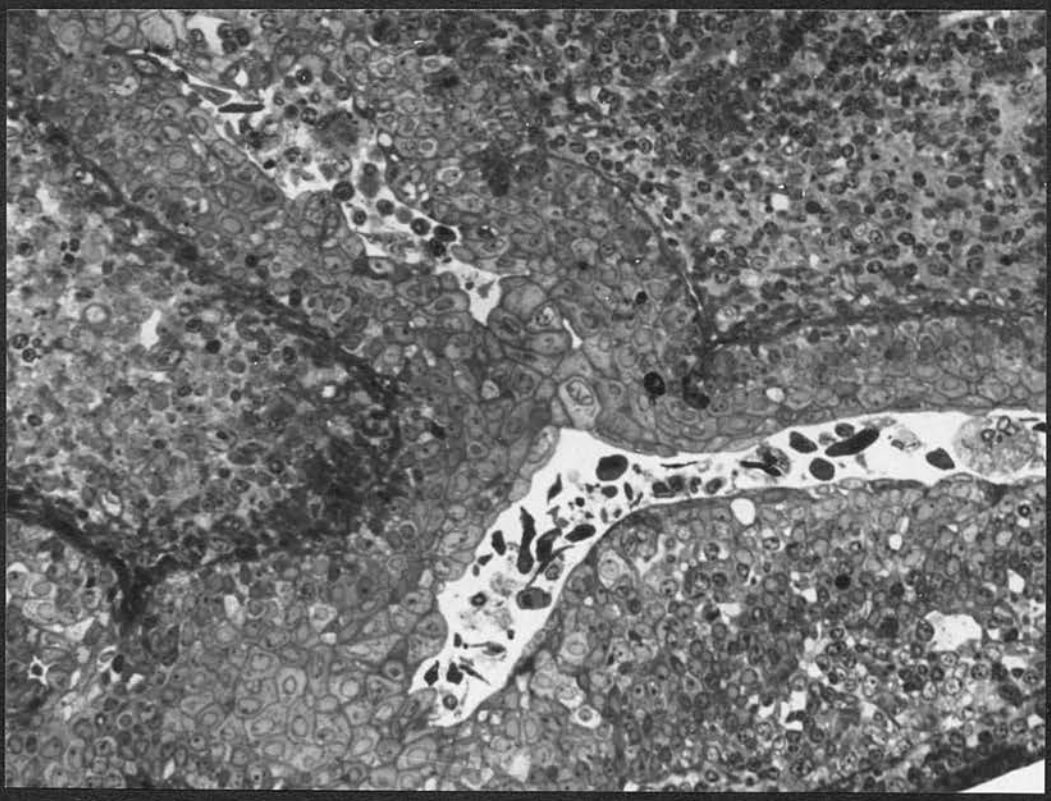
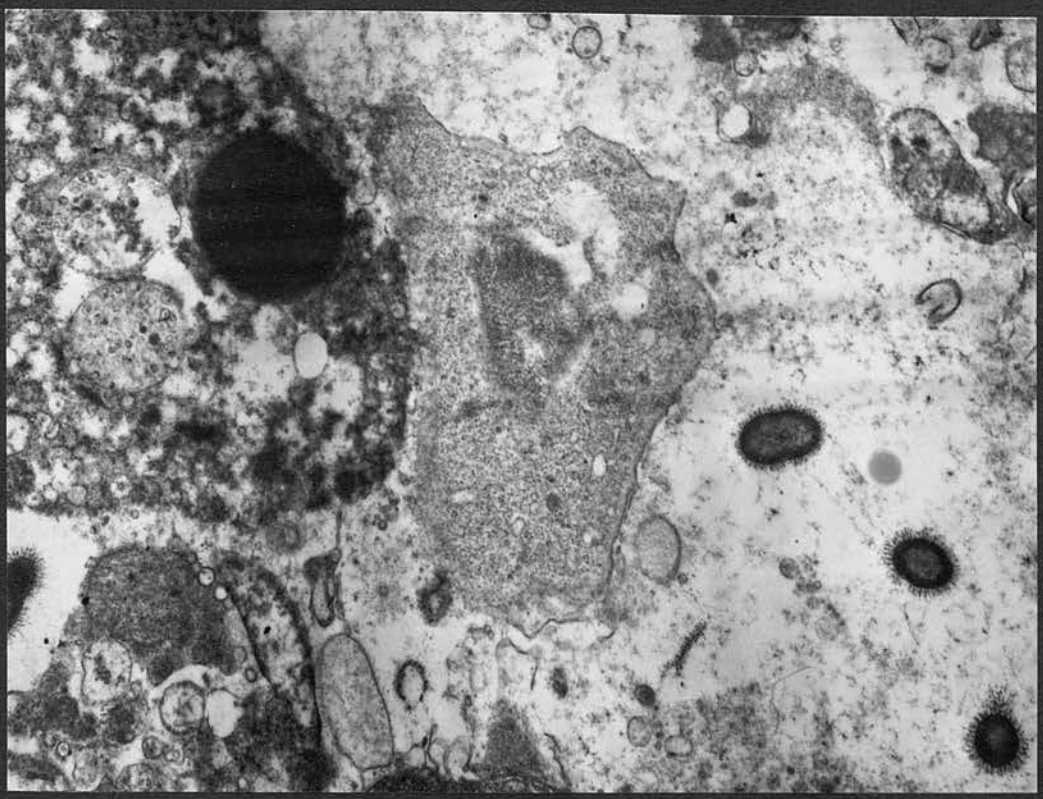


Fig. VII.7: Tonsil. Crypt luminal detritus containing bacteria resembling Strep. suis.

