IMMUNOCHEMICAL ANALYSIS OF FACTORS AFFECTING FERTILITY

(Volume 2 - Figures and Tables)

M.H. Johnson

Christ's College, Cambridge.

1969.

Index to tables.

Figure immediately preceeding the table is indicated.

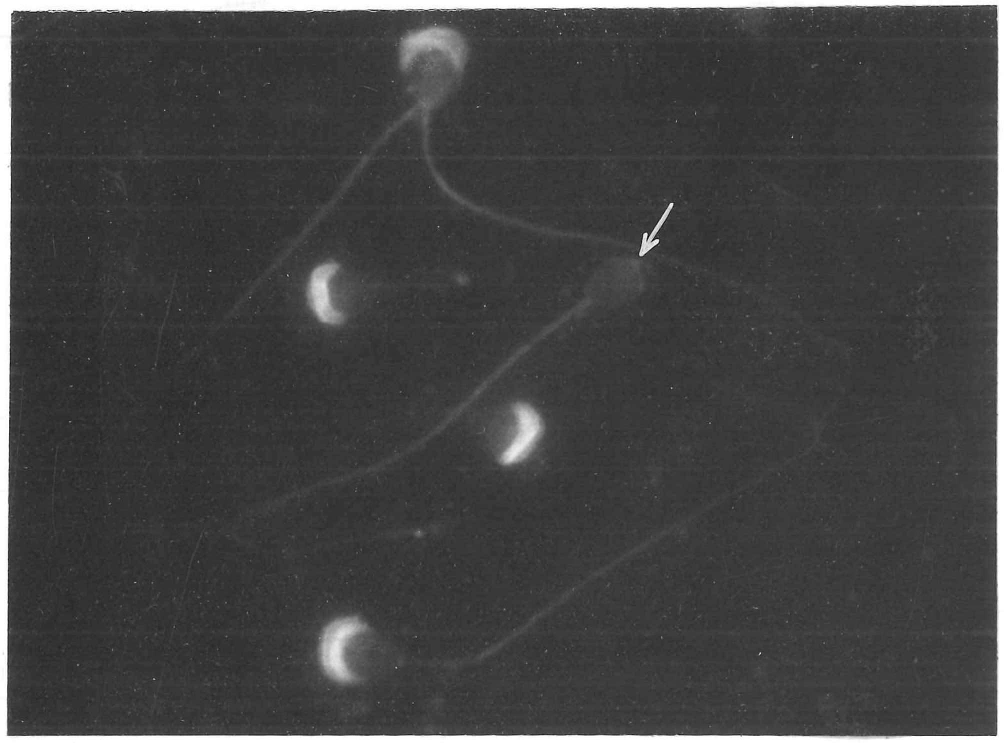
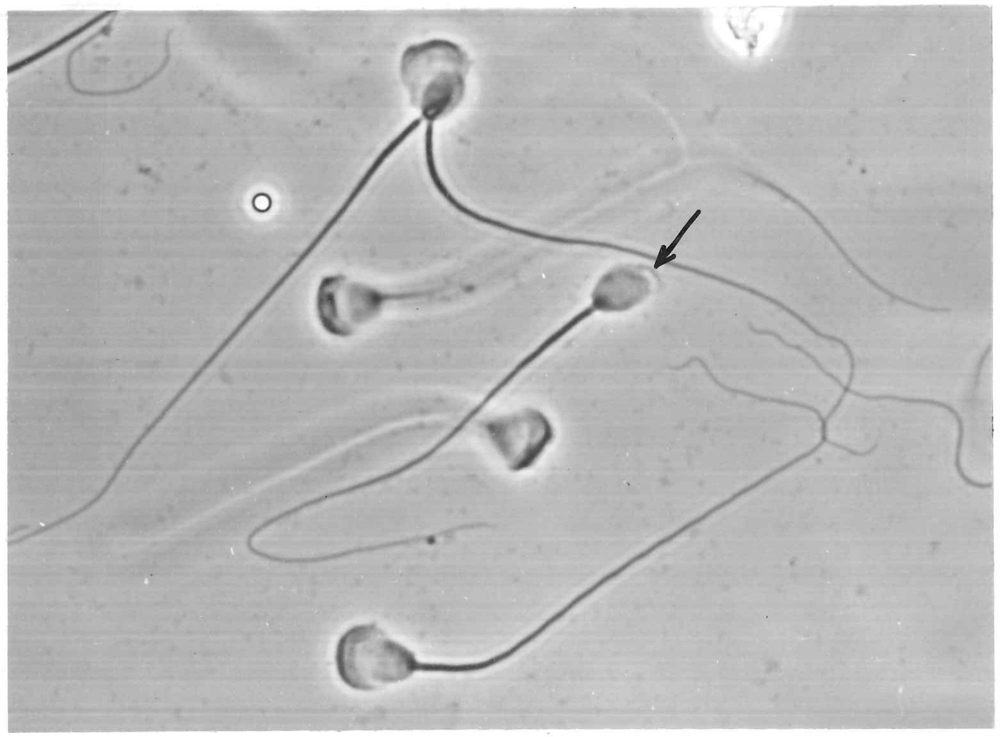


Figure 1.

Guinea-pig spermatozoa treated with normal guinea-pig gamma-globulin followed by fluorescein-conjugated antiserum to guinea-pig globulin. Note acrosomal fluorescence and absence of fluorescence on spermatozoon devoid of acrosome (arrowed). X 720.



Phase picture of same field. X 720.

Table 1. Reaction of acrosome with various test sera using two-step immune fluorescence.

Test solution	Fluorescein conjugate	Acrosomal fluorescence*
Normal male guinea-pig serum	Anti-guinea-pig globulin	+ + + + +
Normal female guinea-pig serum	Anti-guinea-pig globulin	+ + + + +
Normal guinea-pig serum	Anti-guinea-pig gamma-globulin	+ + + +
Guinea-pig serum followed by non-conjugated anti-guinea-pig globulin	Anti-guinea-pig globulin	+ +
Buffered saline	Anti-guinea-pig globulin	-
Normal rabbit serum	Anti-guinea-pig globulin	±
Normal guinea-pig serum	Anti-guinea-pig albumin	-
Normal guinea-pig serum	Normal rabbit serum	±
Normal guinea-pig serum	Non-conjugated fluorescein	-

*Brightness coded + to +++++, ± is dubious fluorescence, - is negative

Table 2. Acrosomal fluorescence after incubation of spermatozoa with absorbed sera.

Serum absorbed by	Acrosomal fluorescence.
Saline	+++++
spleen	++++ and +
kidney	++++
brain	++++
liver	++++
testis	++
spermatozoa	±
sheep red blood cells	++++

F2

Figure 2.

Histogram showing effect of pH on reaction between normal serum and spermatozoa.

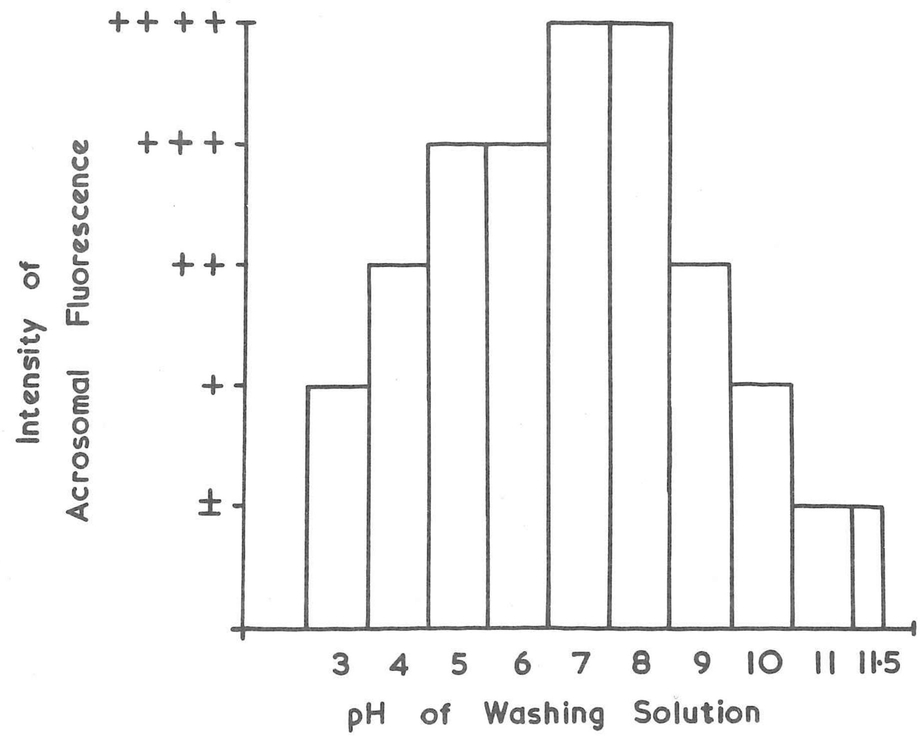
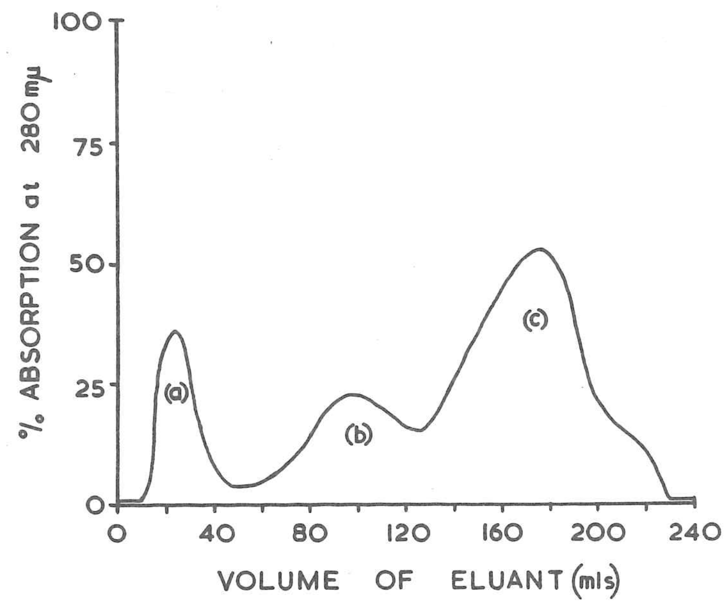
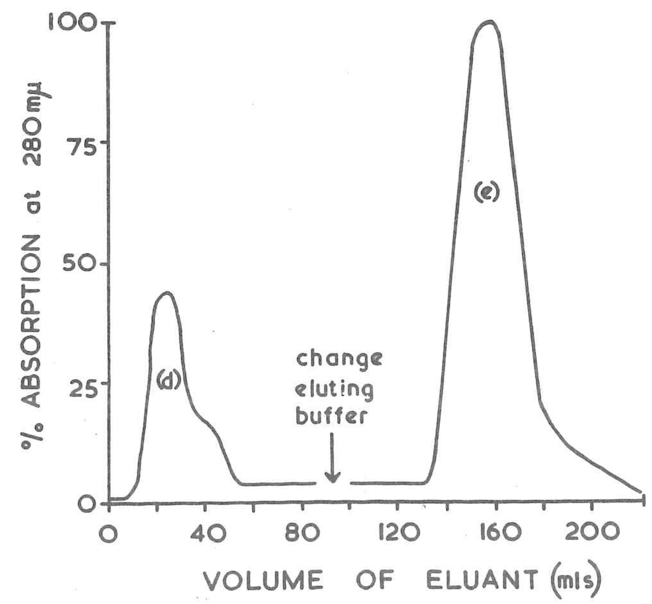


Figure 3.

Distribution of proteins from normal guinea-pig serum following fractionation by gel filtration and ion exchange chromatography.



Sephadex G-200 Fractionation of Guinea-pig Serum



DEAE-Cellulose Fractionation of Guinea-pig Serum

Figure 4.

The fractions shown in figure 3 were concentrated and analysed by immunoelectrophoresis. The activity against spermatozoa is indicated.

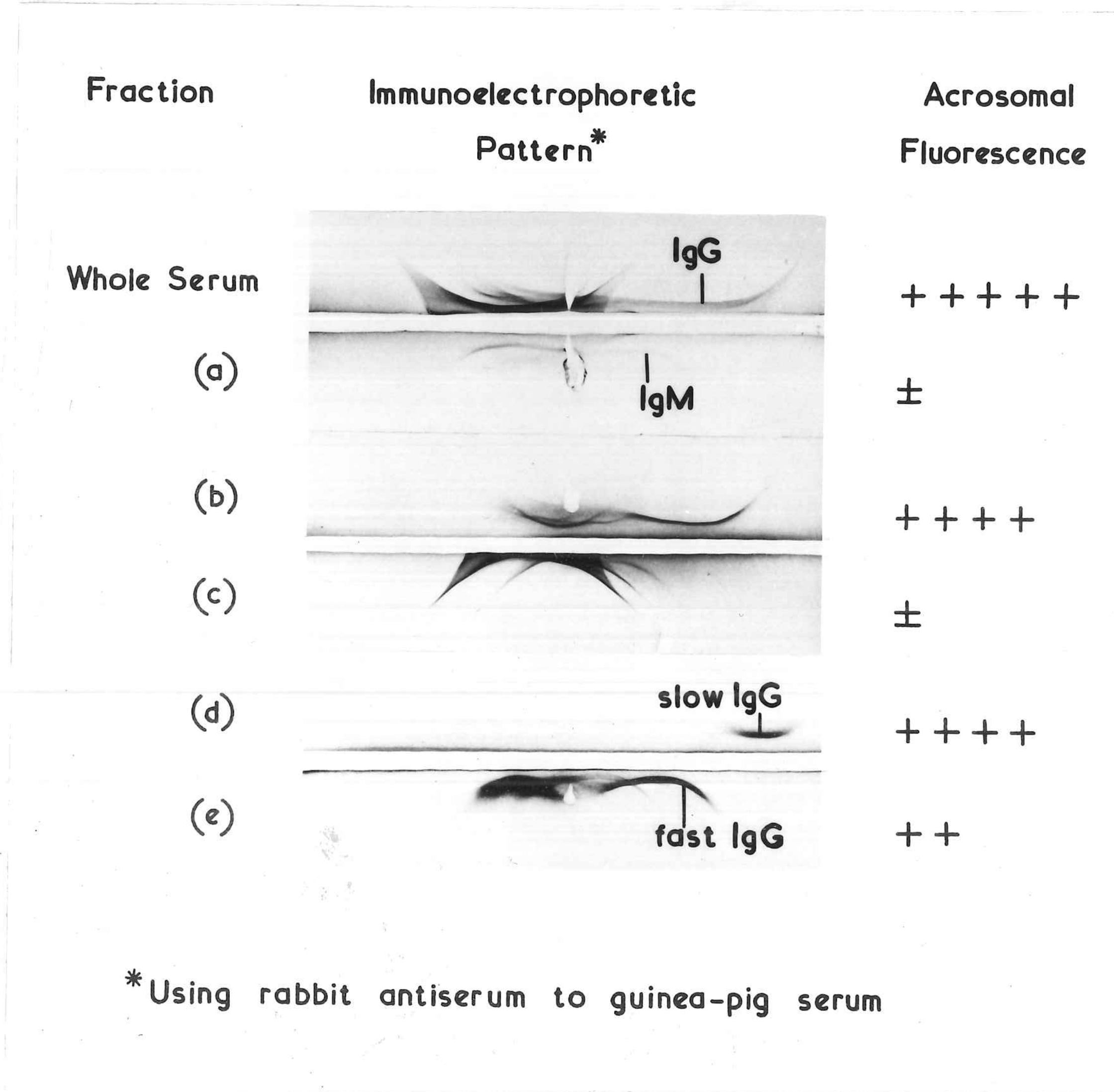


Figure 5.

Caput epididymidis of ram testis exposed.

Figure 6.

Caput epididymidis seperated from ram testis exposing ductuli efferentes connecting them (indicated with scalpel).

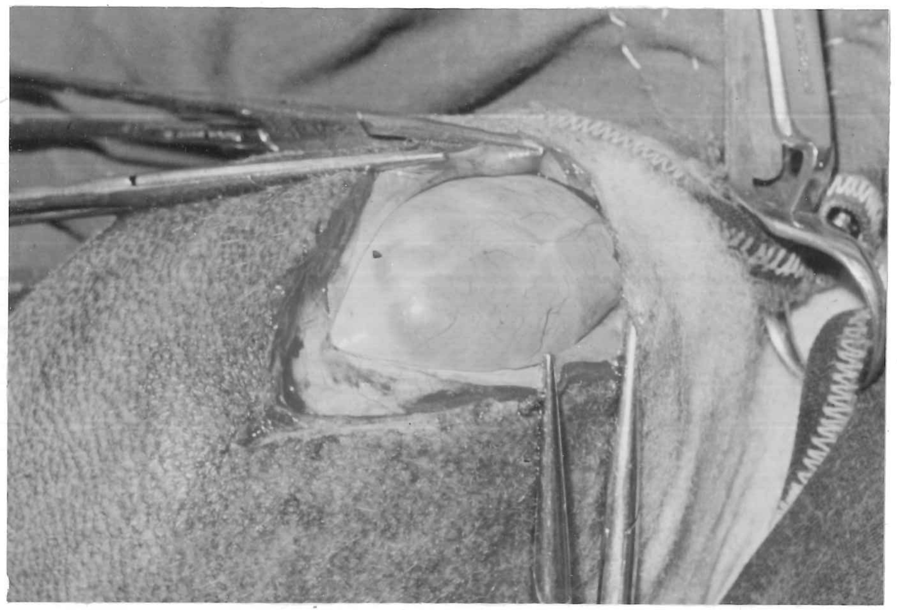


Figure 7.

Shaft of T-piece cannula inserted into ductuli efferentes. Cross-bar of cannula lies in crevice between caput epididymidis and testis.

Figure 8.

Caput epididymidis replaced and skin sutured. Cannula in place connected to tubing carrying away rete testis fluid from conscious ram.

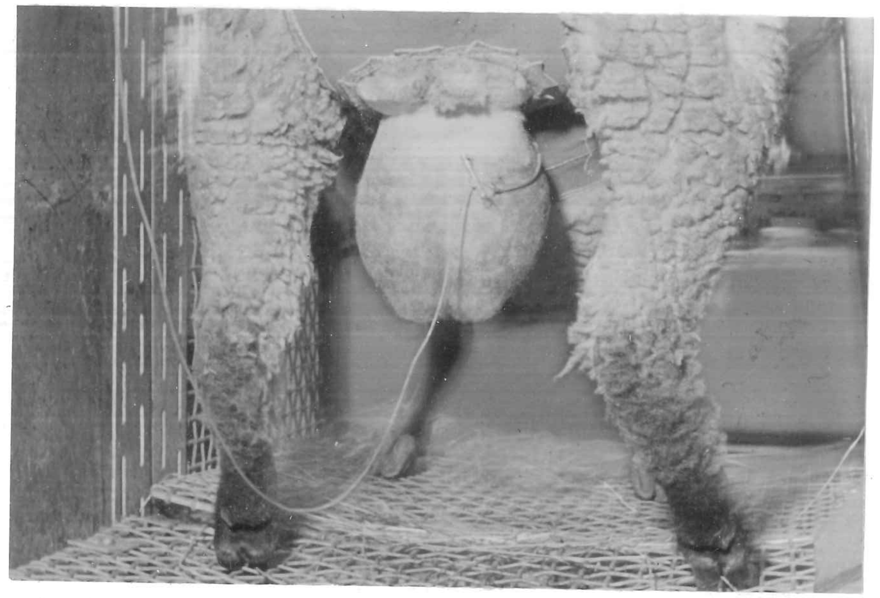


Table 3. Protein and immunoglobulin concentration in ram serum and rete testis fluids.

Sample	Protein concentration (Biuret) mgm/ml ± S.E.	Immunoglobulin concentration (radial immuno-diffusion) mgm/ml ± S.E.
Rete testis fluid from rams with daily spermatozoal output		
< 0.5 x 10^9	1.10 ± 0.26 [x]	0.04 ± 0.01 (both rows)
> 3. x 10^9	1.02 ± 0.32 [x]	
Ram serum [*]	89.00 ± 0.75	19.28 ± 0.15 [⊛]

x Values not significantly different (P = 0.01) by Mann-Whitney U test for nonparametric small samples.

* The protein concentration of ram testicular lymph is 40-60 mgm/ml (Cowie, Lascelles and Wallace, 1964).

⊛ Confirmed by cellulose acetate electrophoresis and analytical centrifugation.

Figure 9.

Phoroslide electrophoresis trace of

(a) Normal ram serum

(b) Concentrated rete testis fluid of ram.

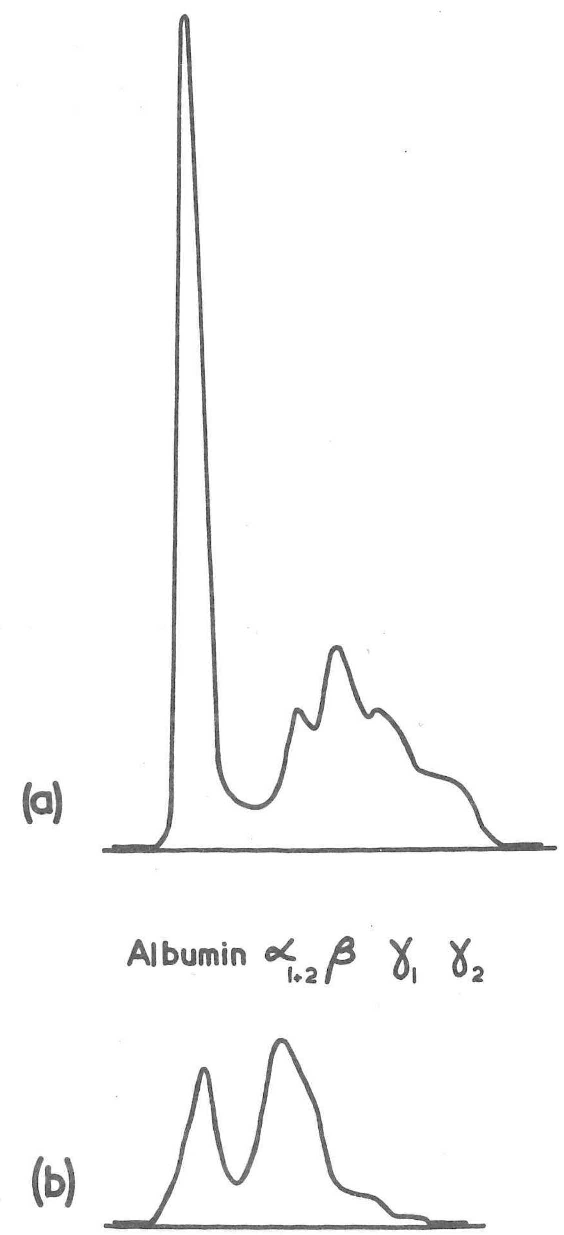


Figure 10.