Uranyl acetate, lead citrate. Top x 13,000.

Bottom x 35,700.



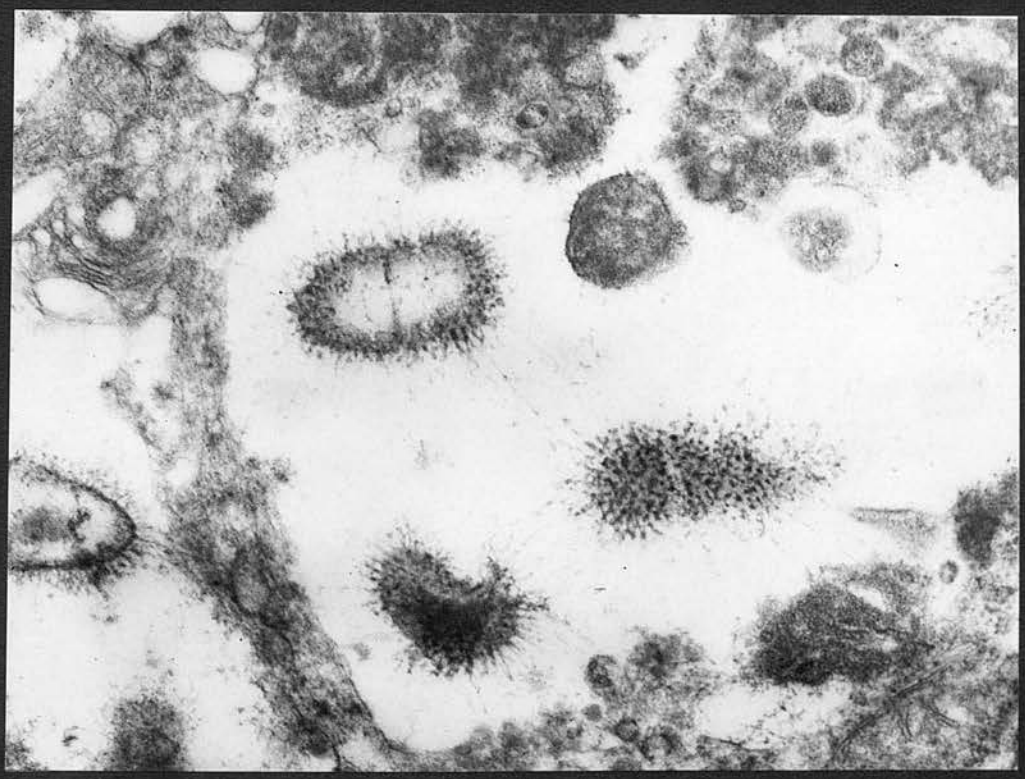


Fig. VII.8: Tonsil. Crypt epithelial cell containing heterogeneity of intracellular foreign matter. Uranyl acetate, lead citrate. x 8,250.