Immunoelectrophoresis.

Troughs

Upper two - antiserum to ram serum (rabbit)

lower one - antiserum to rete testis fluid of ram (rabbit)

Wells

(a) and (e) Normal ram serum

(b) Unconcentrated ram rete testis fluid

(c) and (f) Concentrated ram rete testis fluid

(d) Ram seminal plasma

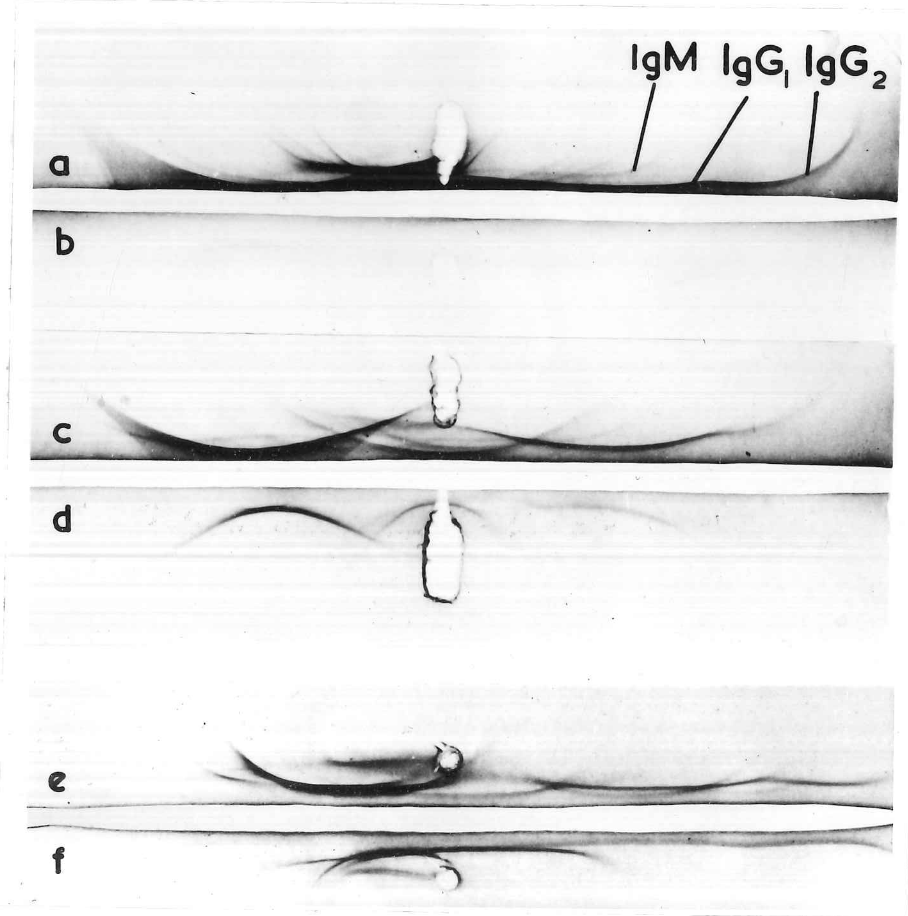


Table 4. Classification of stages of spermatogenesis in the guinea-pig

Stage	Cell types at each stage			
1 (figure 11)	spermatogonia A	resting primary spermatocyte	pachytene primary spermatocyte	pale round spermatid
2 (figure 12)	spermatogonia A	leptotene primary spermatocyte	pachytene primary spermatocyte	oval spermatid
3 (figure 13)	spermatogonia A	leptotene primary spermatocyte	pachytene primary spermatocyte	elongating spermatid
4 (figure 14)	spermatogonia A	zygotene primary spermatocyte	dividing and secondary spermatocytes	elongating spermatid
5 (figure 15)	spermatogonia A	pachytene primary spermatocyte	dark round spermatid	deeply embedded elongated spermatid
6 (figure 16)	spermatogonia A and Intermed-iate	pachytene primary spermatocyte	dark round spermatid	embedded elongated spermatid
7 (figure 17)	spermatogonia A and B	pachytene primary spermatocyte	dark round spermatid	luminally embedded elongated spermatid
8 (figure 18)	spermatogonia A and B	pachytene primary spermatocyte	pale round spermatid	luminal and free spermatozoa

Spermatogenic stages in the guinea-pig

X480

Figure 11.

Stage 1.

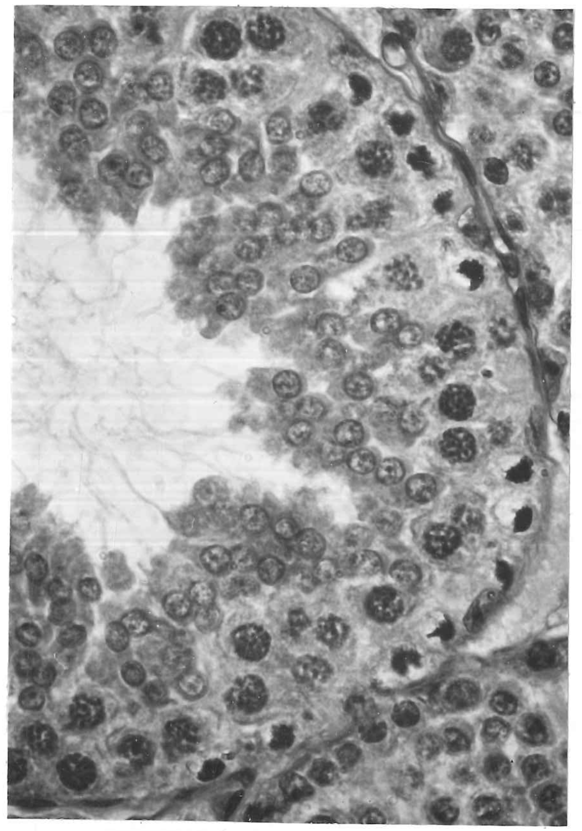


Figure 12.

Stage 2.

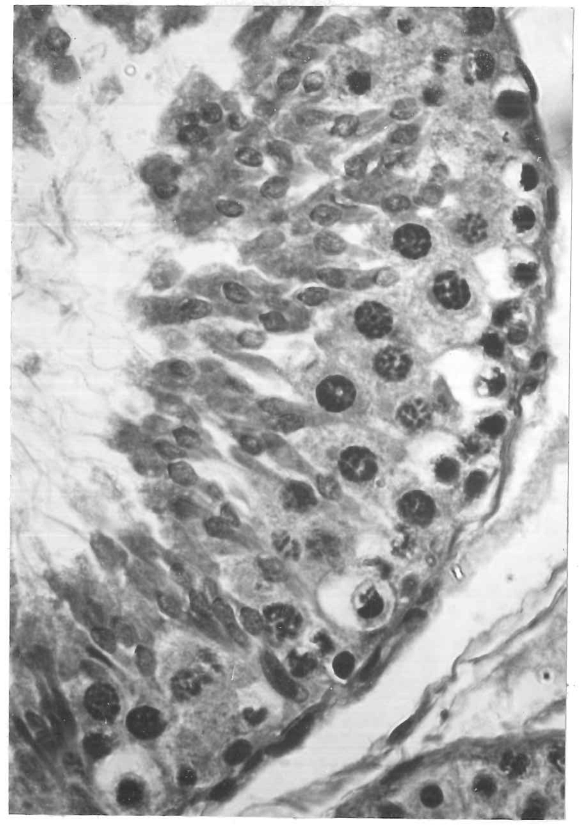


Figure 13.

Stage 3.

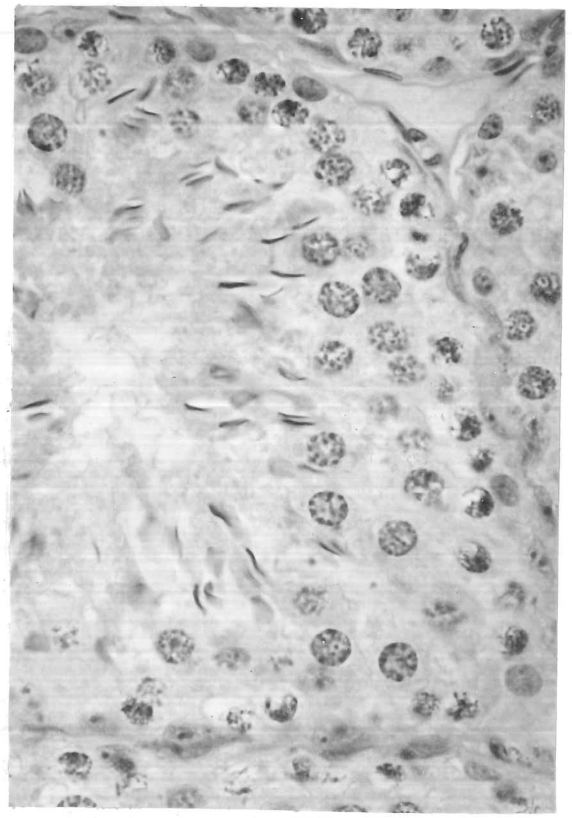
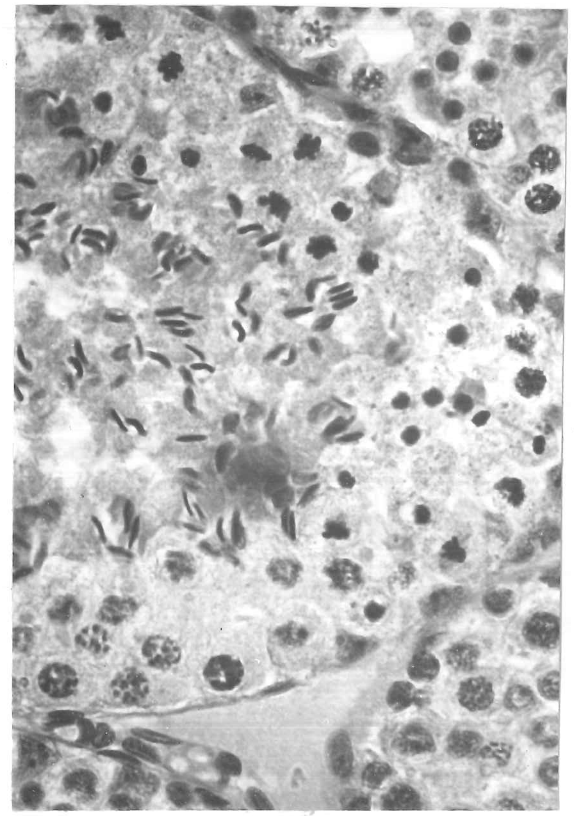


Figure 14.

Stage 4.



Spermatogenic stages in the guinea-pig

X480

Figure 15.

Stage 5

Figure 16.

Stage 6

Figure 17.

Stage 7

Figure 18.

Stage 8

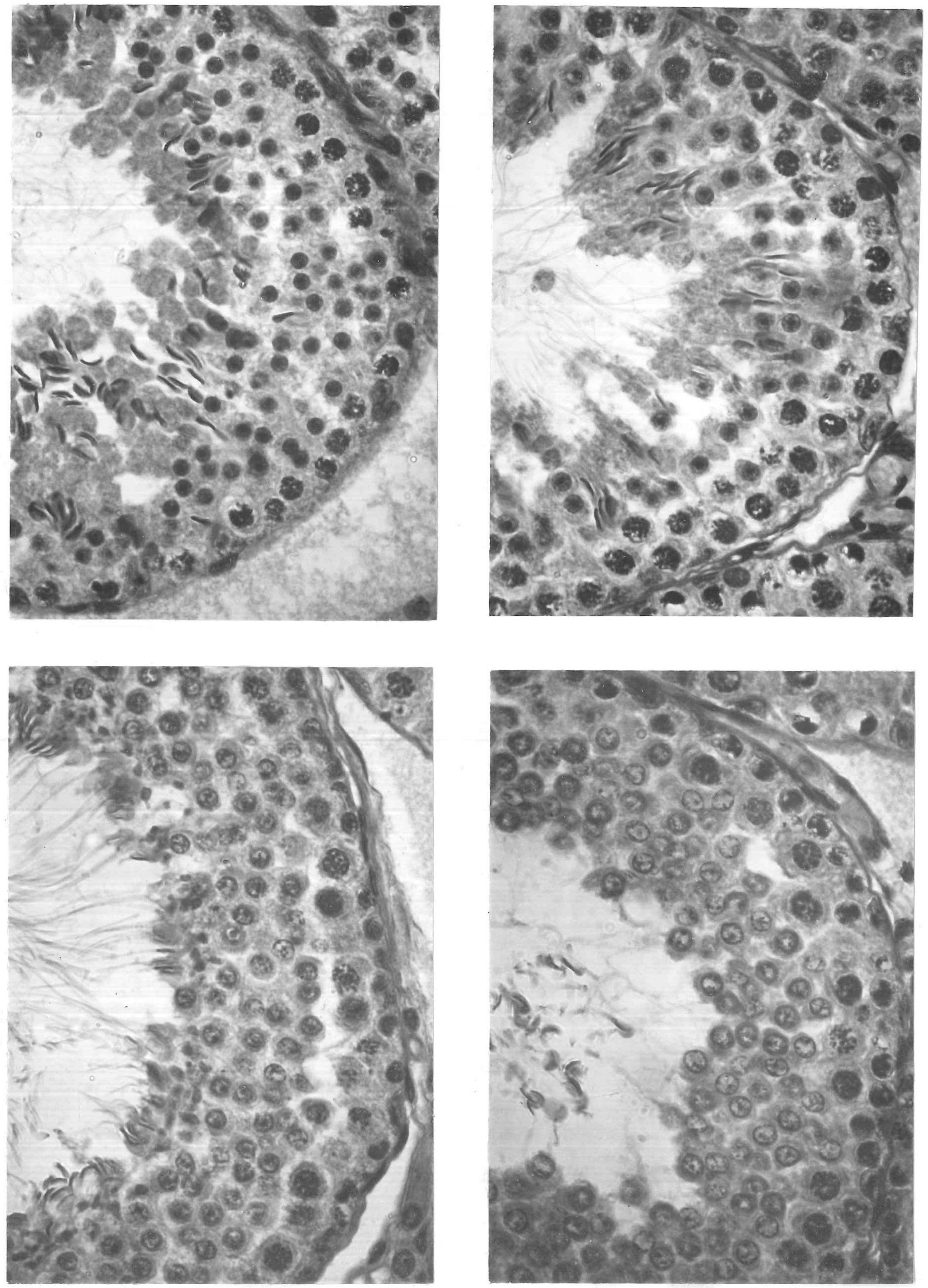
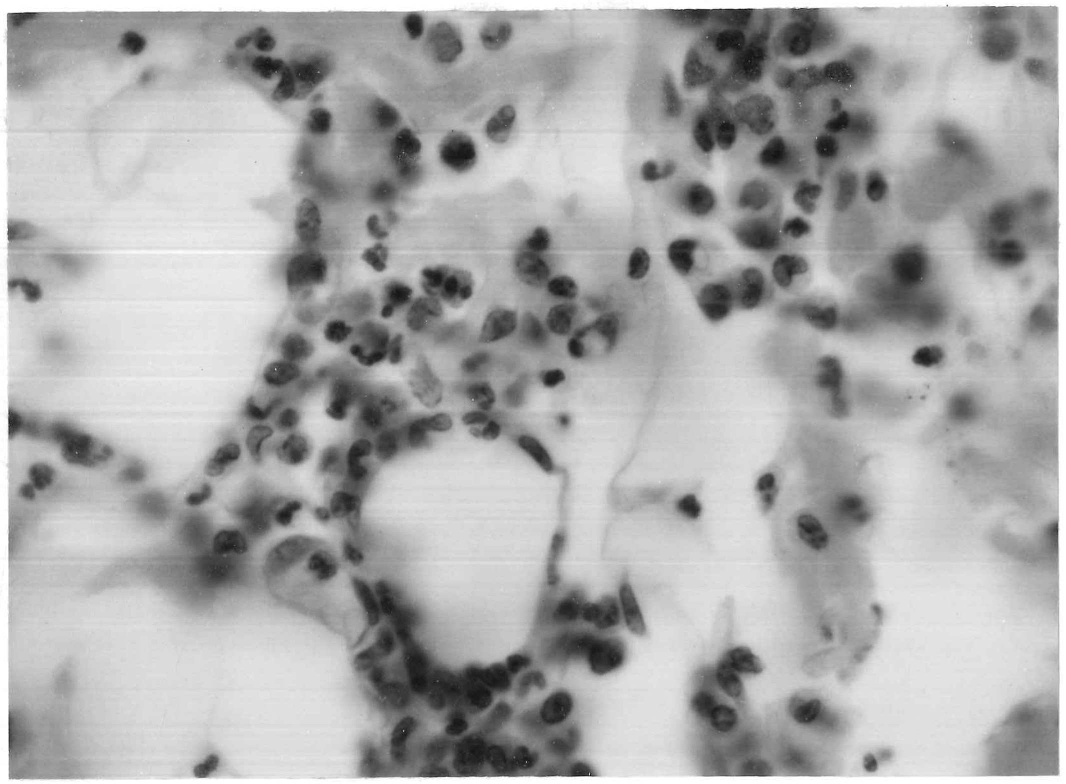


Figure 19.

Histology of skin response 24 hours after intradermal injection of soluble sperm antigen (guinea-pig) into guinea-pig immunized with isologous testis. X720

Figure 20.

Low power view of rete testis tubules in testis of guinea-pig isoimmunized with testis. Note leucocytic invasion. X180



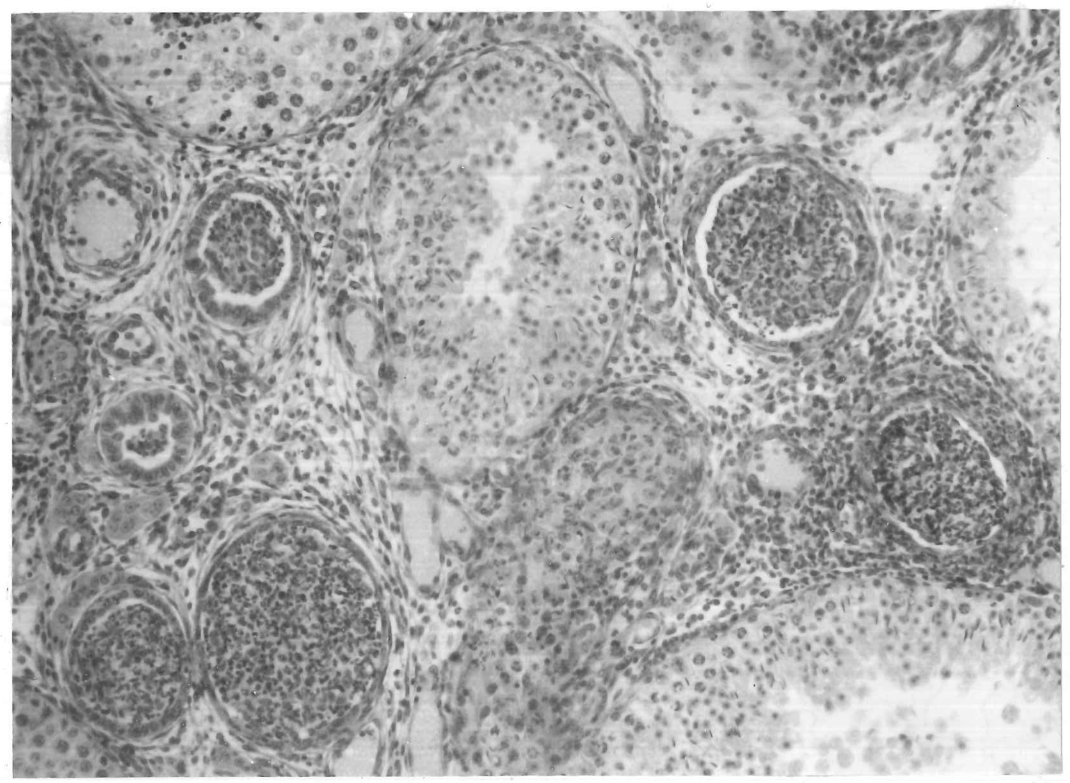
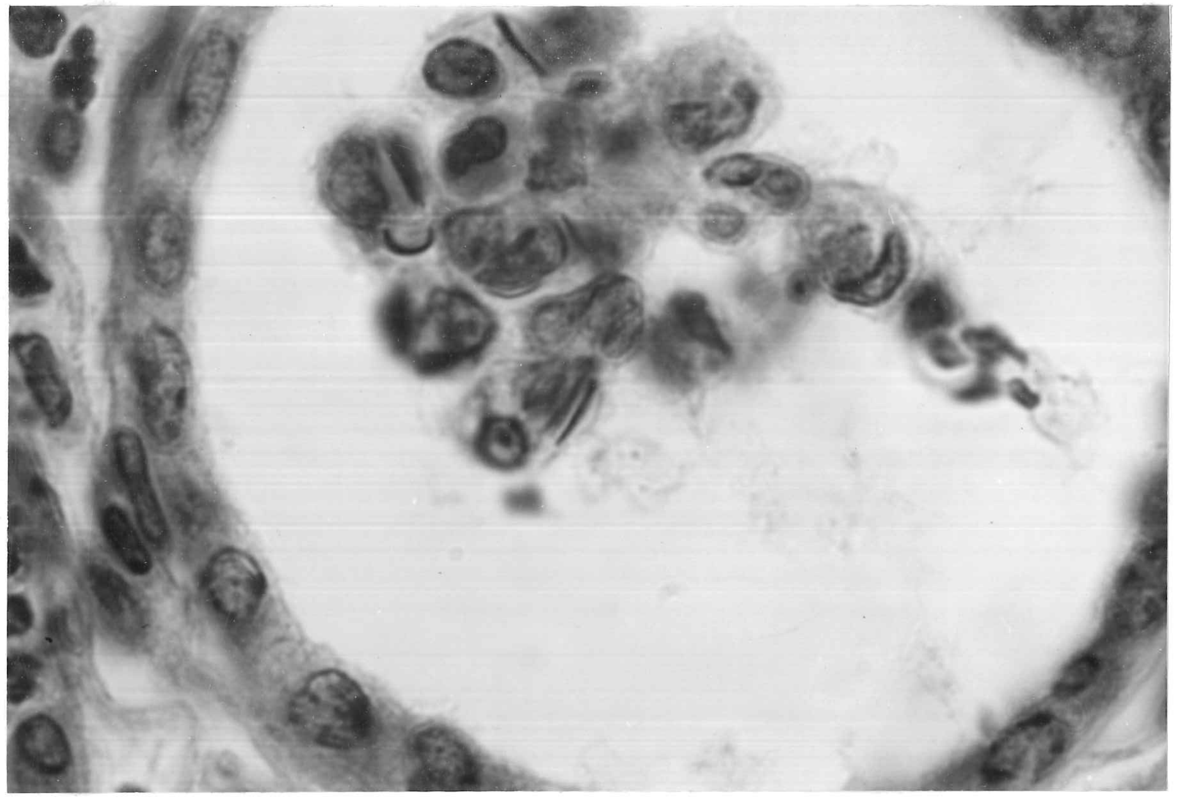


Figure 21.

High power view of part of figure 20. Note phagocytosed spermatozoa within rete testis. ~~X720~~ X 1800

Figure 22.

Ductuli efferentes containing leucocytes. X720



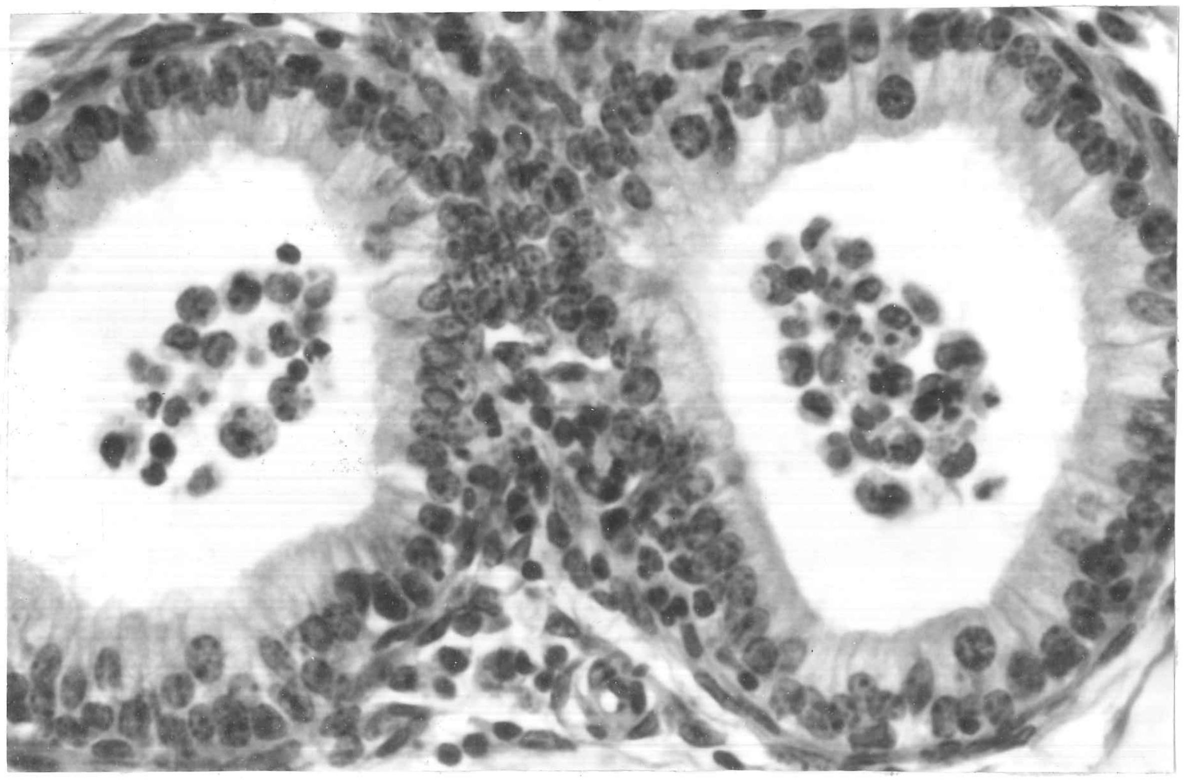
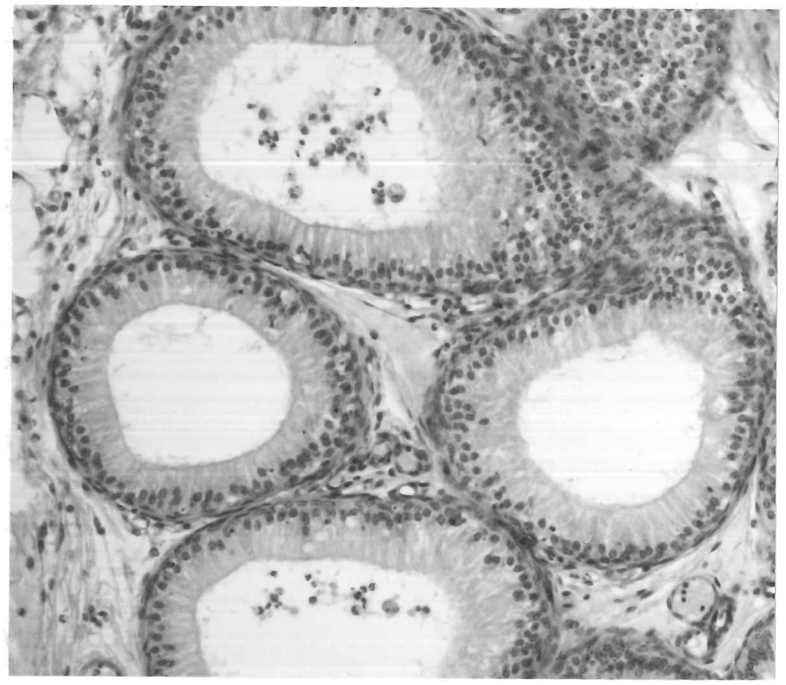


Figure 23.

Leucocytes in lumen of epididymis. Note absence of inflammation interstitially. X290

Figure 24.

Interstitial granuloma in epididymis. X290



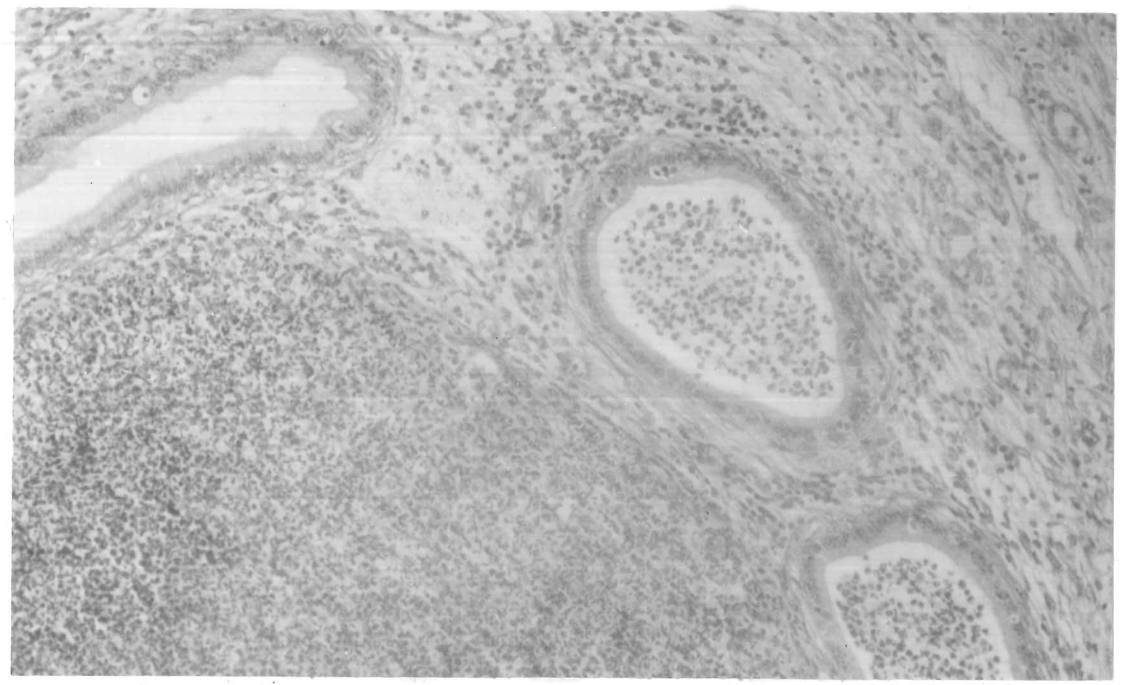
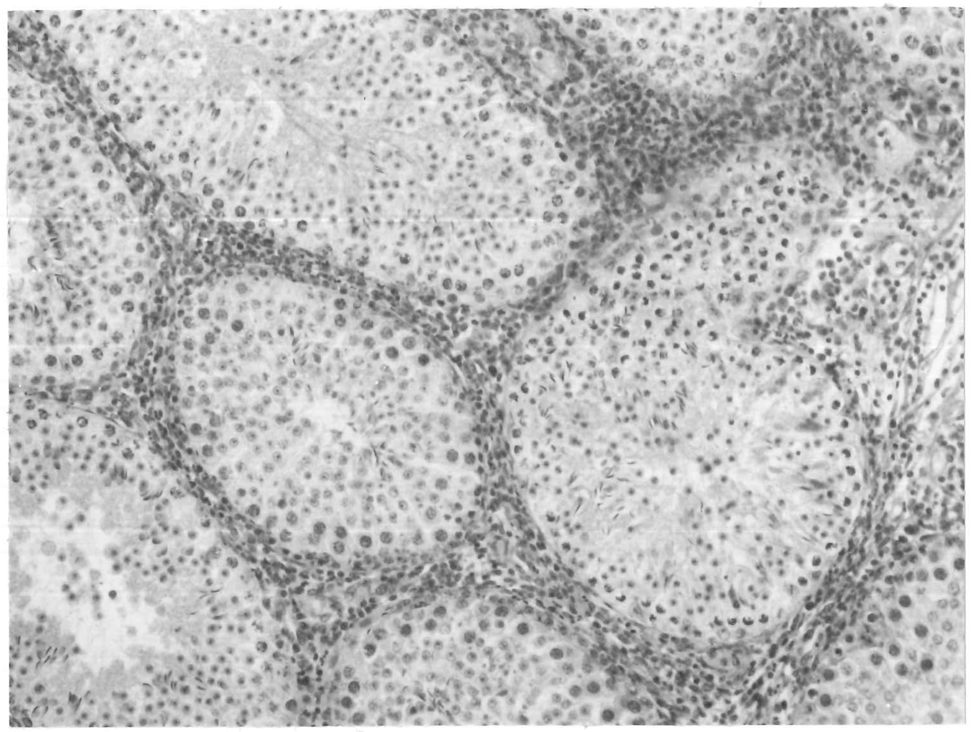


Figure 25.

Interstitial inflammation around tubules showing normal spermatogenesis. X180

Figure 26.

Leucocytes within seminiferous tubule near rete testis. X180



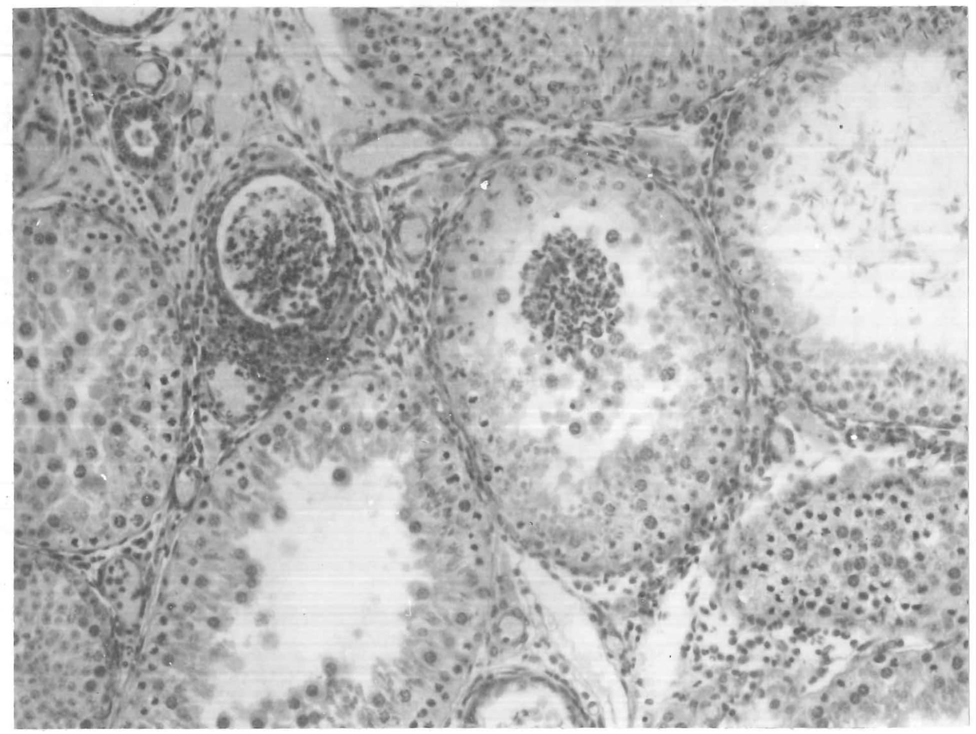


Figure 27.

Eosinophils within seminiferous tubule. ~~X480~~
X 1600

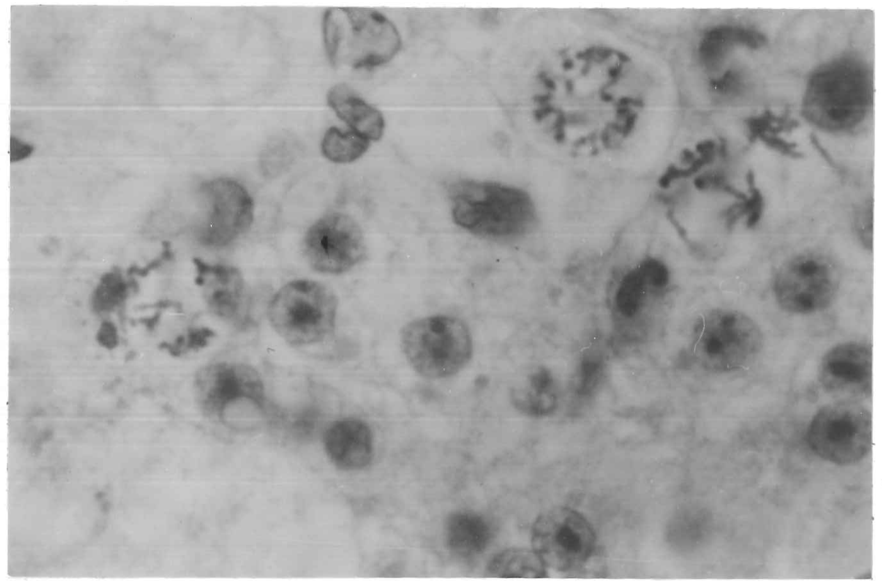


Figure 28.

Phagocytic mononuclear cells in seminiferous tubule. X720

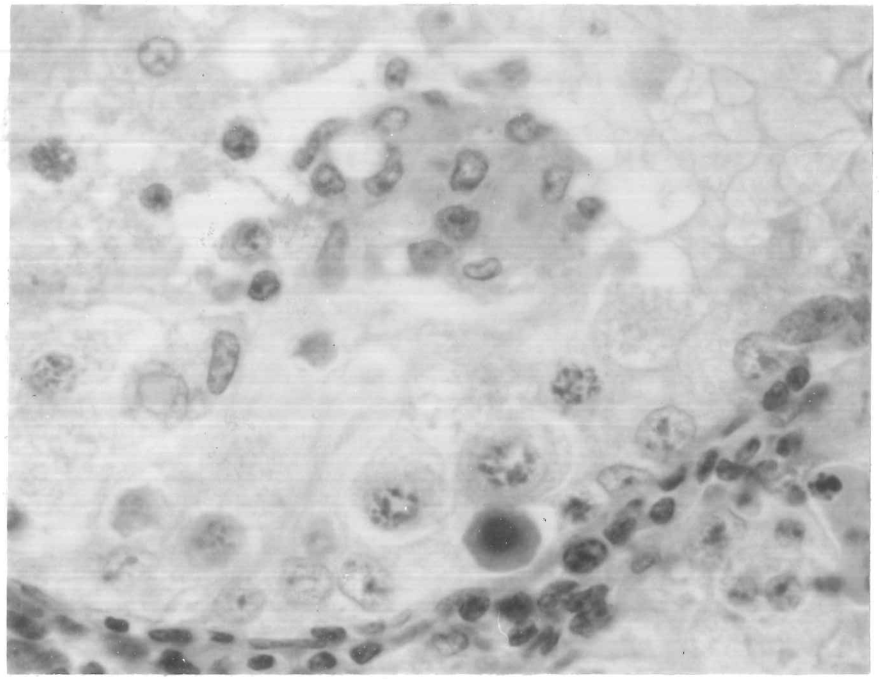


Figure 29.

Eosinophil crossing basement membrane of seminiferous tubule. ~~X480~~ X2520

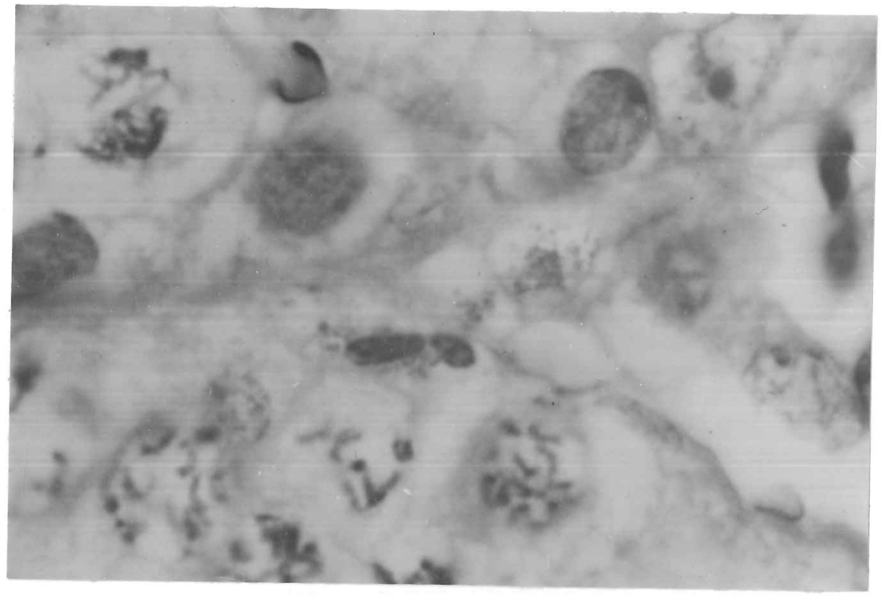


Figure 30.

Lymphatics distended with mononuclear cells. X720

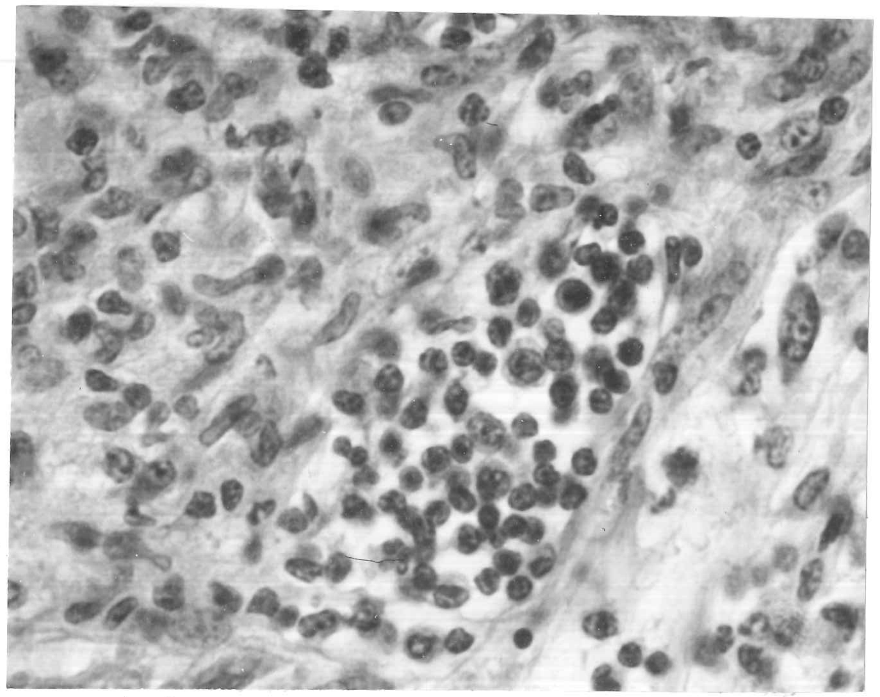


Figure 31.

Tubules distended with fluid unable to flow out at inflamed rete testis. Note atypical presence of free spermatozoa at spermatogenic cell stage 5. X 180

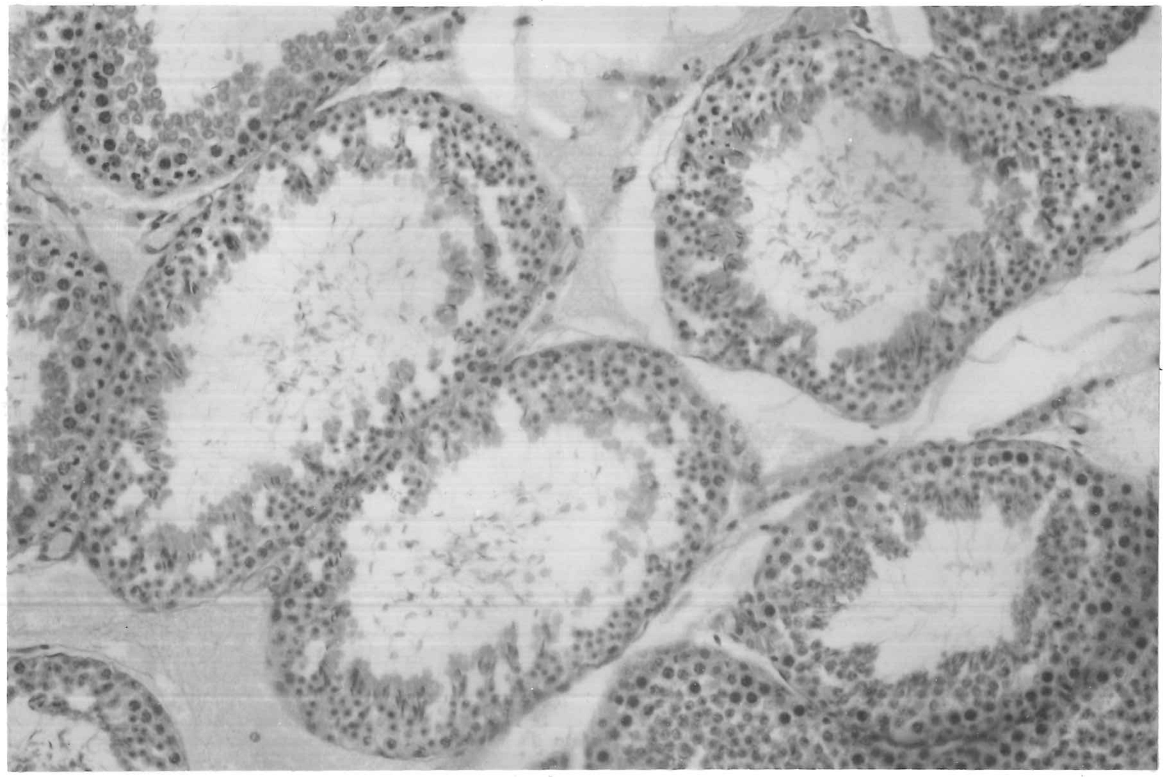


Table 5. Tabulations of damage in one tubule traced from the rete testis, in testis from an animal isoimmunized with testis.