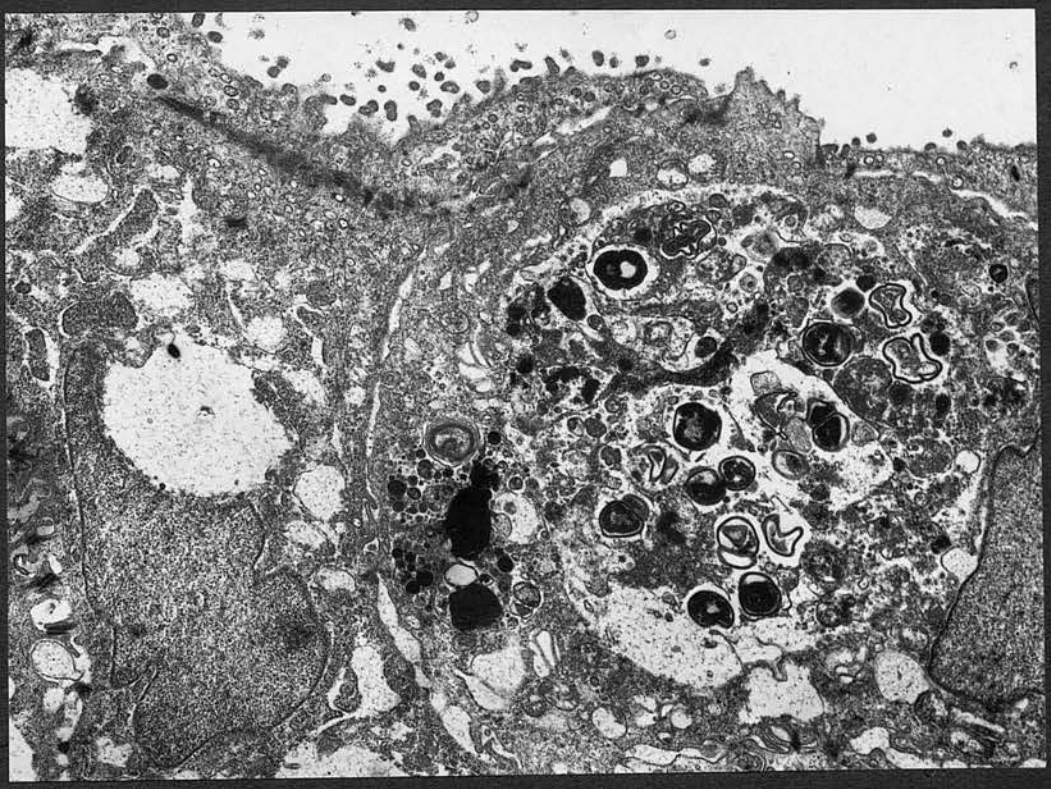


Fig. VII.10: Tonsil. Crypt epithelial cell containing bacteria and bacterial fragments.
Uranyl acetate, lead citrate. x 17,500.

Fig. VII.9: Tonsil. Bacteria within cell of the crypt epithelium.
Uranyl acetate, lead citrate. x 9,150.

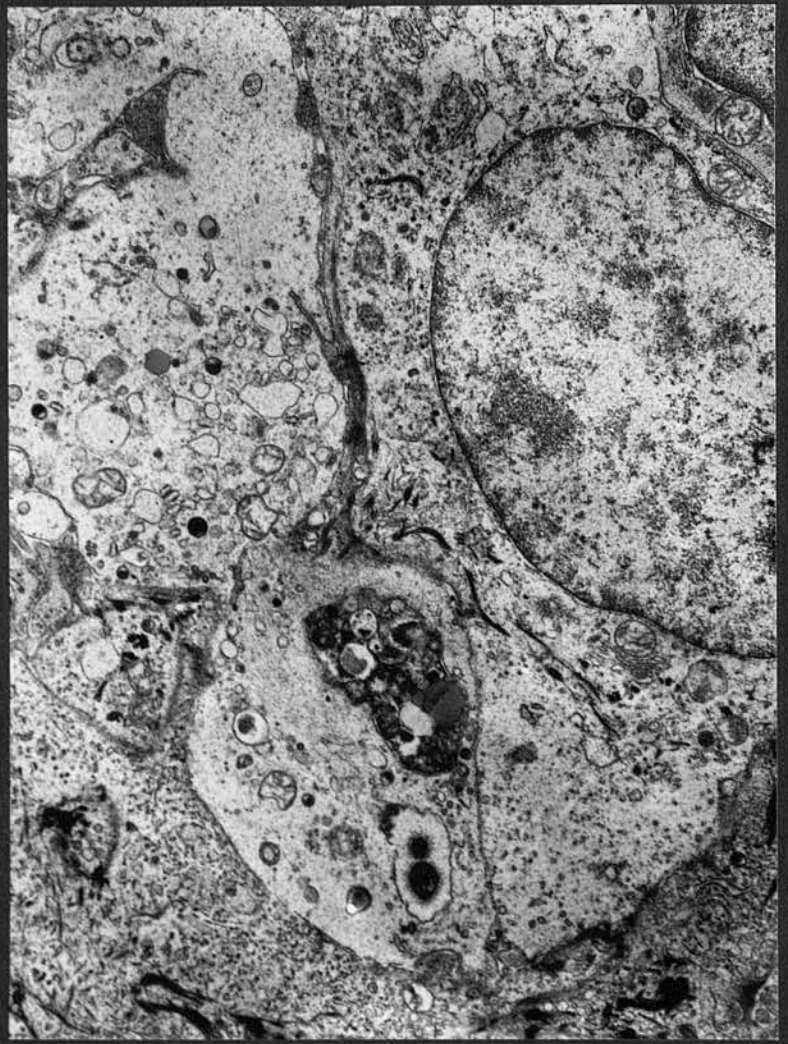
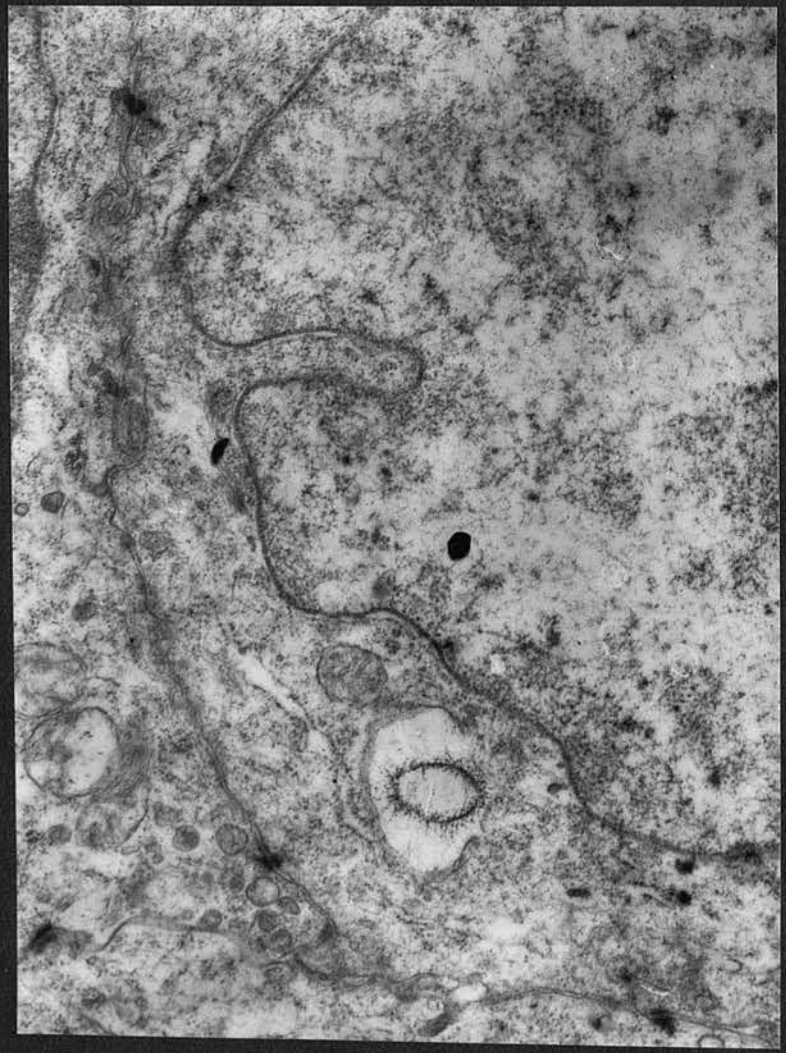