Stage	Rete Testis	6?	7	5/6	1/8	1	1/8		Late 8		Early 8	
Degree of damage	+++	+++	++	++	++	-	-		±		+	1 and 3
											-	
	±	-	-	-	-	-	-		-		-	
1	8	1/2/3/5	1	4/3	5	5	3/4		3		3/4	
	2	2	1	6/7	6/7	6/7	7	7	7		8	
	-	-	-	++	++	++	+	±	±		++	8
											++	
	-	-	-	+	+	±	-	±	+	+	±	
3	2	1	1	5/6	5/6	8	1	1	8	8	8	
	4	6	6/7	7								
	-	-	-	-	undamged from here on							

The solid line represents the tubule which was serially sectioned. In each section (35μ) the stage(s) of spermatogenesis was scored (1-8) and the degree of damage assessed (-, ±, +, ++ or +++).

Table 6. Frequency of occurrence of different spermatogenic stages in testes partially damaged after iso-immunization with testis (expressed as percentage of total tubules counted).

Animal	Stage	1	2	3	4	5	6	7	8	Damaged
Normal data pooled from four animals		5.8	6.6	6.3	6.9	18.3	28.1	12.1	15.8	0.1
Immunized	1	6.5	6.6	7.1	6.8	15.3	13.2	4.6	9.2	30.8
"	2	4.7	5.3	6.3	7.4	10.9	7.7	7.0	6.0	44.7
"	3	7.4	5.1	2.8	4.0	9.7	5.0	1.4	4.9	59.7
"	4	4.7	4.9	3.9	4.0	7.4	4.0	2.4	4.8	64.0
"	5	4.9	3.1	1.8	2.4	5.8	2.7	0.0	2.3	80.9

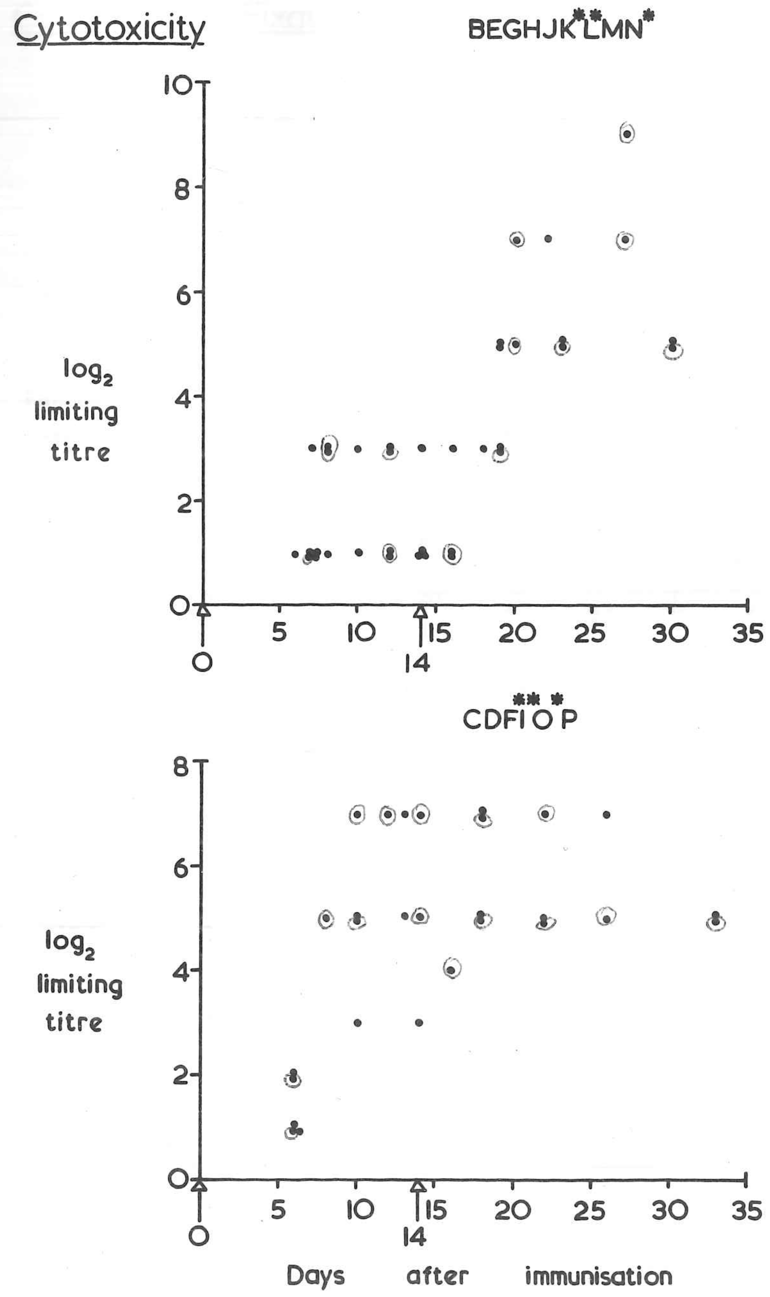
Values to the right of the line are significantly different from control values at a level of P = 0.01 (x^2)

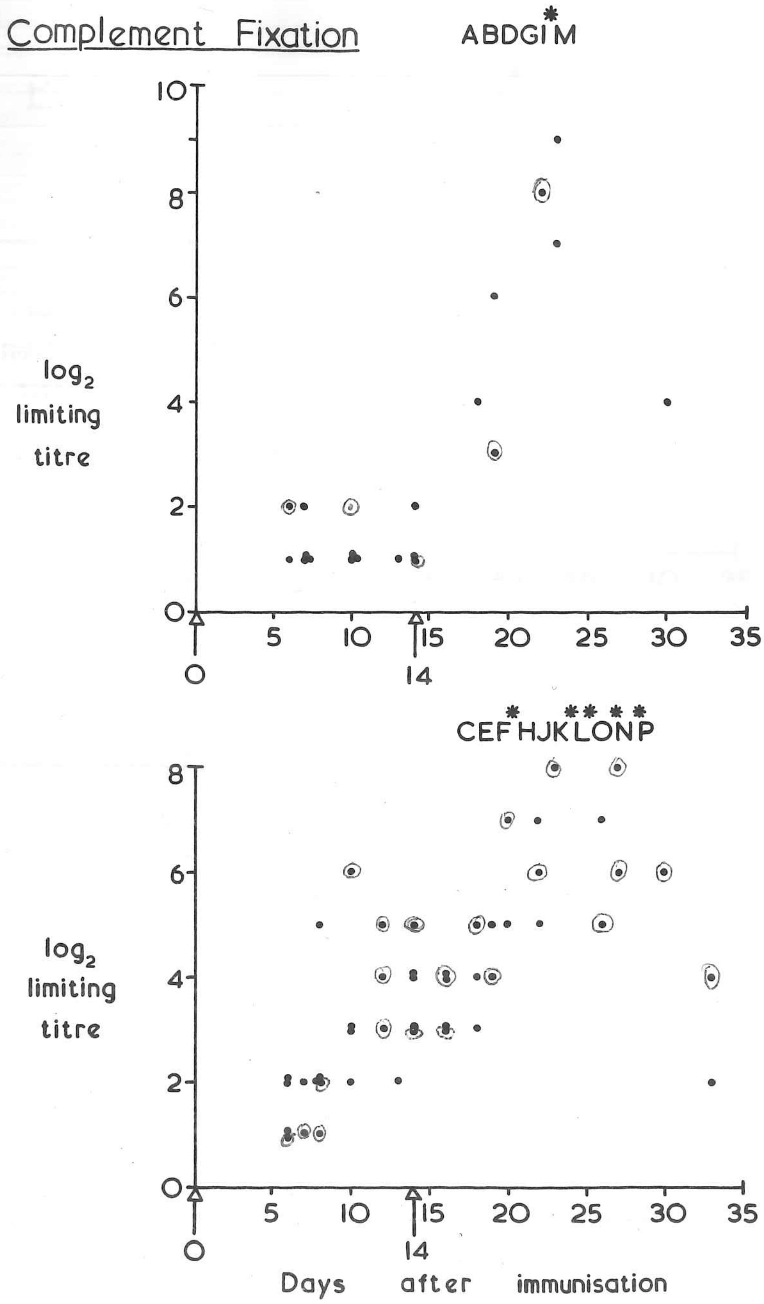
F 32-34

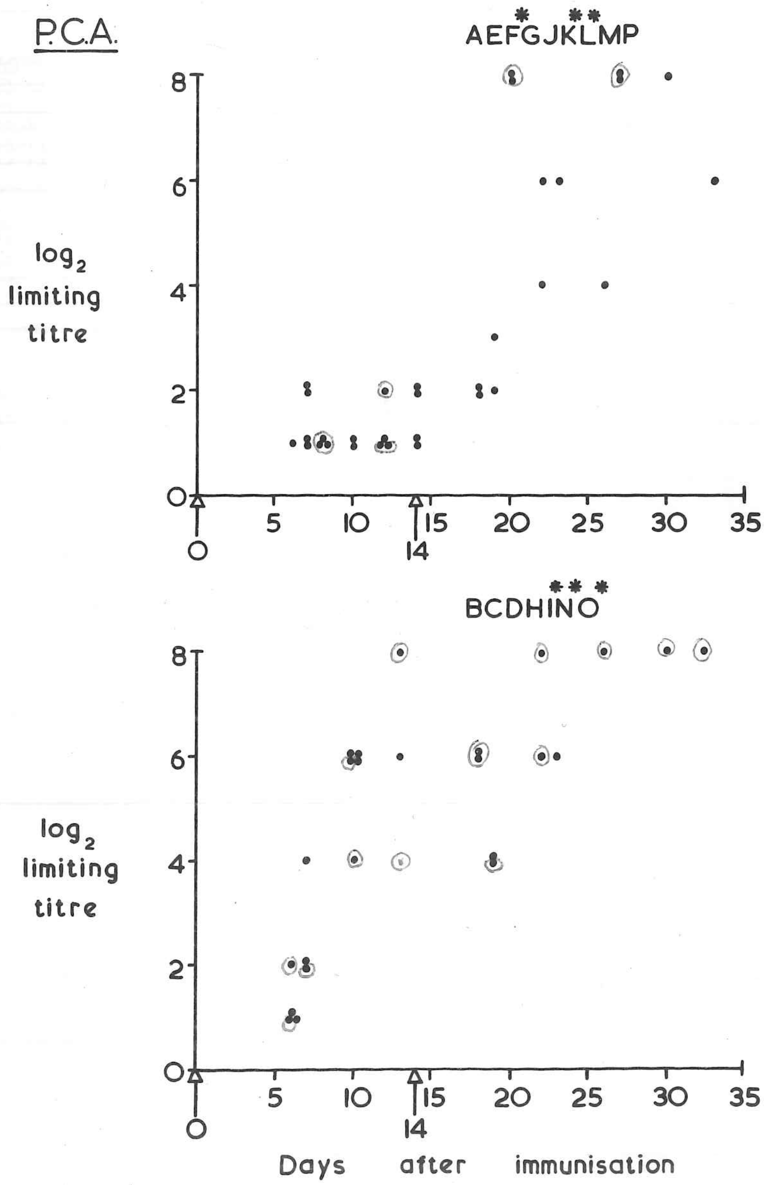
Figures 32 - 34.

Graphs showing rise in titres of cytotoxic, complement - fixing and passive cutaneous anaphylactic antibodies following isoimmunization with testis. Upper graph shows results from animals responding to secondary immunization and lower graph results from animals responding to primary immunization. Asterisks indicate animals which developed testicular lesions.

The points representing values from these animals are ringed.




Complement Fixation
ABDGI*M
log2 limiting titre
CEF*HJ**KL**O**N*P
log2 limiting titre
Days after immunisation



P.C.A.
AEFGJKLMP
log2 limiting titre
8
6
4
2
0
5
10
15
20
25
30
35
0
14
BCDHINO
log2 limiting titre
8
6
4
2
0
5
10
15
20
25
30
35
0
14
Days after immunisation

Table 7. Testicular damage, delayed skin reaction and antibody titres in guinea-pigs isoimmunized with testis.

Animal	Day of sacrifice	Testicular damage	Delayed Hypersen-sitivity	Final PCA titre	Final complement fixing titre	Final cytotoxi-city titre
A	7	-	-	4	8	8
B	10	-	-	64	2	2
C	13	-	-	256	8	128
D	13	-	-	64	8	32
E	16	-	-	4	16	8
F	16	+	-	4	16	32
G	19	-	+	4	128	32
H	19	-	±	16	64	32
I	22	+	+	256	512	128
J	22	-	+	64	64	128
K	27	+	+	256	512	128
L	27	+	+	256	128	524
M	30	-	+	256	32	32
N	30	+	+	256	128	32
O	33	+	-	256	32	32
P	33	-	+	64	32	32

Table 8. Testicular damage and immune responsiveness in maturing male guinea-pigs isoimmunized with testis.

Group	Age at death (days)	No. of animals with damaged testes	Most advanced cell type.	Mean of delayed skin response	Mean of early skin response	γ_2 response. Mean $\log_2$ limiting diln ± S.E.	γ_1 response. Mean $\log_2$ limiting diln ± S.E.	Presence of antigen in testis.	Presence of auto-reactive antibody.
1	54	0/8	Embedded elongated spermatid (stage 5/6)	++	+++	6.0 ± 0.5⊠	9.0 ± 0.1	+	+
2	64	4/10*	Long spermatids (stage 5/6/7)	+++	+++	4.5 ± 1.0⊠	8.0 ± 0.7⊛	+	+
3	74	0/8	Long spermatids luminal but not released (stage 6/7)	+++	++++	4.0 ± 1.0⊠	7.7 ± 0.9⊛	+	+
4	84	6/8	Spermatozoa in rete testis and vasa efferntia (stage 8)	++	+++	5.0 ± 1.0⊠	7.3 ± 0.8⊛	+	+
5	> 84	16/19	Spermatozoa in epididymis	++	+++	3.9 ± 0.8⊠	6.8 ± 0.9⊛	+	+

* Two damaged testes had spermatozoa in the excurrent ducts and were therefore mature. The other two were inflamed only at the rete testes which contained sloughed spermatocytes and early spermatids.

⊠ & ⊛ Values within vertical columns not significantly different by the Mann-Whitney U test for non parametric small samples.

Figures 35 - 37.

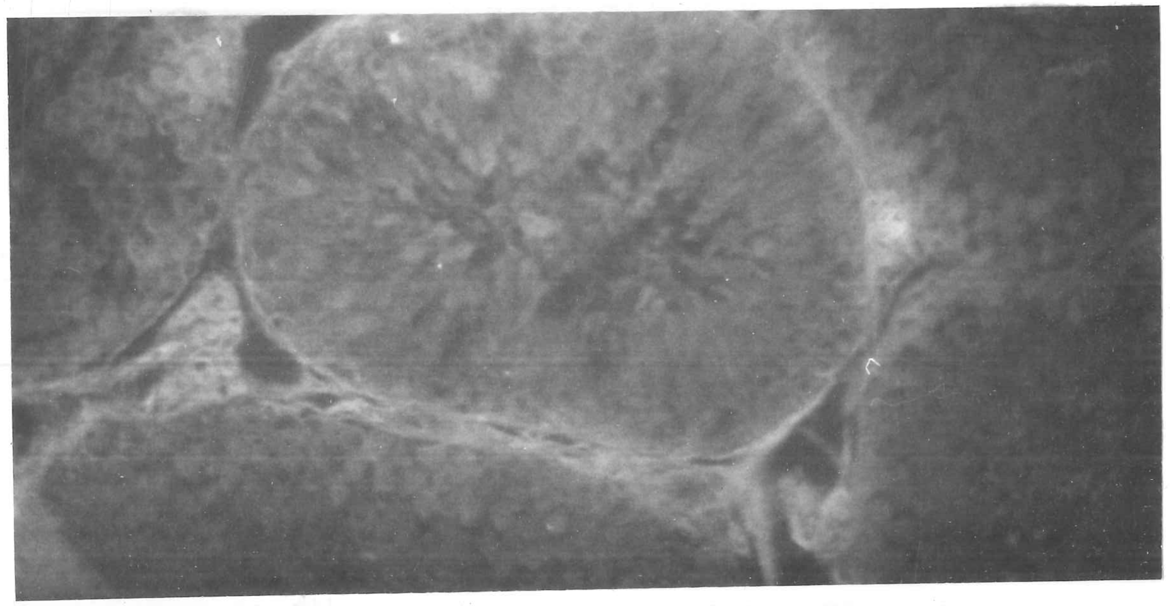
Sections of testis from a 74 day old guinea-pig twenty days after isoimmunization with testis following incubation with

35) autologous serum ($\frac{1}{10}$) followed by by conjugated anti gamma-globulin.

36) control serum ($\frac{1}{10}$) followed by conjugated anti gamma-globulin,

37) saline followed by conjugated anti gamma-globulin

X 180



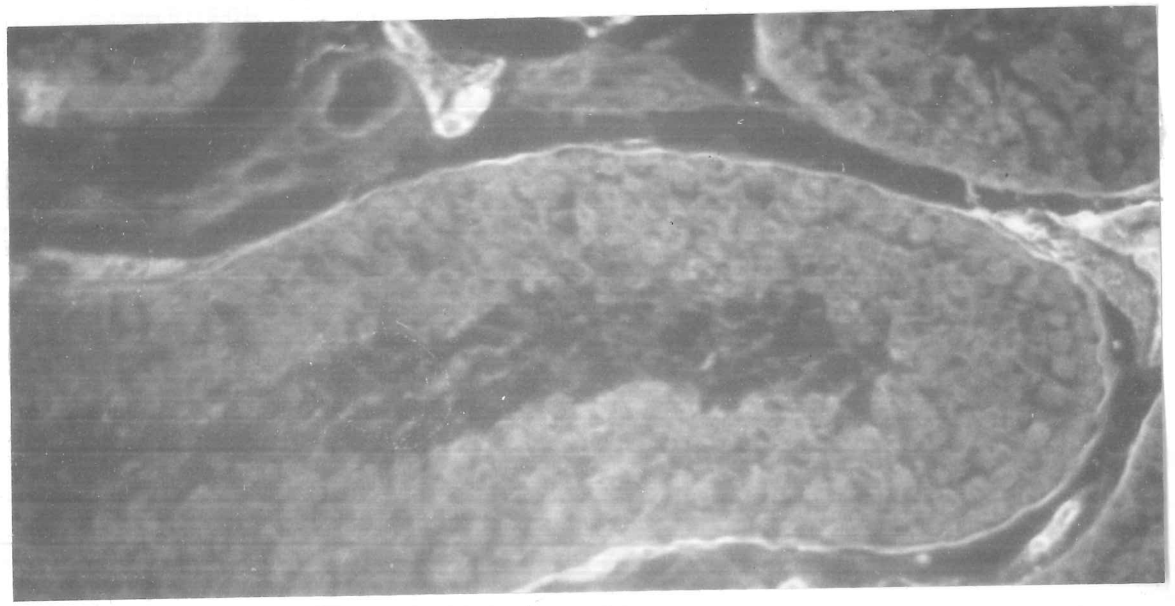


Table 9. Relative cytotoxicity of antisera for spermatids and spermatozoa.

Antiserum	Cell type	Number of dead cells at each dilution in 100 cells of same type						Diluent alone
		1/6	1/12	1/24	1/48	1/92	1/196	
1.	spermatozoa	100	100	78	51	24	29	20
	spermatids	80	65	41	35	39	40	38
2.	spermatozoa	100	100	100	70	34	30	33
	spermatids	91	80	65	40	35	43	40
3.	spermatozoa	100	100	100	100	98	64	20
	spermatids	100	90	79	67	58	50	38

Figure 38.

Graph showing relative cytotoxicity of antisera for spermatozoa and spermatids (data pooled from fiftenn antisera).

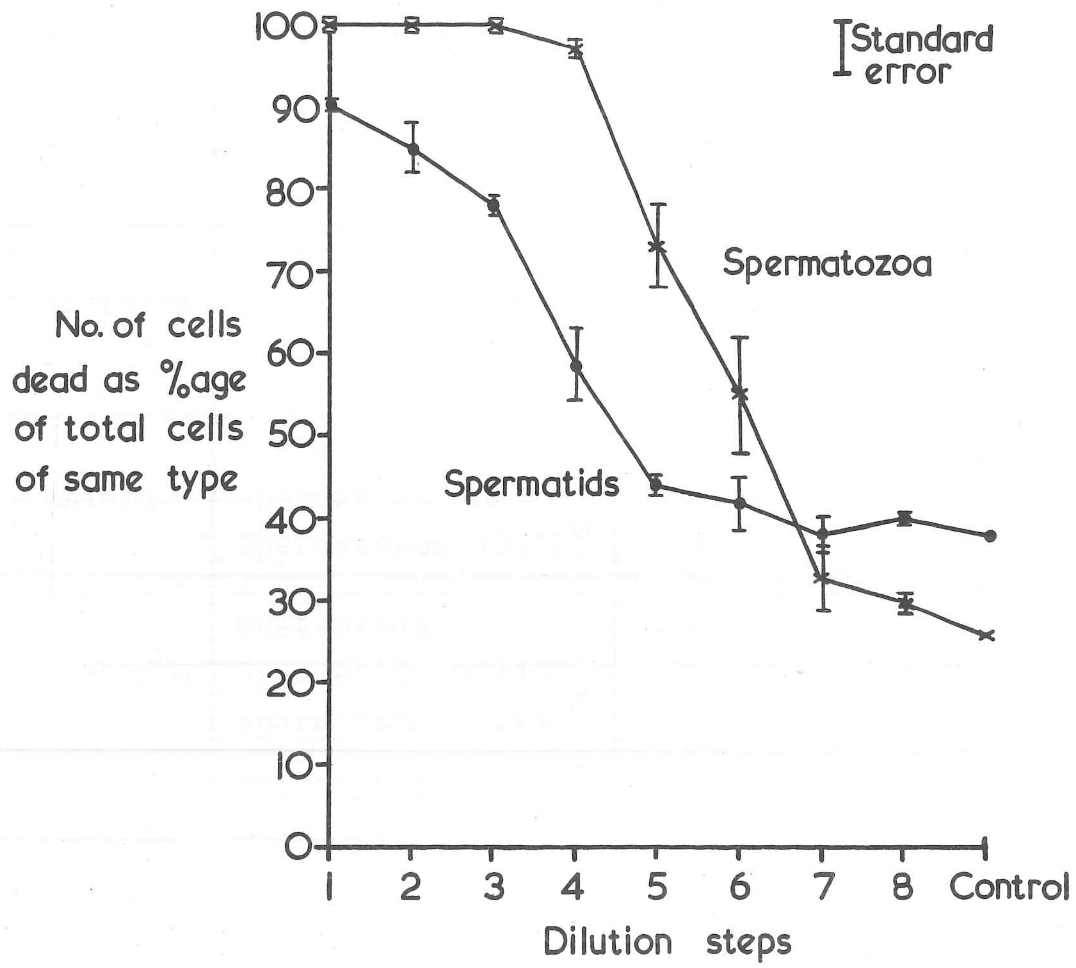


Table 10. Cytotoxicity of antisera after absorption with cells.

Anti-serum	Test cell	Absorbed by	Number of dead cells at each dilution in 100 cells of the same type					
			1/6	1/12	1/24	1/48	1/96	1/192
(a)	Spermatozoa	Unabsorbed	100	92	56	50	26	N.D.
		Spermatids 10×10^6	99	100	74	28	40	N.D.
		Spermatozoa 10×10^6	36	42	39	35	37	N.D.
	Spermatid	Unabsorbed	77	41	40	48	32	N.D.
		Spermatids 10×10^6	40	50	28	28	25	N.D.
		Spermatozoa 10×10^6	31	40	40	50	55	N.D.
(b)	Spermatozoa	Unabsorbed	100	100	99	89	64	40
		Spermatids 40×10^6	98	100	98	82	60	40
		Spermatozoa 40×10^6	96	90	69	49	44	40
	Spermatids	Unabsorbed	85	82	65	47	50	40
		Spermatids 40×10^6	60	70	72	68	40	37
		Spermatozoa 40×10^6	66	61	52	60	45	40
(c)	Spermatozoa	Unabsorbed	100	95	83	68	45	32
		Spermatids 40×10^6	100	80	75	44	38	18
		Spermatozoa 40×10^6	42	29	41	22	39	28
	Spermatids	Unabsorbed	84	60	42	45	37	40
		Spermatids 40×10^6	65	56	42	44	36	38
		Spermatozoa 40×10^6	39	38	36	29	43	26

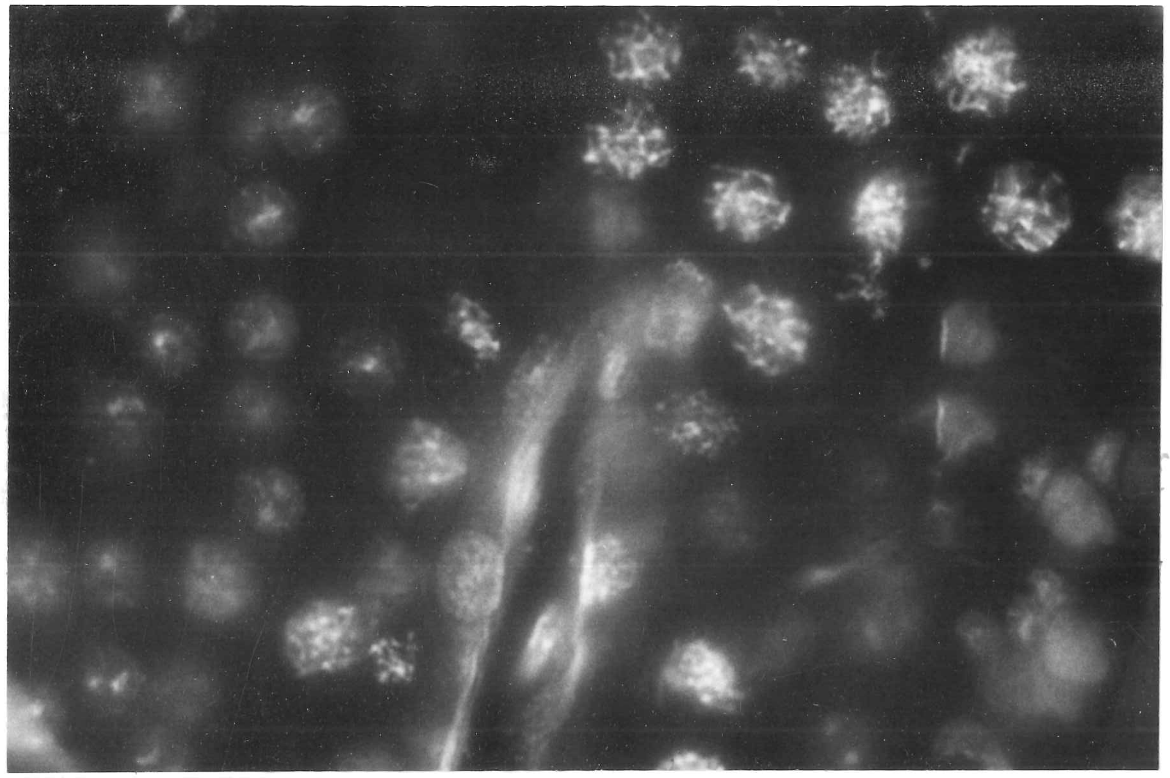
N.D. not done

Figure 39.

Guinea-pig testis following intratesticular injection of acriflavine. Note intratubular staining. X720

Figure 40.

Testis from 3 day rat after subcutaneous injection of acriflavine. Note intratubular staining. X720



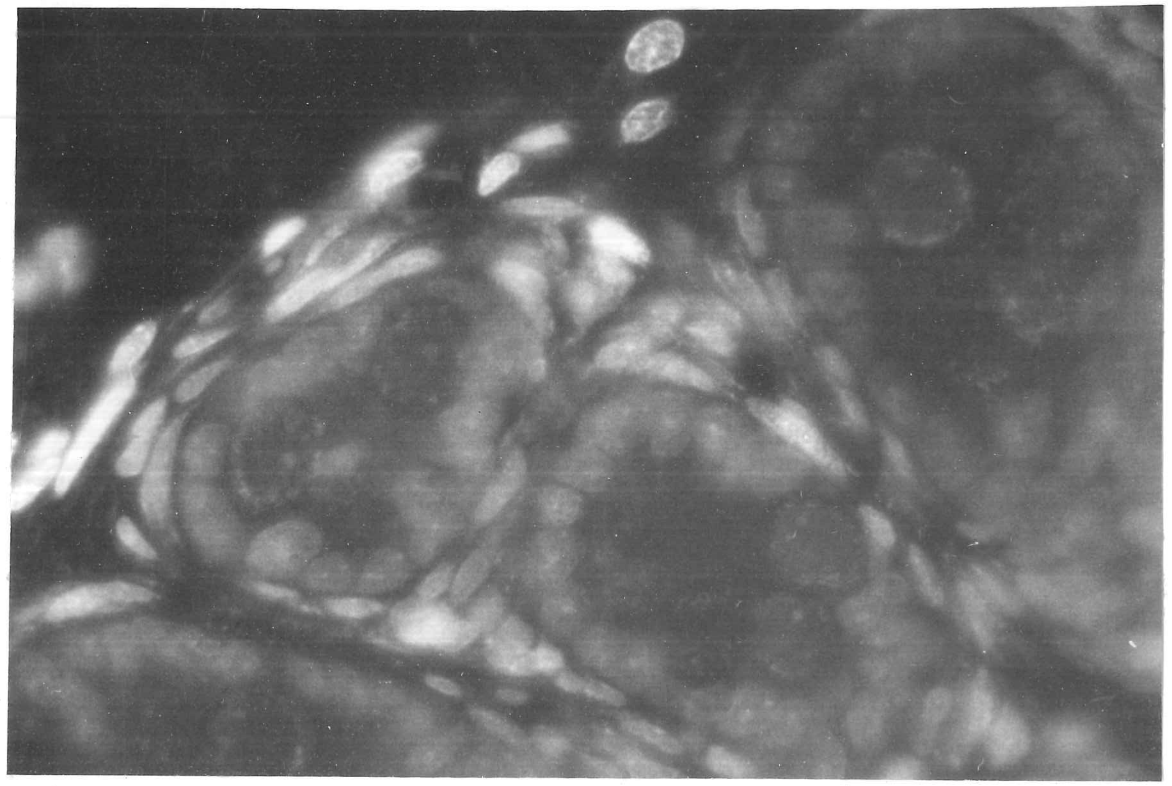


Table 11. Effect of daily administration of oestradiol benzoate to neonatal male rats on some features of testicular development.

Age at sampling (days	Experi-mental group	No. of animals	Acri-flavine* staining V I T	Tubular lumen	Most mature sperma-togenic stage	Mean testis length (mm) ± S.E.	Position* of testis	Presence* of alka-line phos-phatase V P.
3	All	5 in each	++ ++ +	-	sperma-togonia	1.8± 0.2	A	- -
15	(1)	9	++ ++ -	-	lepto-tene/ zygotene	5.6± 0.2	S	+ +
	(2)	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	(3)	5	++ ++ -	-	lepto-tene/ zygotene	3.4± 0.1	A	+ +
	(4)	5	++ ++ -	-	lepto-tene	2.8± 0.1	A	+ -
25	(1)	9	++ ++ -	+	late pachy-tene	10.0± 0.2	S	+ +
	(2)	6	++ ++ -	+	pachy-tene	4.1± 0.2	A	+ +
	(3)	7	++ + -	+	lepto-tene/ zygotene	3.6± 0.2	A	+ +
	(4)	4	++ ++ -	±	lepto-tene	3.8± 0.2	A	+ +
50	(1)	3	++ + -	+	Mature sperma-tids	15.2± 0.1	S	+ +
	(2)	3	++ ++ -	+	zygotene /pachy tene	4.6± 0.2	A	+ +

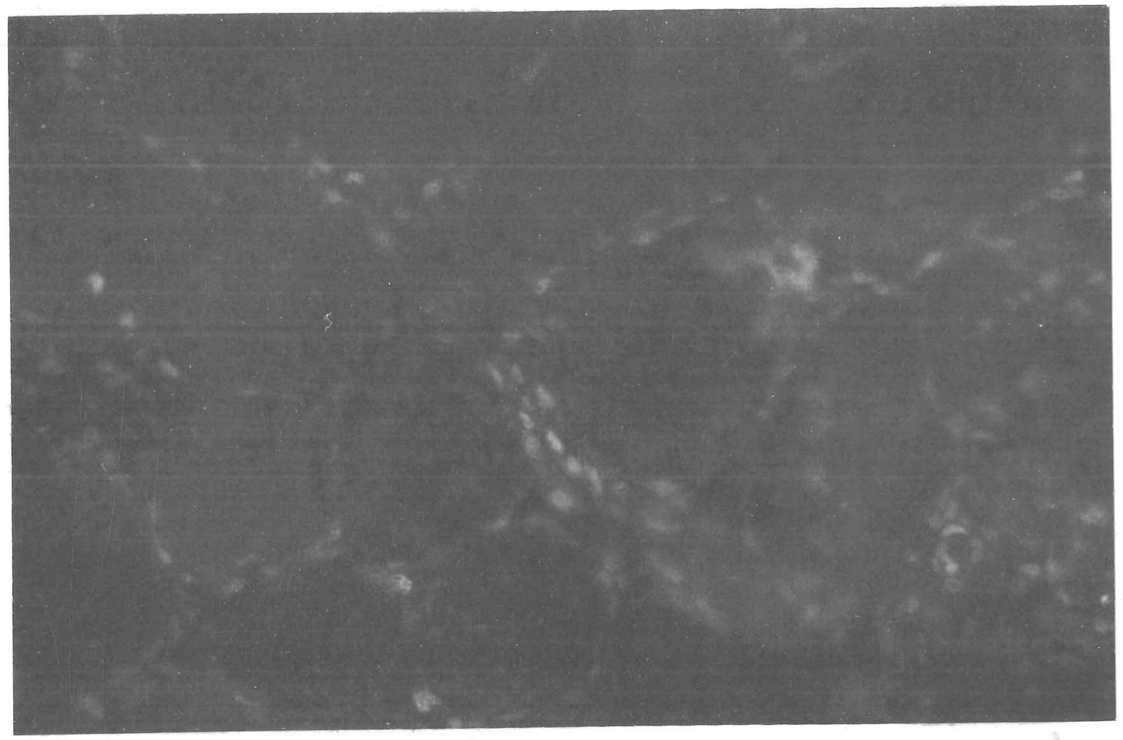
* V = Vascular   A = Abdominal
I = Interstitial   S = Scrotal
T = Intratubular   P = Peritubular

Figure 41.

Testis from 15 day rat treated with oestrogens (group 4) and injected subcutaneously with acriflavine. Note absence of intratubular staining. X290

Figure 42.

Testis from 15 day rat treated with arachis oil (group 1) and injected subcutaneously with acriflavine (control for fig,41) X290



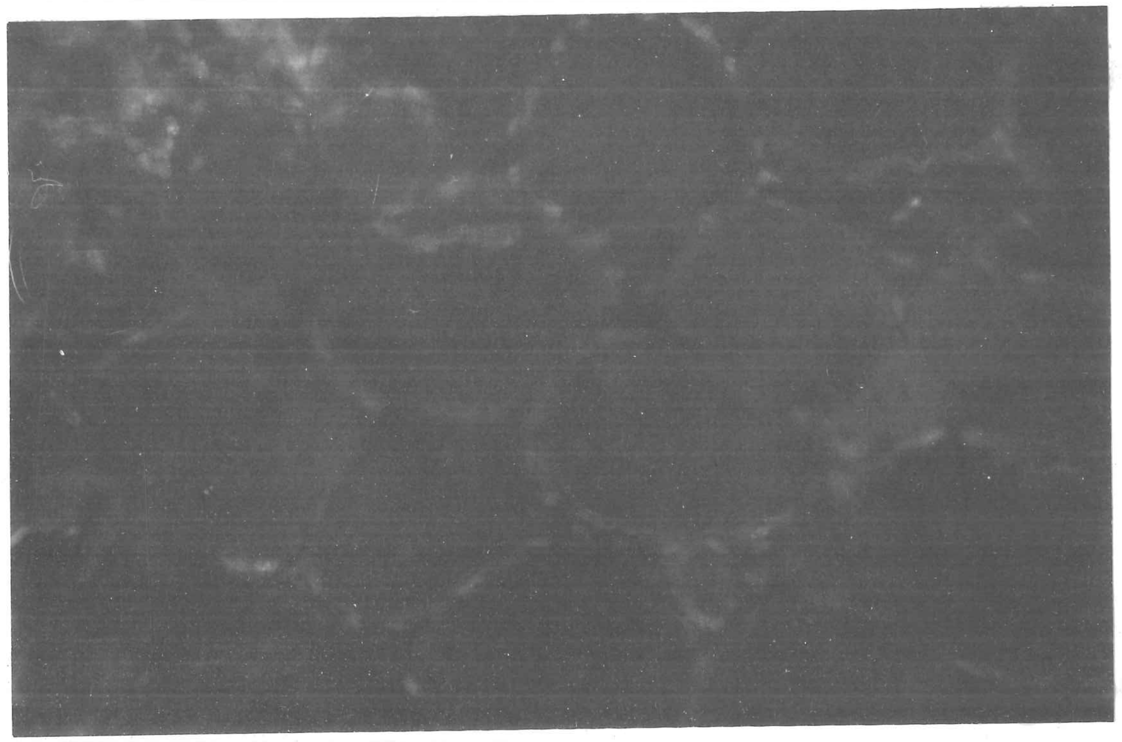


Figure 43.

Testis from 3 day rat (Eosin and haematoxylin).

Tubular lumen absent. X720

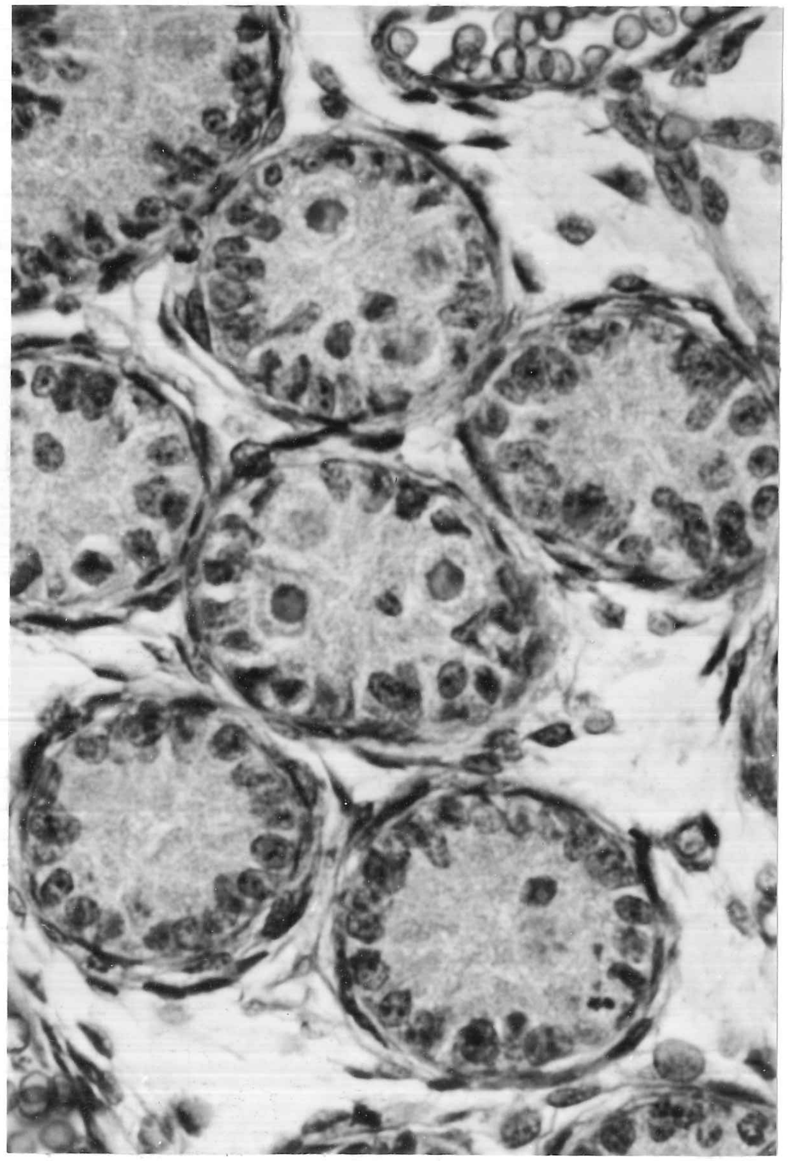
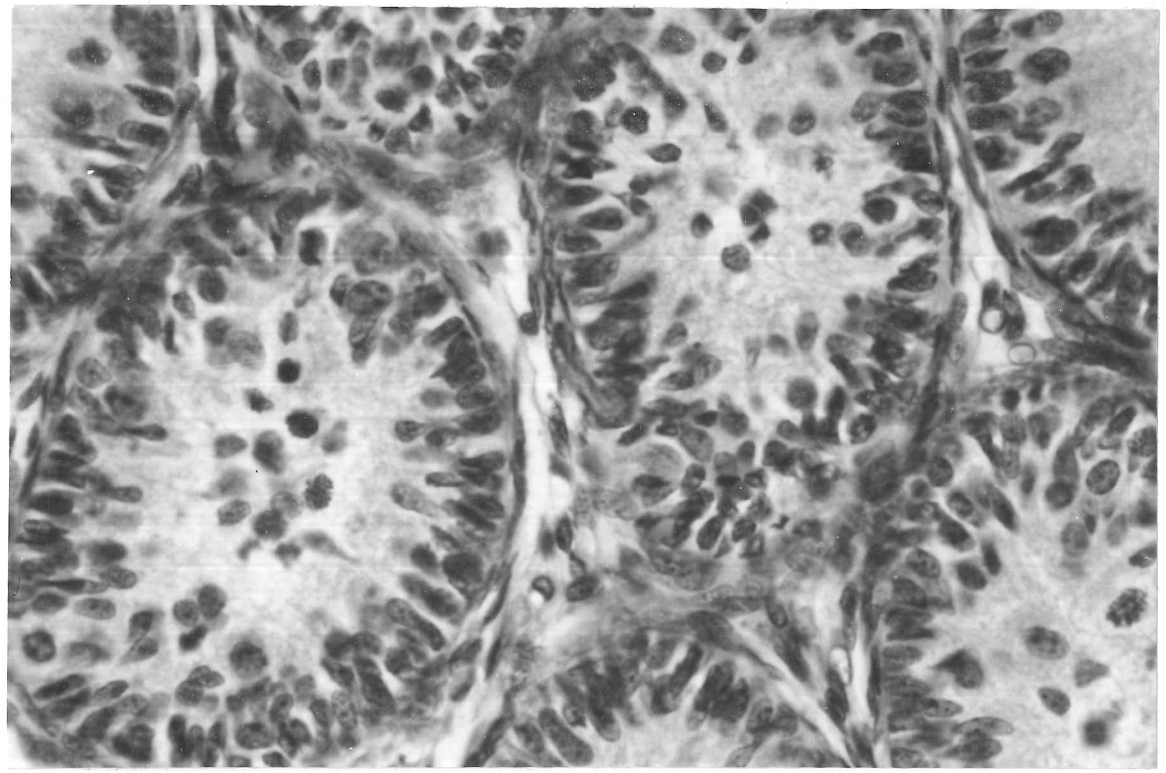


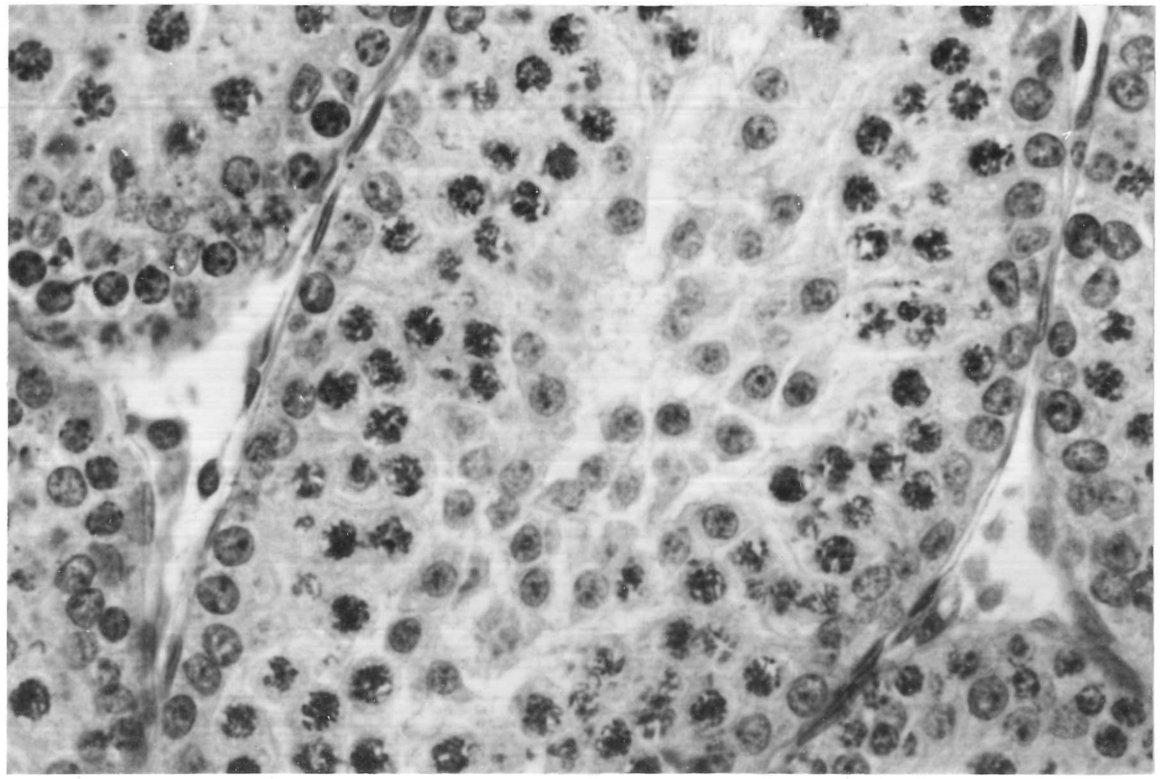
Figure 44.