Fig. VII.11: Tonsil. Crypt epithelial cell with intracellular bacteria.

Uranyl acetate, lead citrate. Top x 9,000.

Bottom x 20,000.



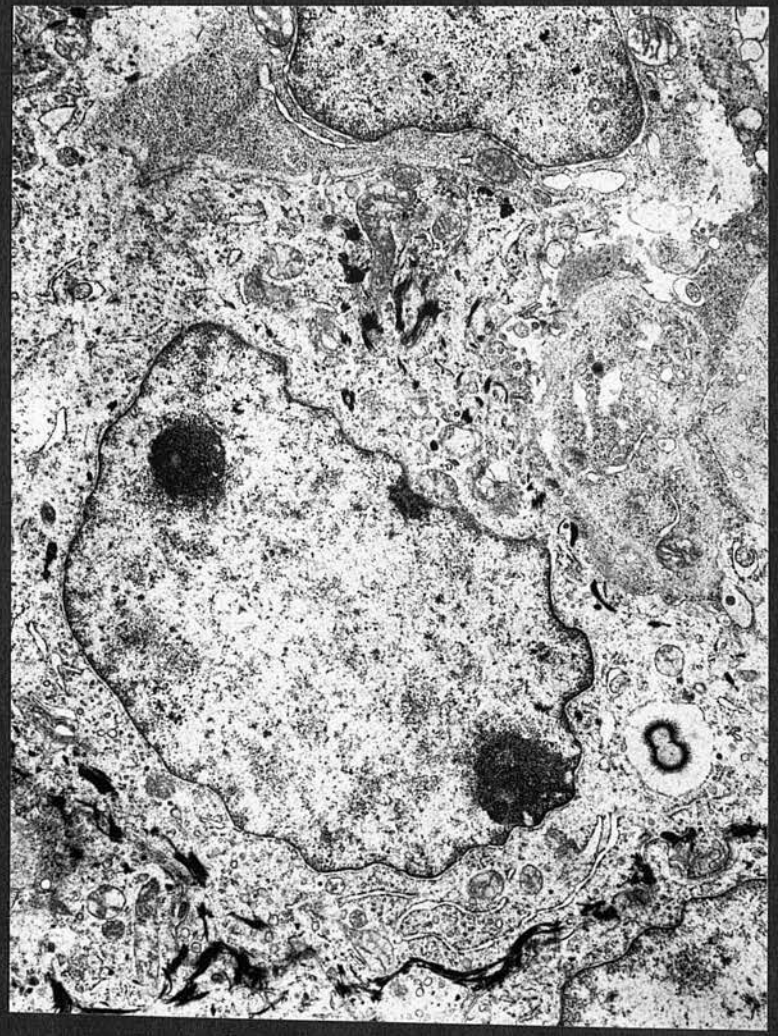
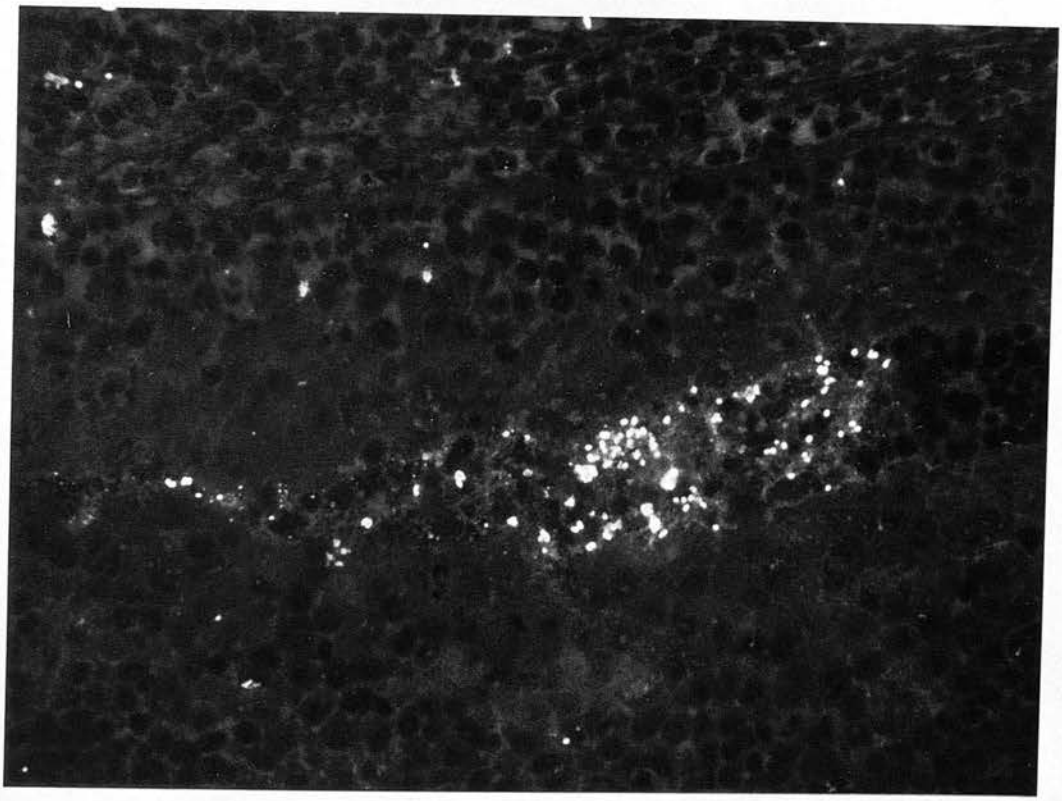