Testis from 25 day old rat treated with oestrogen (group 3). Eosin and haematoxylin. Note absence of tubular lumen and small tubular diameter. X720

Figure 45.

Testis from 25 day old rat treated with arachis oil (group 1). Eosin and haematoxylin. Control for figure 44. X720





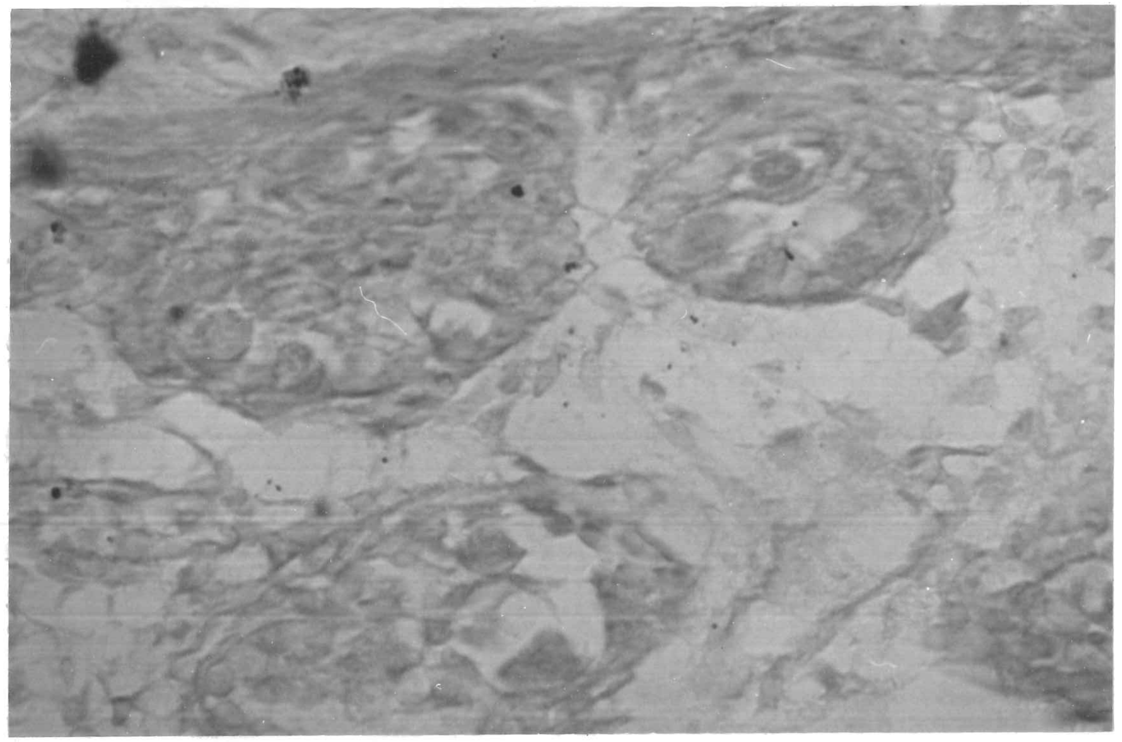


Figure 46.

Alkaline phosphatase staining absent from 3 day rat testis. X720

Figures 47, 48 and 49.

Staining for alkaline phosphatase in

47) 15 day rat testis treated with oestrogens (group 4). Note absnece of tubular staining and presence of vascular staining. X180

48) 15 day rat testis treated with arachis oil (group 1). Control with both tubular and vascular staining. X180

49) As for fig. 48, but β-glycerophosphate was omitted from incubation medium. No staining. X180

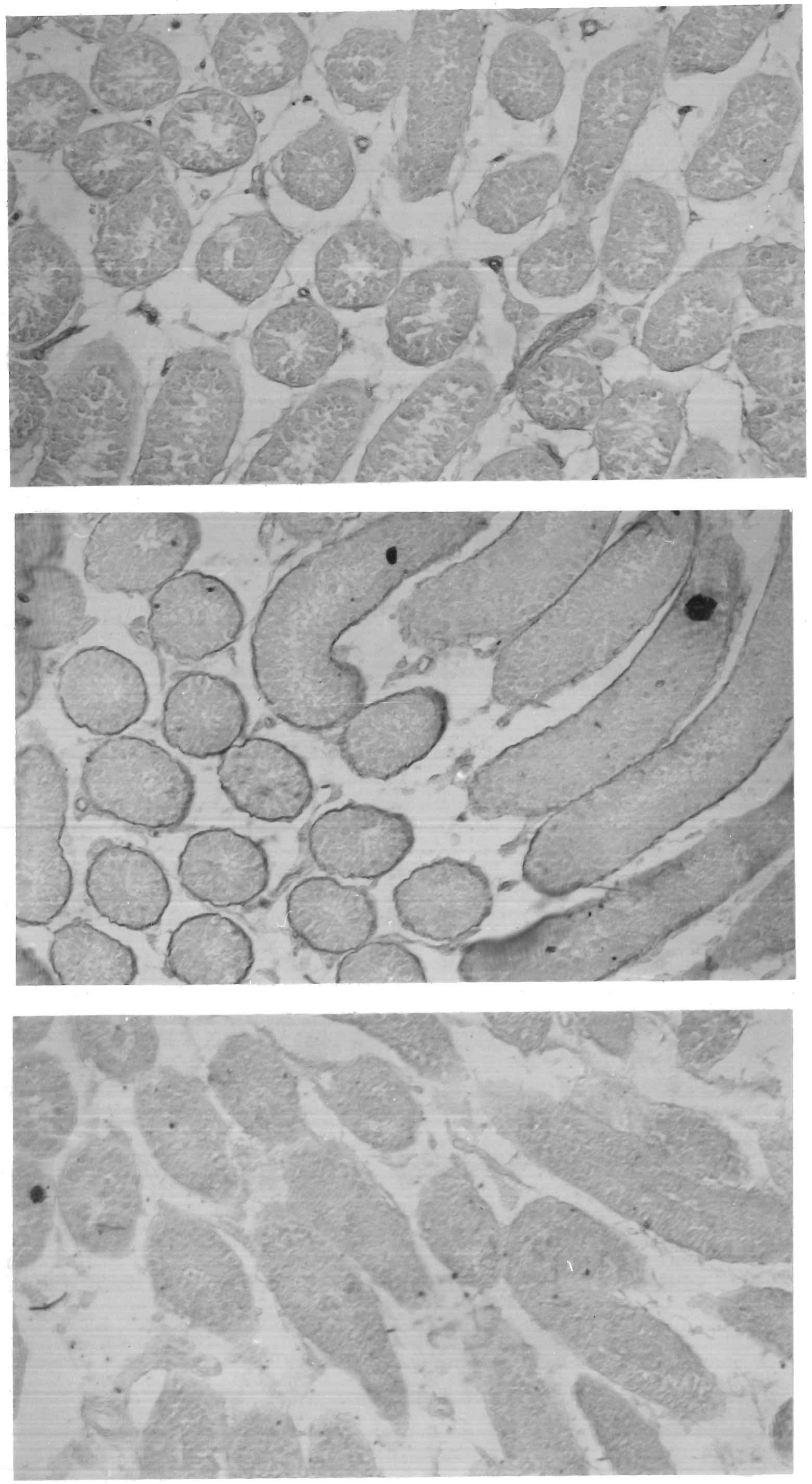


Table 12. Changes in the testis and body weights, and in the distribution of alkaline phosphatase and acriflavine after hypophysectomy of adult rats.

	Hypophysectomised				Sham-operated			
Days after operation	Acriflavine Staining pattern * V I T	Mean body weight gms ± S.E.	Mean testis weight gms ± S.E.	Alkaline phosphatase	Staining pattern * V I T	Mean body weight gms ± S.E.	Mean testis weight gms ± S.E.	Alkaline phosphatase.
2	++ + -	N.D.	N.D.	+	++ + -	N.D.	N.D.	+
3	++ + -	341 ± 9	1.39$^+$±.05	+	++ + -	372 ± 7	1.53 ±.04	+
10	++ + -	285$^+$± 4	1.12$^+$±.02	+	++ + -	386 ± 8	1.59 ±.03	+
25	++ + -	271$^+$± 9	0.45$^+$±.06	+	++ + -	415 ± 9	1.56 ±.05	+
35	++ + -	270$^+$± 7	0.34$^+$±.02	+	++ + -	454 ± 8	1.61 ±.05	+

*

V = Vascular

I = Interstitial

T = Intratubular

\+ significantly different from control value (P = 0.01 Mann-Whitney U test for nonparametric small samples)

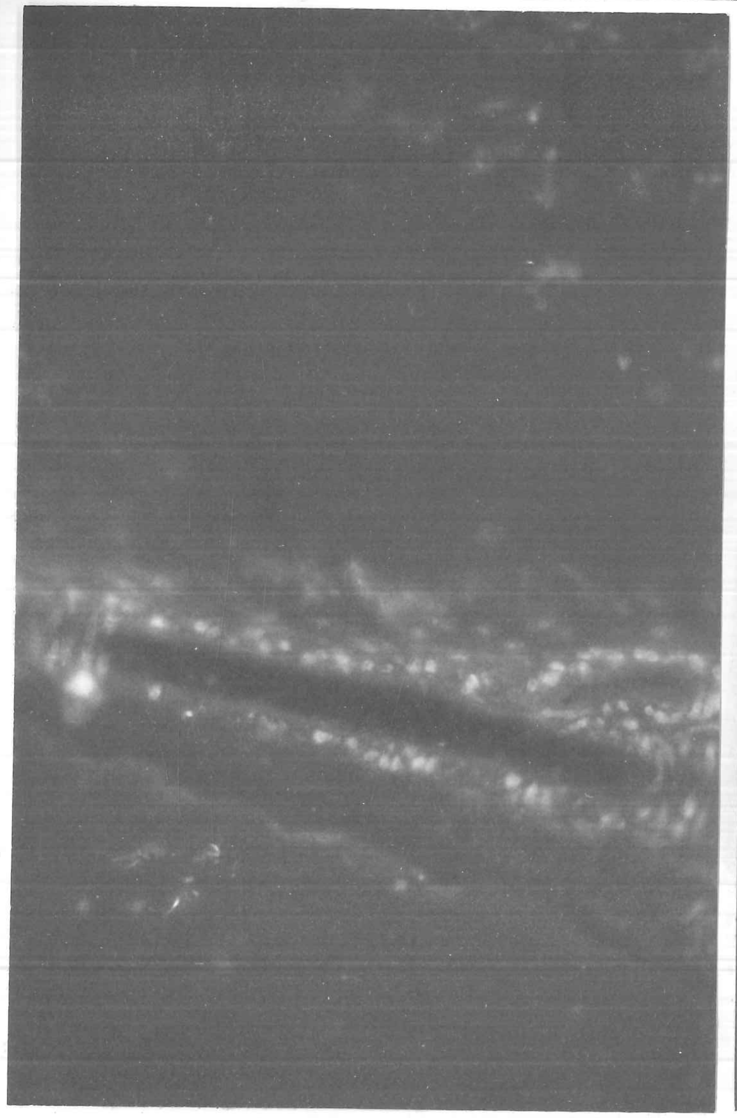
Figures 50, 51 and 52.

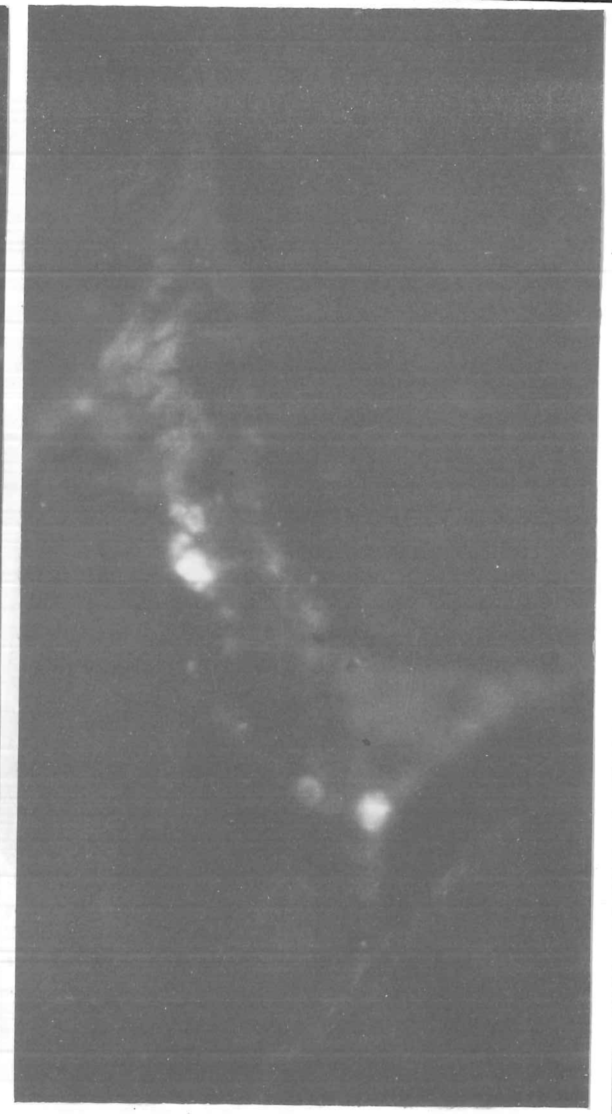
Testes from rats 35 days after operation.

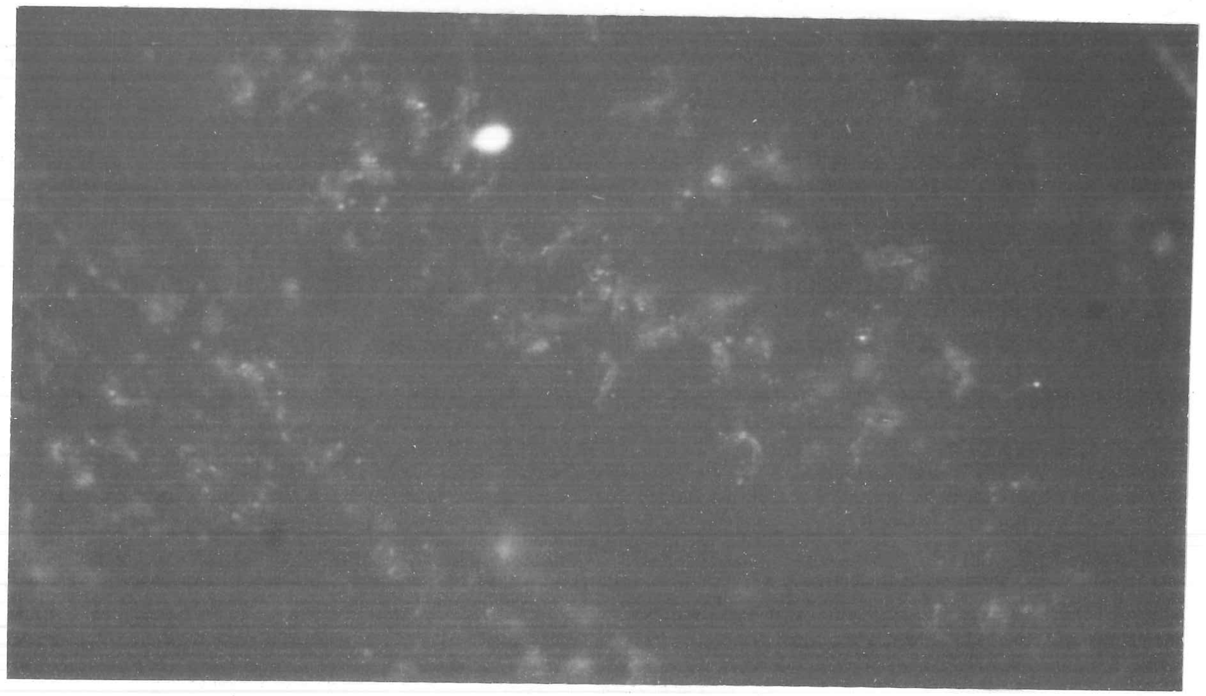
50) Hypophysectomised, and treated with acriflavine. Extratubular fluorescence due to acriflavine is mainly vascular. Intratubular fluorescence is nonspecific (see 52). X720

51) Sham operated, and treated with acriflavine. Extratubular fluorescence is mainly vascular. X720

52) Hypophysectomised but not treated with acriflavine. Nonspecific yellow autofluorescence within tubules. X720







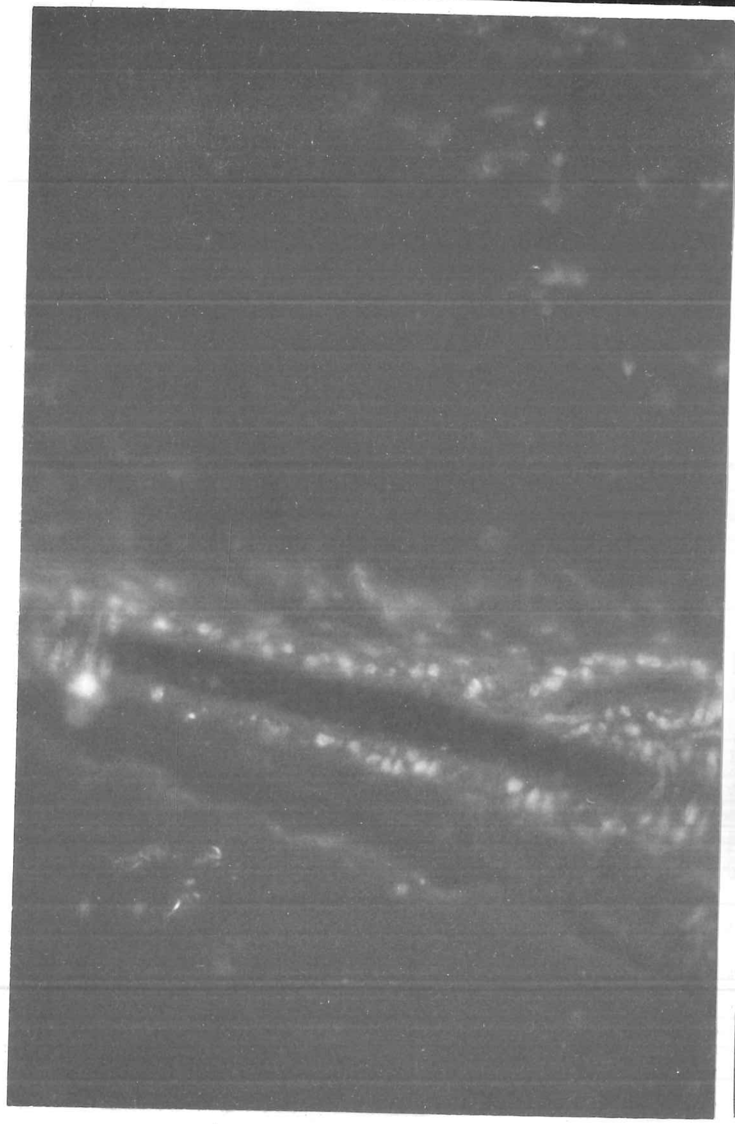
Figures 50, 51 and 52.

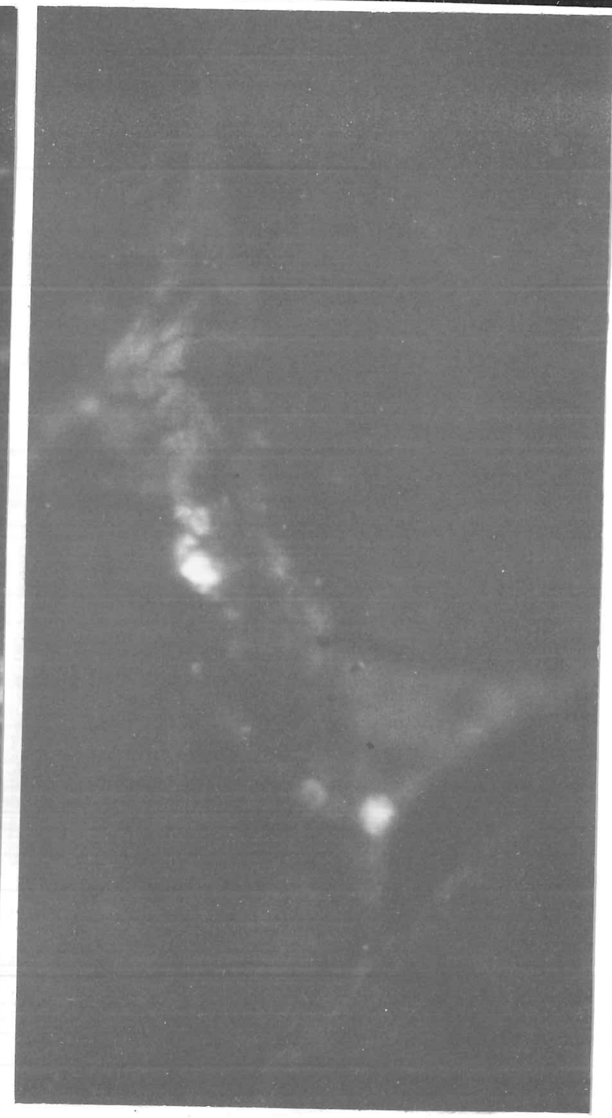
Testes from rats 35 days after operation.