Fig. VIII.1: Tonsil. *Strep. suis* bacteria within crypt lumen.

Indirect stain. PM34/B, FITC-GAR. x 250.

Fig. VIII.2: Tonsil. Crypt lumen and epithelium showing *Strep. suis* bacteria and antigen.

Indirect stain. PM34/B, FITC-GAR. x 250.



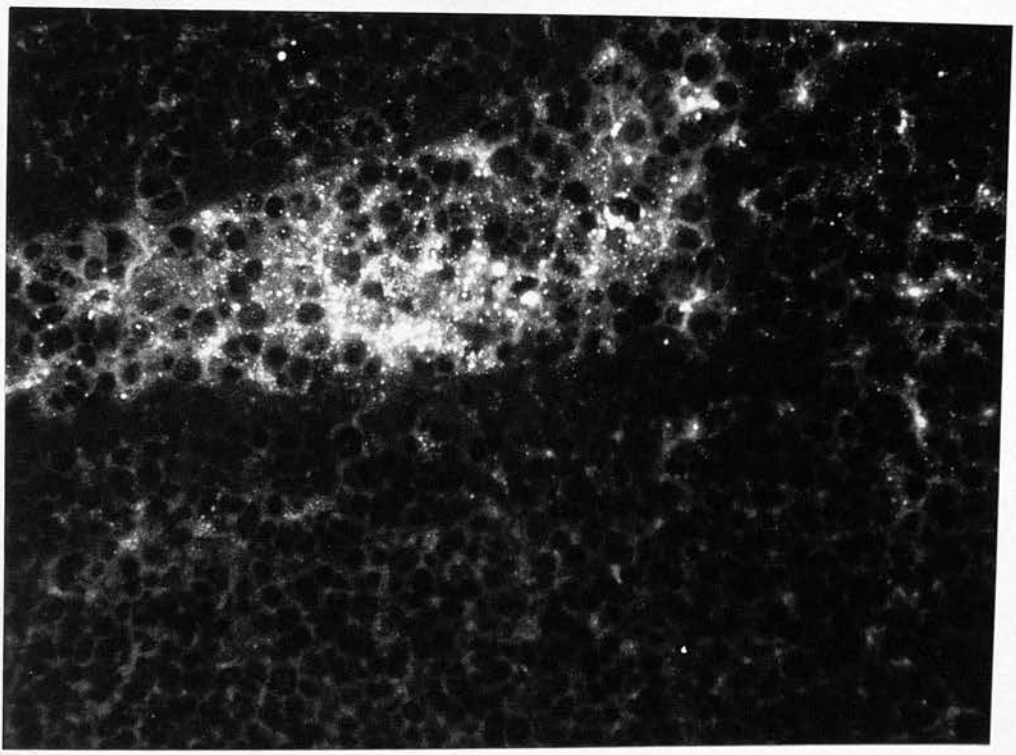


Fig. VIII.3: Tonsil. Crypt epithelium and subepithelial zone containing *Strep. suis* antigen. Indirect stain. PM34/B, FITC-GAR. x 500.