50) Hypophysectomised, and treated with acriflavine. Extratubular fluorescence due to acriflavine is mainly vascular. Intra-tubular fluorescence is nonspecific (see 52). X720

51) Sham operated, and treated with acri-flavine. Extra-tubular fluorescence is mainly vascular. X720

52) Hypophysectomised but not treated with acriflavine. Nonspecific yellow auto-fluorescence within tubules. X720





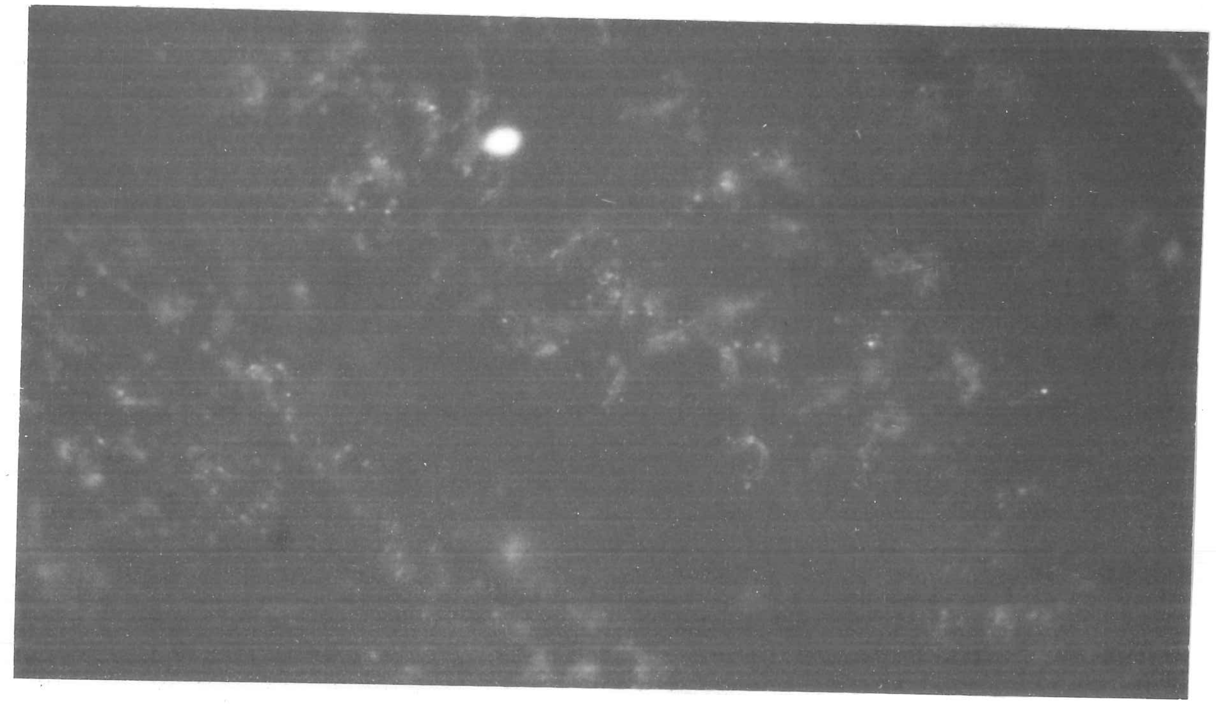
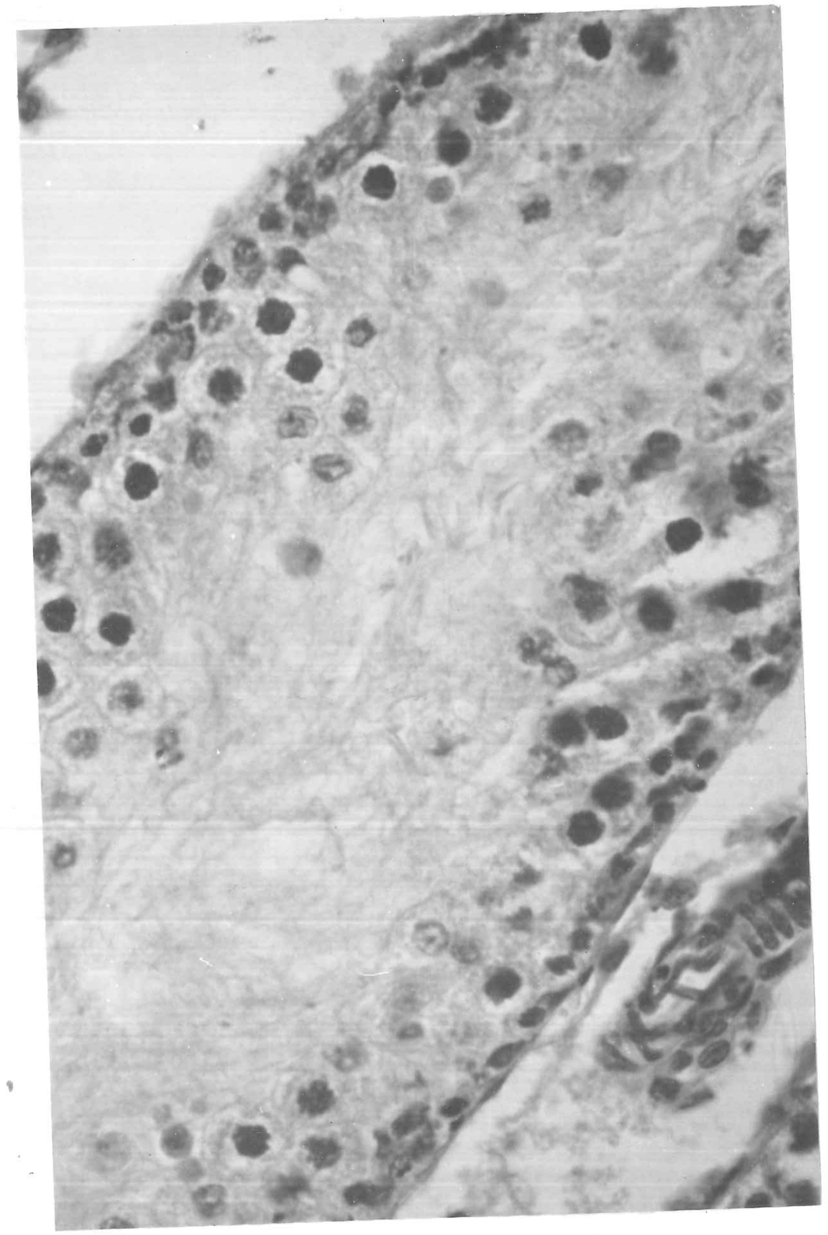


Figure 53.

Testis from rat 35 days after hypophysectomy. Eosin and haematoxylin. X720



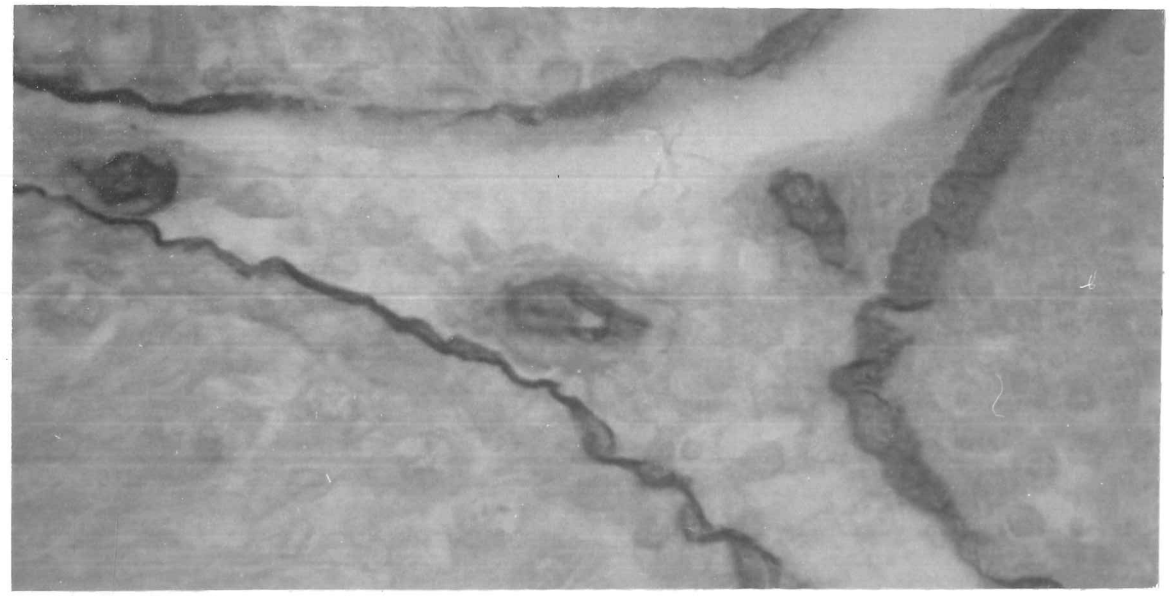
Figures 54, 55 and 56.

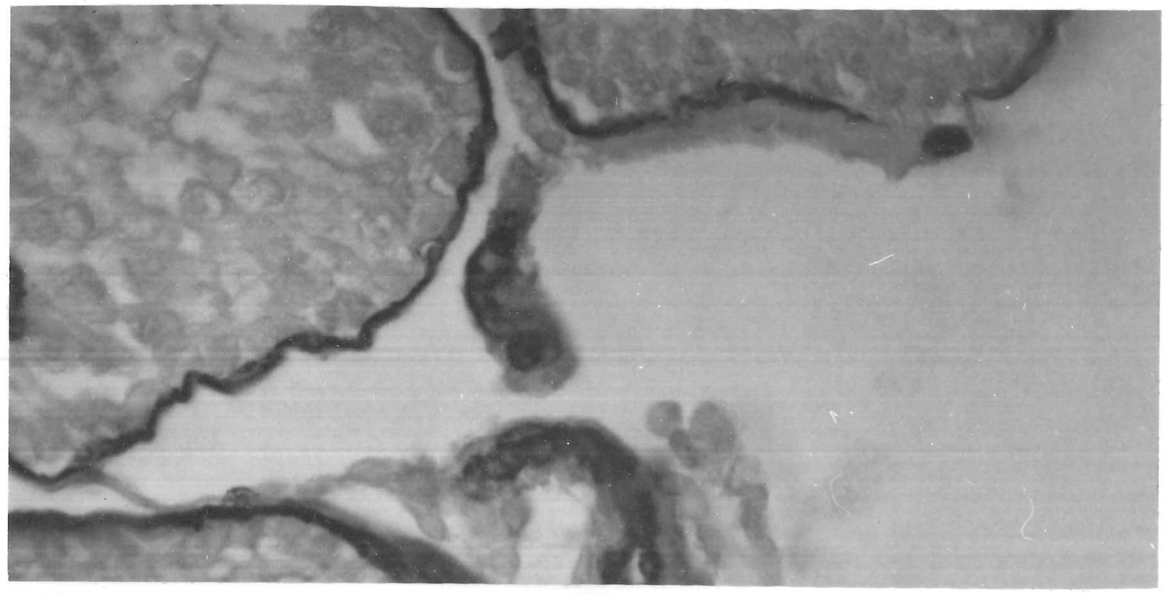
Staining for alkaline phosphatase in animals operated 35 days previously

54) Sham operated. X720

55) Hypophysectomised - note same staining pattern as figure 54. X720

56) Sham operated but with β-glycerophosphate absent from incubating medium. X720





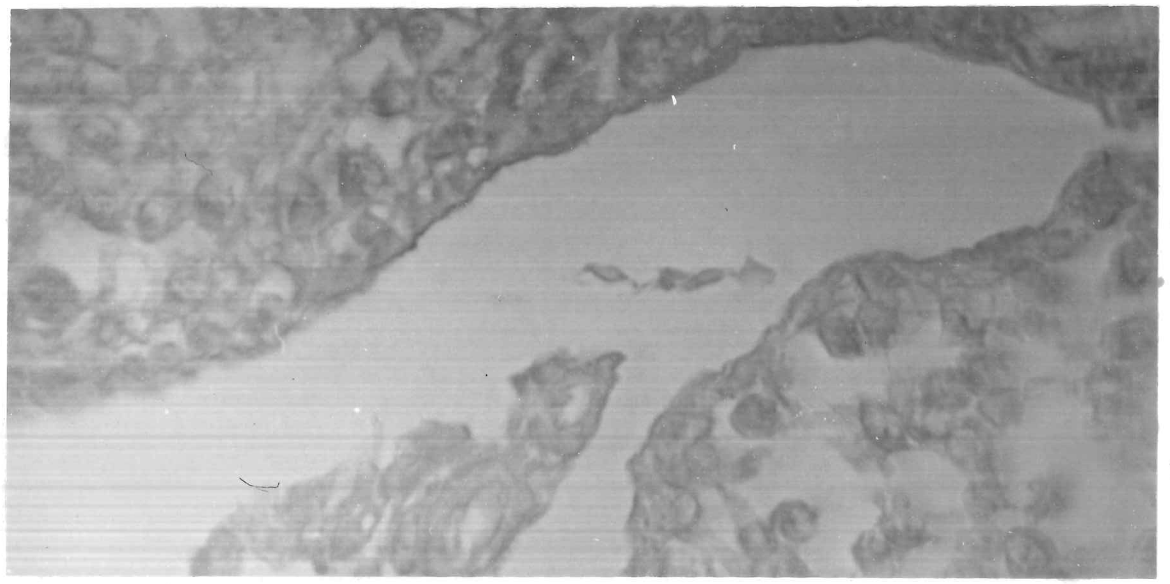


Table 13. Effect of $CdCl_2$ injection on the acriflavine staining pattern of the guinea-pig testis.

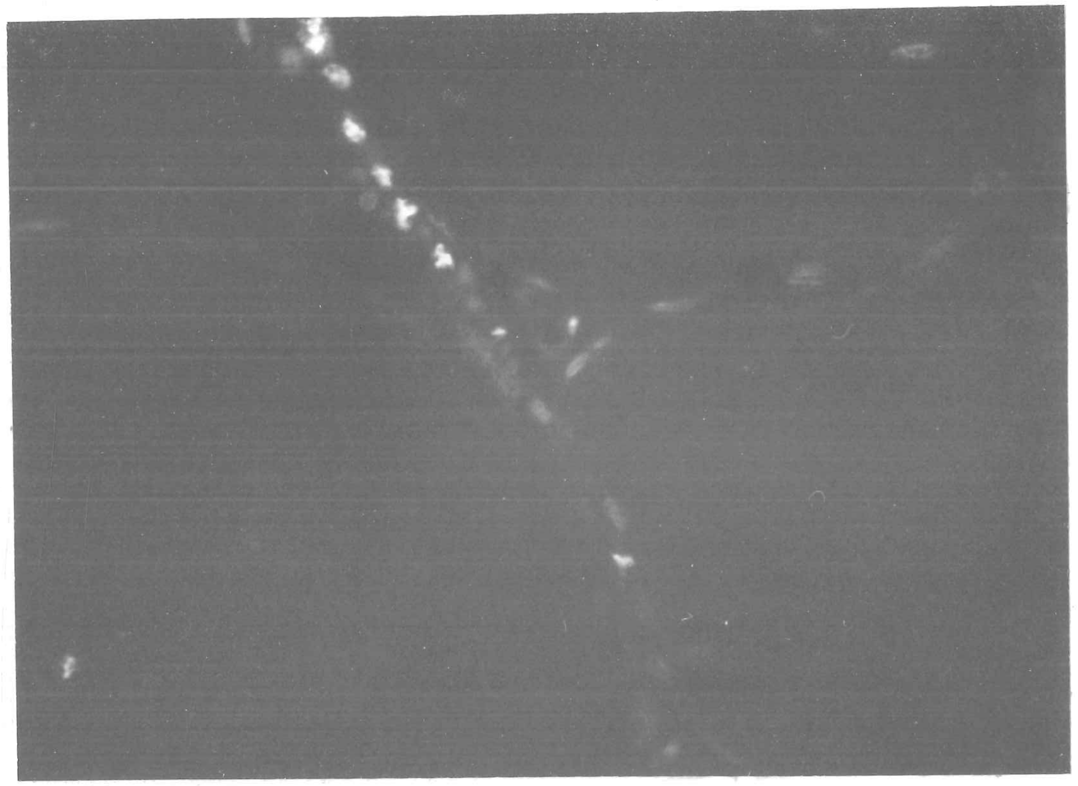
Time after $CdCl_2$ injection hrs	Number of animals	Vascular staining	Interstitial staining	Intratubular staining	Histology
0 (Control (2))	5	++	- to +		Normal
5	2	++	± to +	-	No obvious damage
12	5	++	++	-	No obvious damage
24	6	++	++	- or focal ± or +	Haemorrhage into interstitial spaces. Not much tubular damage.
36	6	++	++	-/±/+/++	Focal areas of damage, corresponding with increased acriflavine staining
48	2	++	++	++	Complete necrotic degeneration

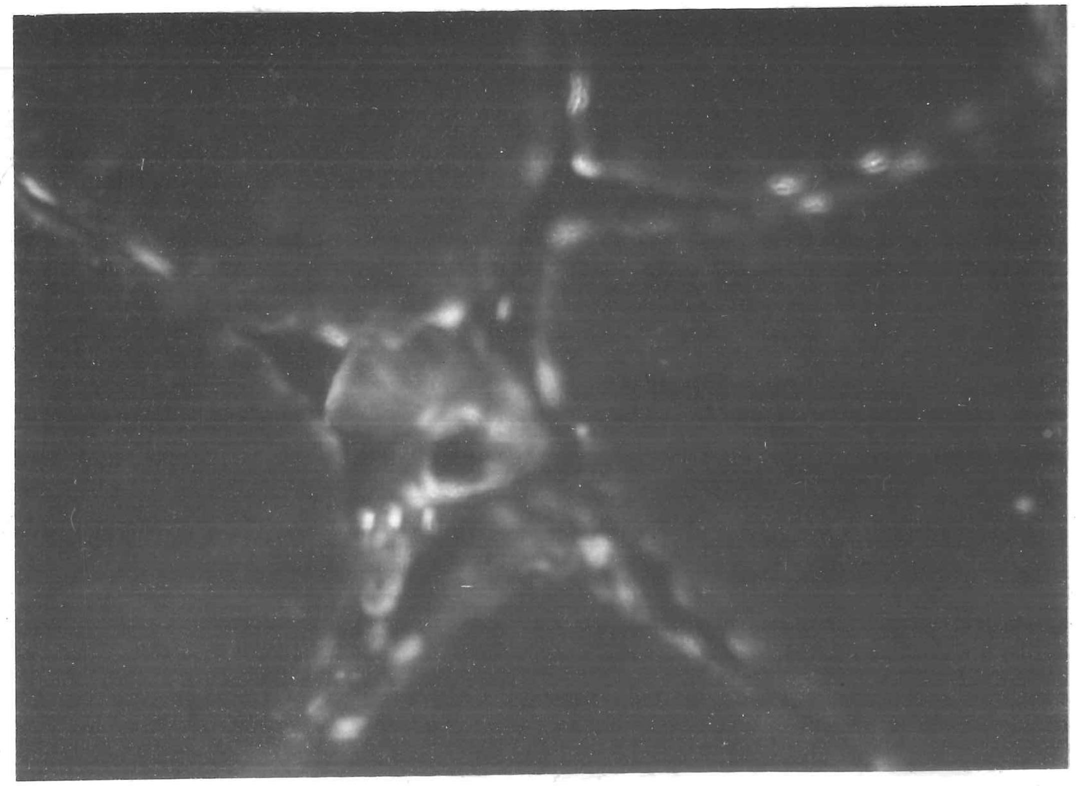
Figures 57 and 58.

Testes from guinea-pigs injected five hours previously with acriflavine.

57) Normal guinea-pig. Strong vascular, weak interstitial and no intratubular staining. X290

58) Guinea-pig injected with $CdCl_2$ twelve hours previously. Strong interstitial staining. X180



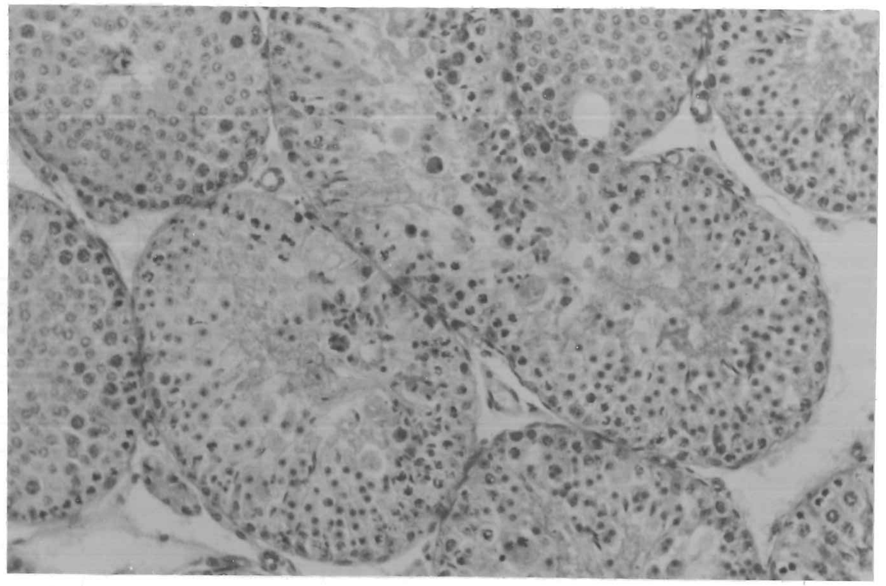


Figures 59 and 60.

Section of guinea-pig testes damaged by $CdCl_2$. Eosin and haematoxylin. X120

59) 24 hours after $CdCl_2$ injection.