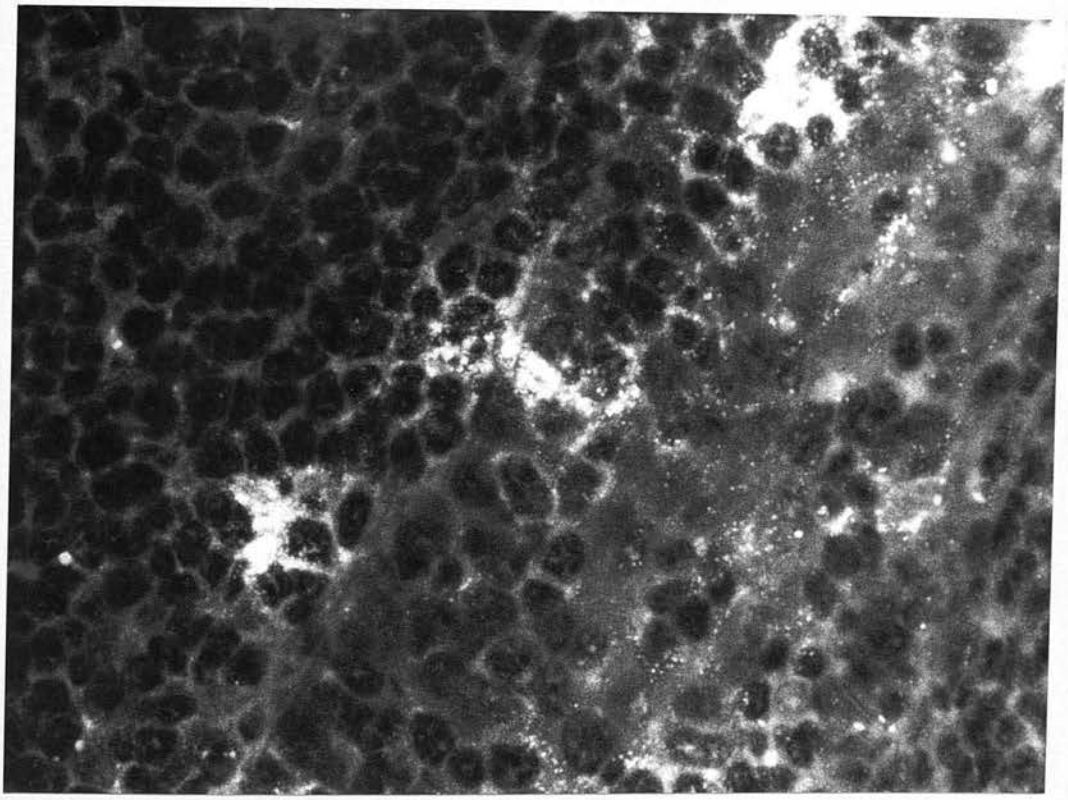


Fig. VIII.4: Tonsil. Gram-positive bacteria within crypt lumen, epithelium and subepithelial zone.

Gram's stain. x 350.

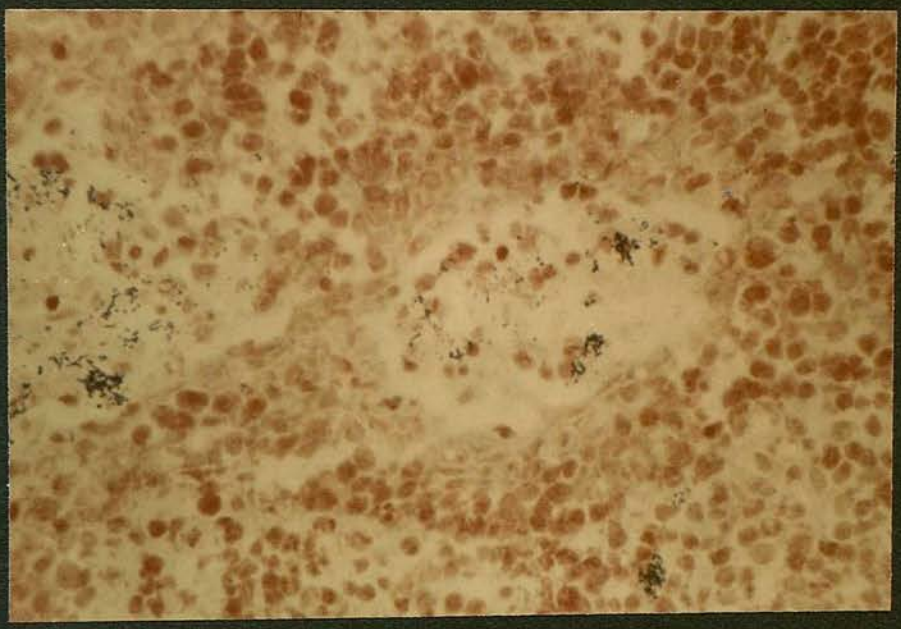
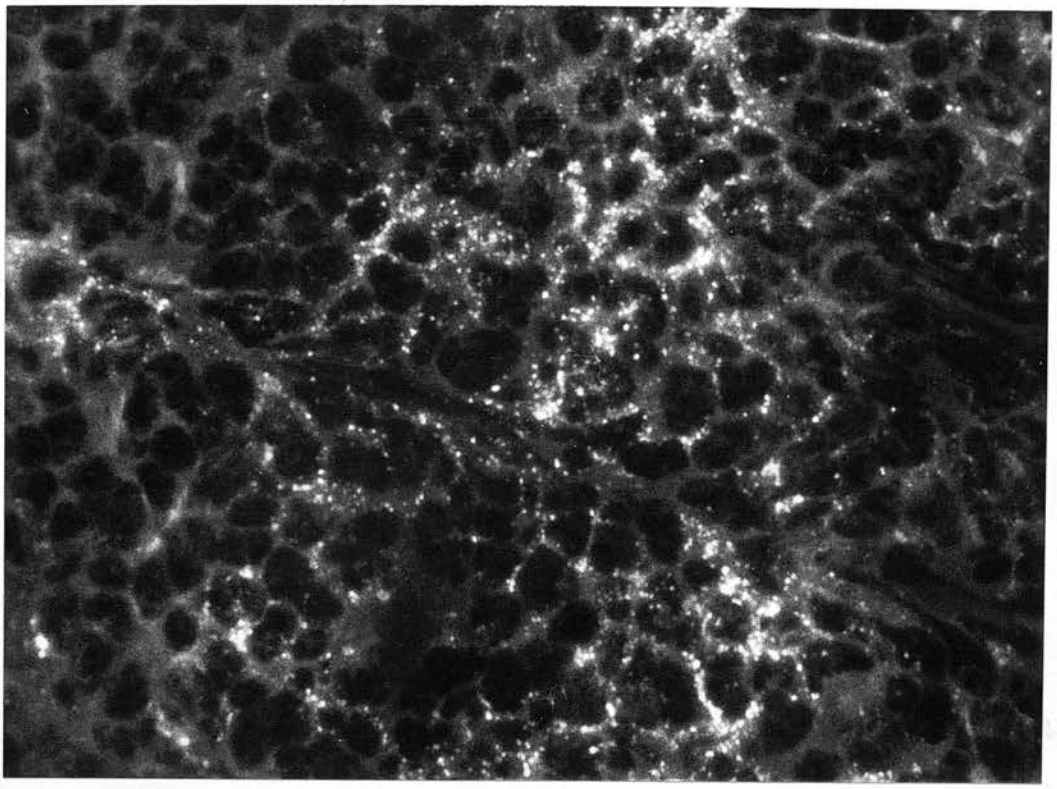


Fig. VIII.5: Tonsil. Interfollicular tissue showing Strep. suis antigen.

Indirect stain. PM34/B, FITC-GAR. x 500.

Fig. VIII.6: Tonsil. Macrophages in interfollicular tissue containing diffusely fluorescing Strep. suis antigen.

Indirect stain. PM34/B, FITC-GAR. x 500.



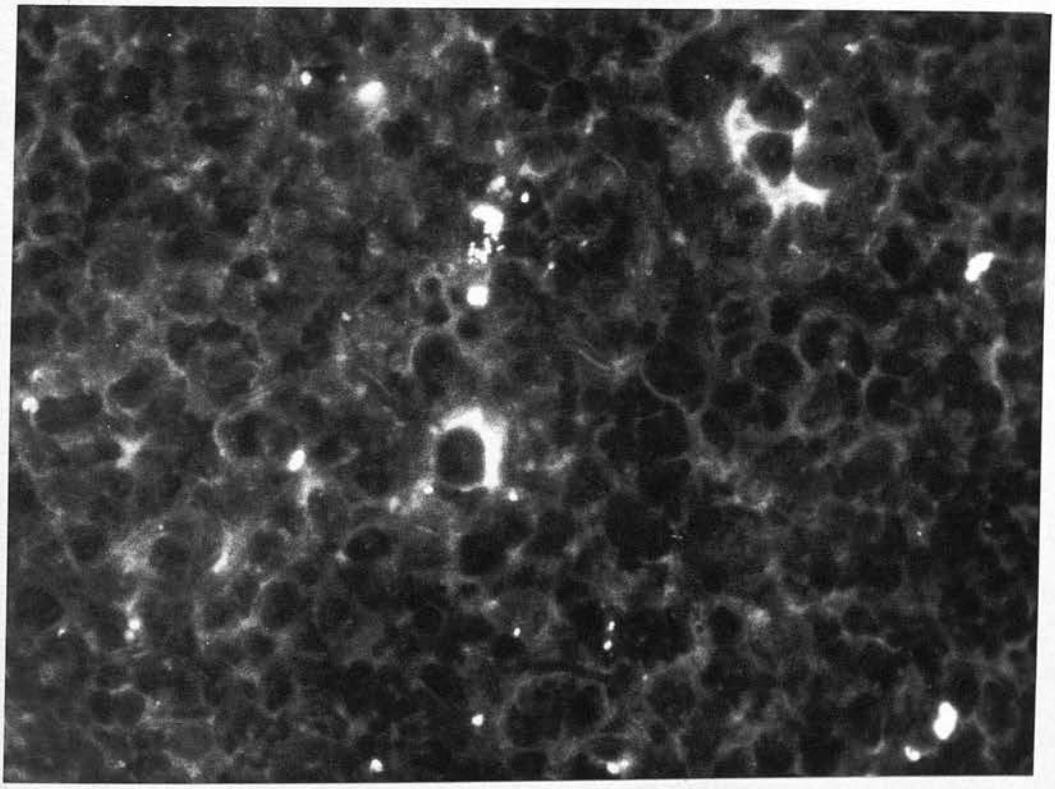
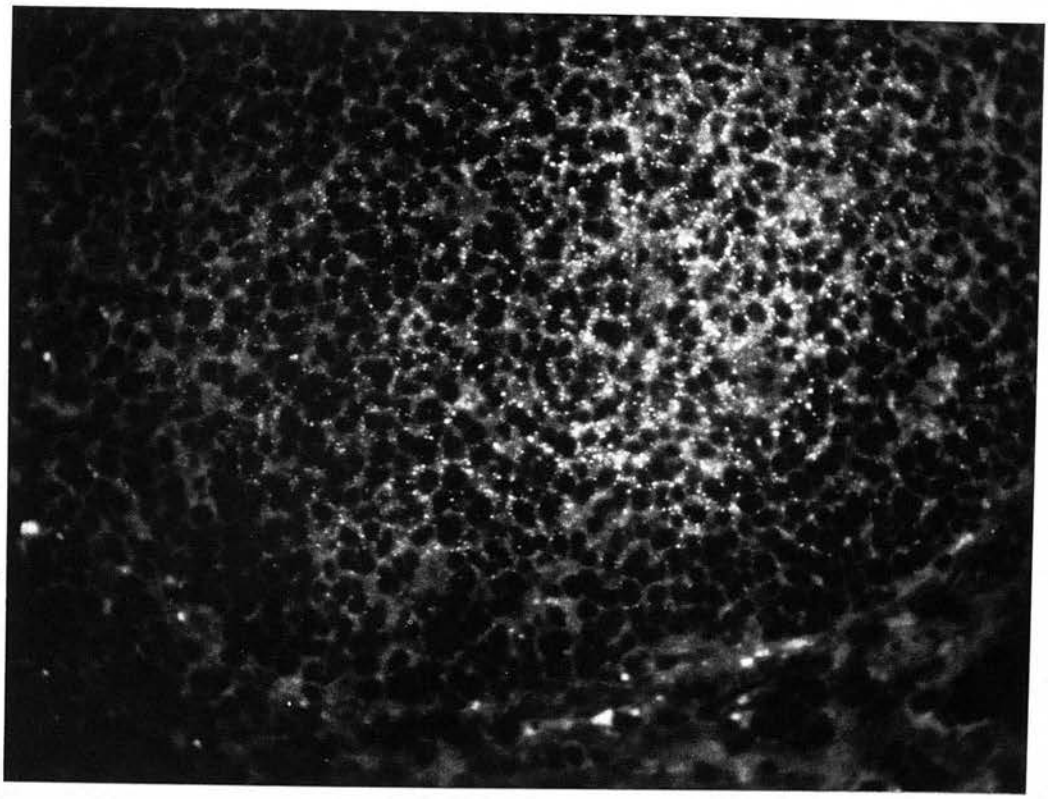


Fig. VIII.7: Tonsil. Germinal centre containing *Strep. suis* antigen.

Indirect stain. PM34/B, FITC-GAR. x 250.

Fig. VIII.8: Tonsil. Germinal centre with *Strep. suis* antigen.

Indirect stain. PM34/B, FITC-GAR. x 500.



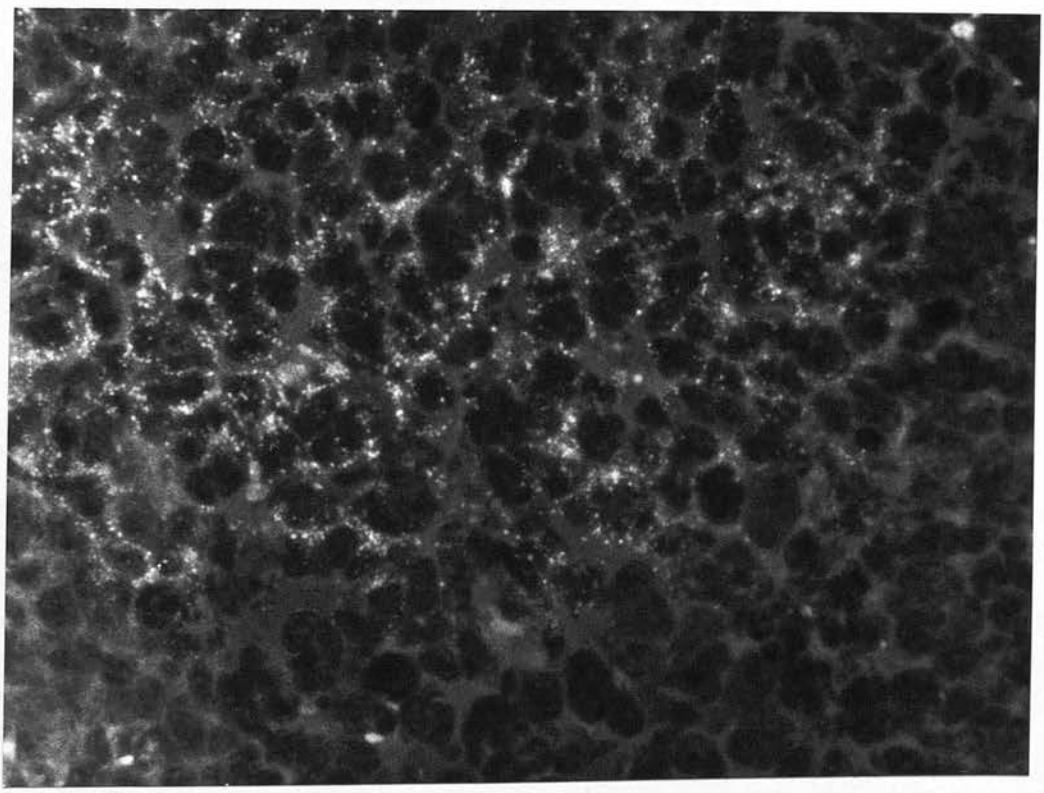


Fig. VIII.9: Tonsil. Germinal centre containing globulin. Direct stain. FITC-rabbit anti-pig globulin. x 440.