60) 48 hours after $CdCl_2$ injection. X120



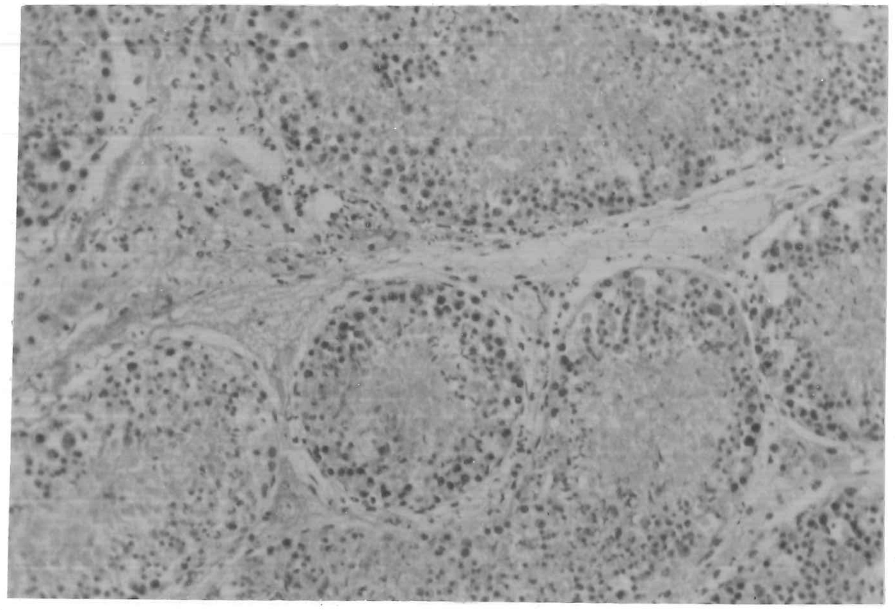
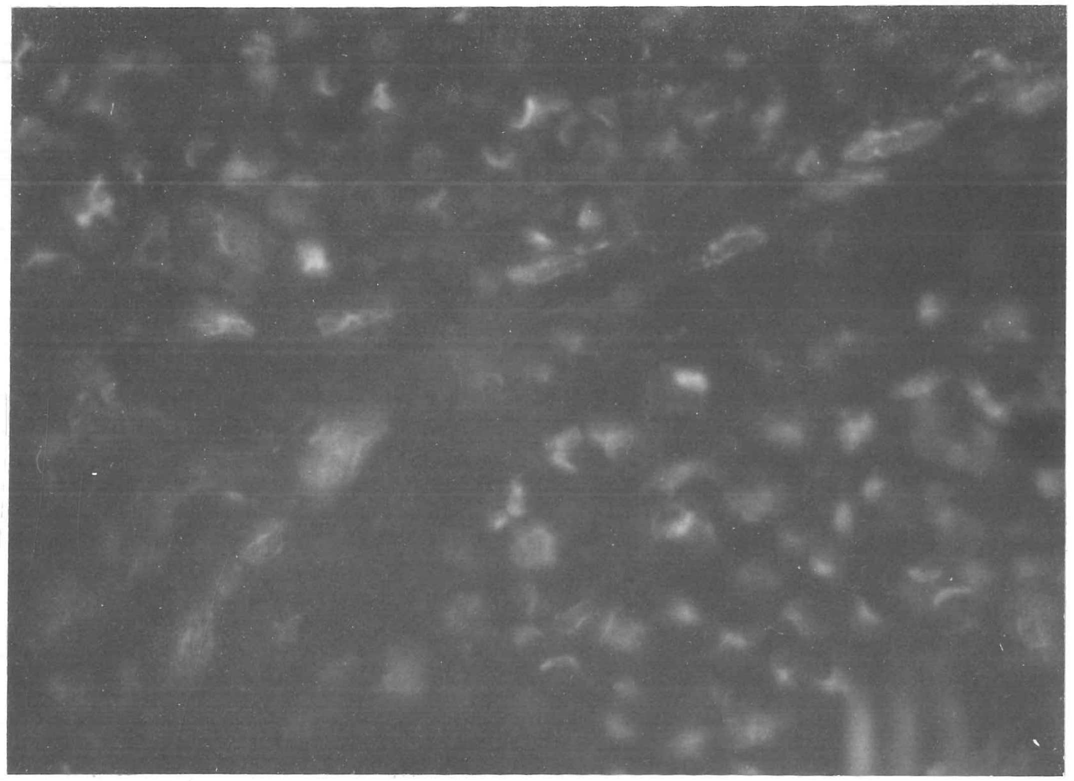


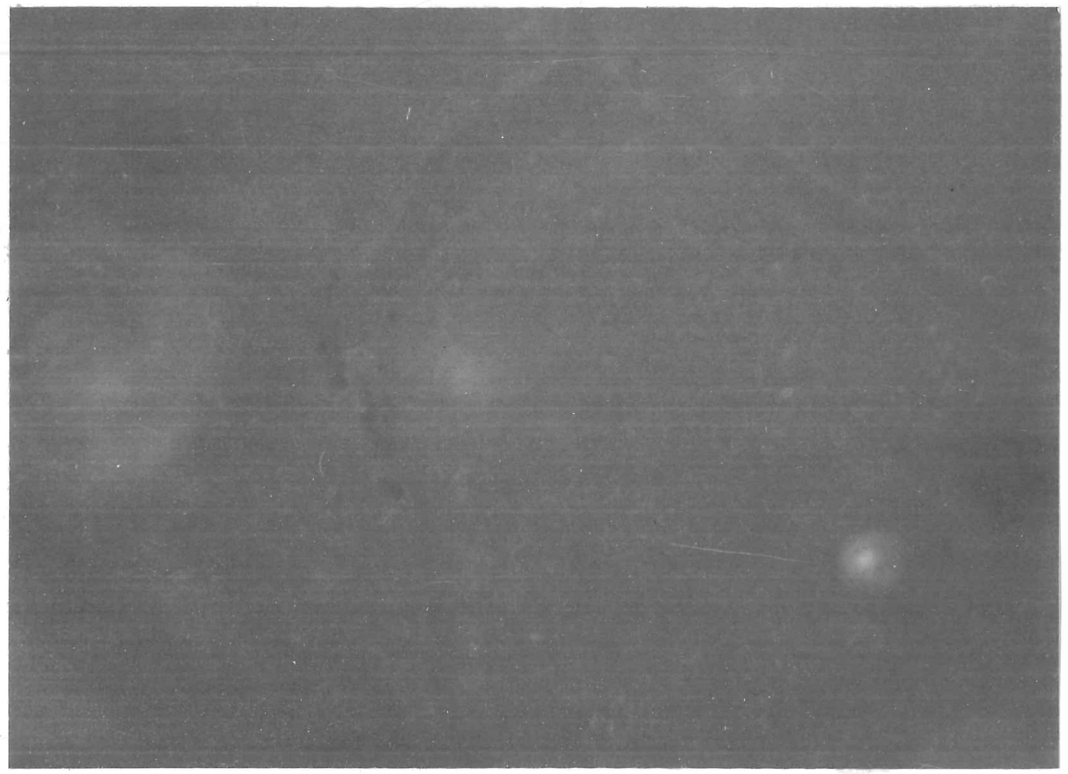
Figure 61.

Guinea-pig testis from animal treated 48 hours previously with $CdCl_2$ and 5 hours previously with acriflavine. Note intratubular fluorescence. X720

Figure 62.

Guinea-pig testis from animal treated 36 hours previously with $CdCl_2$ but not with acriflavine. X290



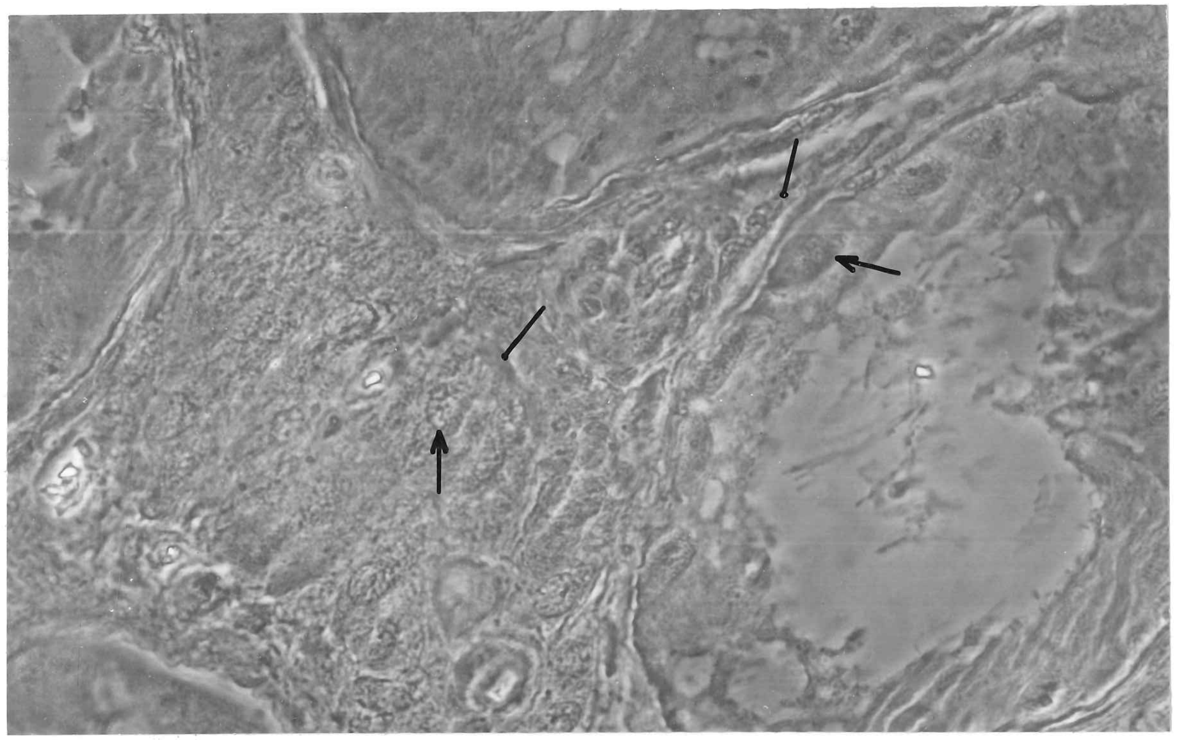


Figures 63 and 64.

Testis from a guinea-pig injected with isologous testis 40 days previously, and acriflavine 5 hours previously.

63) Phase contrast view. X290

64) Same field viewed by fluorescence. Note staining of interstitial nuclei (lined) but not of intratubular nuclei (arrowed). X290



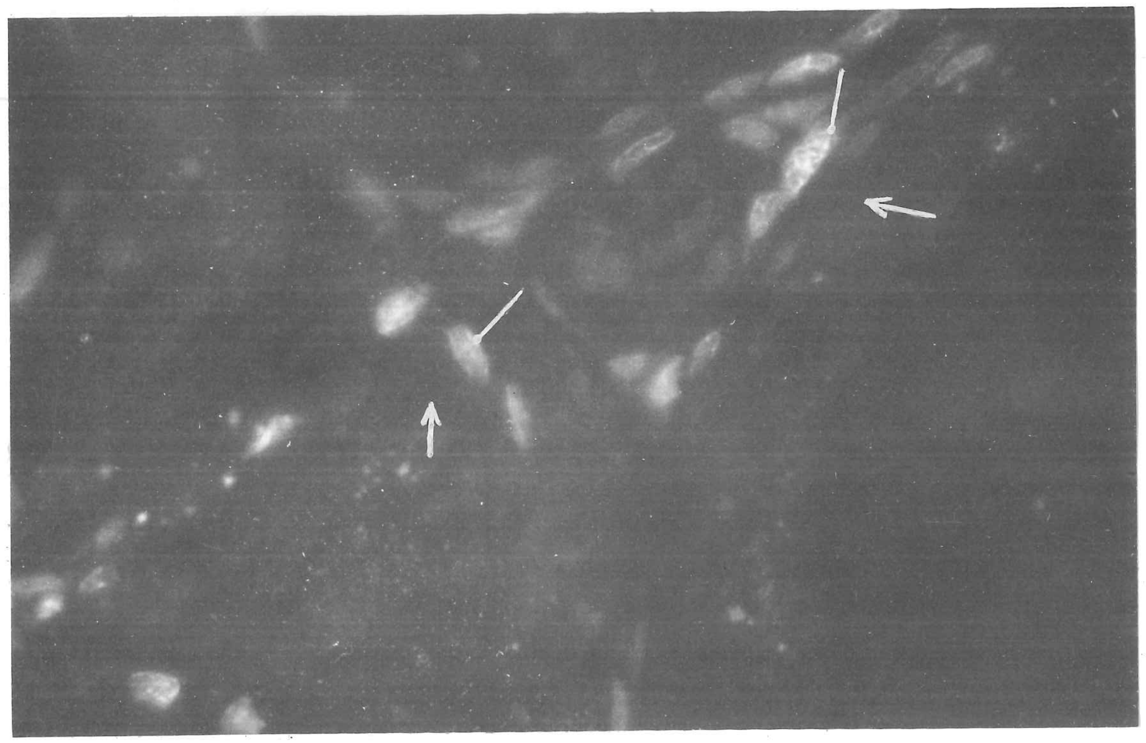
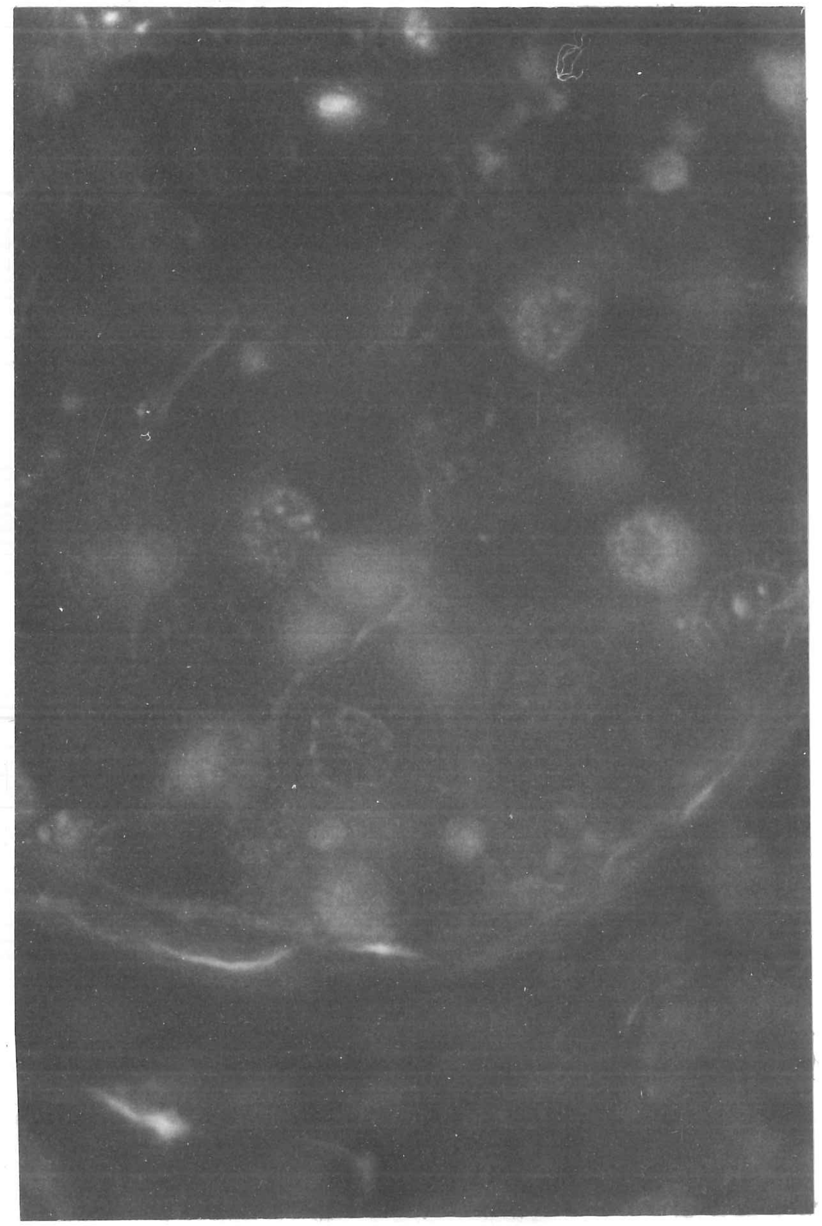


Figure 65.

Testis from a guinea-pig injected with isologous testis 18 days previously and with acriflavine 5 hours previously. Note staining of intratubular nuclei. X720

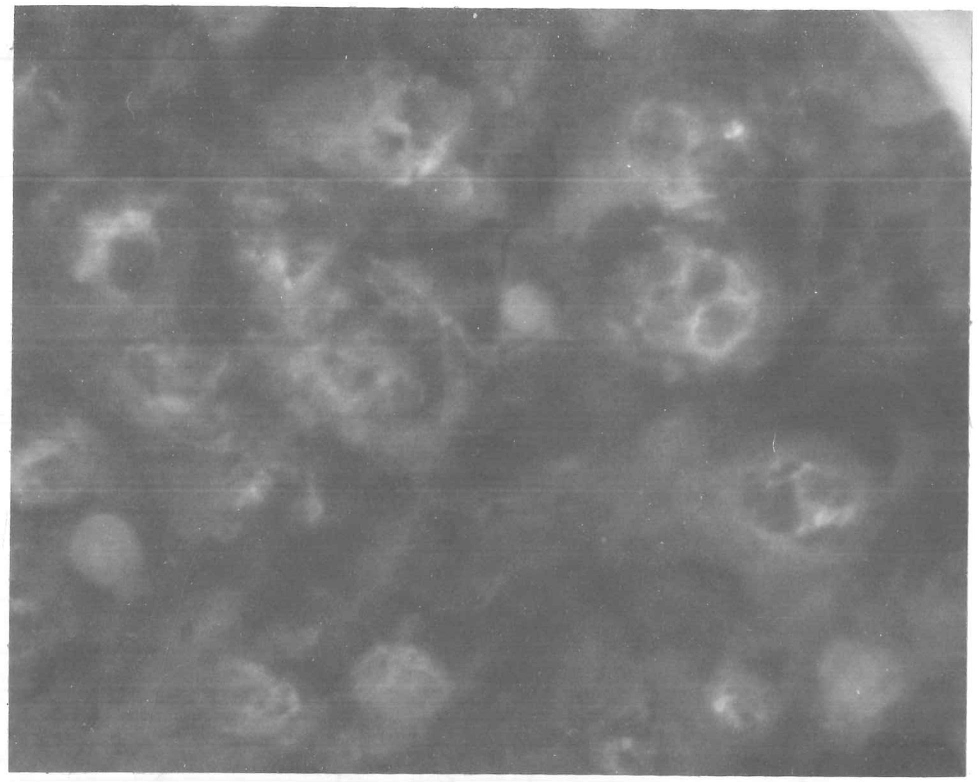


Figures 66 and 67.

Sections, from testis of a guinea-pig isoimmunized with testis, following incubation with fluorescein-labelled anti guinea-pig gamma-globulin.

66) Section from inflamed, acriflavine-permeable region. X720

67) Section from undamaged region at same spermatogenic stage. Note absence of fluorescence. X720



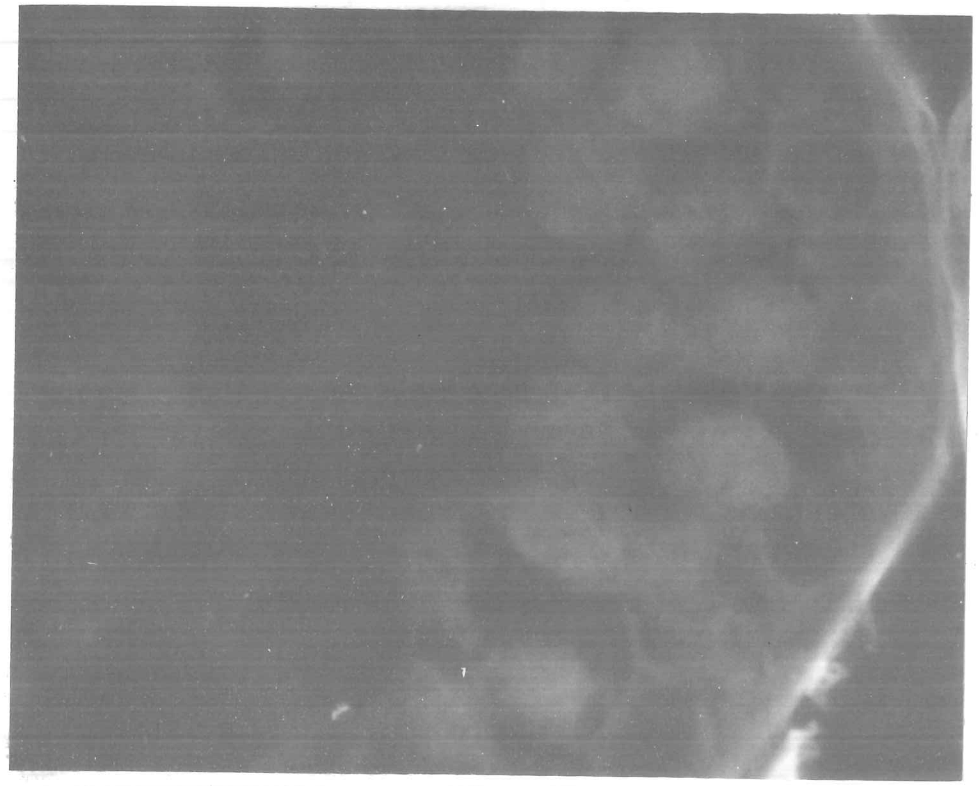


Table 14. The strength of immune response and incidence of testicular lesions in immature guinea-pigs immunized with homologous testis.

Group	Dose of testis mgm/kgm	No. of animals	No. of animals with damaged testis	Delayed skin response	γ_1 response (mean value $\log_2$ of dilution ± S.E.)	γ_2 response (mean value $\log_2$ of dilution ± S.E.)
1	200	8	0	++	7.7 ± 0.9	4.0 ± 1.0
2	400	10	1	++	9.9 ± 0.2	9.6 ± 0.7
3	800	11	2	++	9.9 ± 0.3	9.6 ± 0.7
4	1600	9	0	++	9.6 ± 0.4	11.3 ± 0.4

Table 14. The strength of immune response and incidence of testicular lesions in immature guinea-pigs immunized with homologous testis.

Group	Dose of testis mgm/kgm	No. of animals	No. of animals with damaged testis	Delayed skin response	γ_1 response (mean value $\log_2$ of dilution ± S.E.)	γ_2 response (mean value $\log_2$ of dilution ± S.E.)
1	200	8	0	++	7.7 ± 0.9	4.0 ± 1.0
2	400	10	1	++	9.9 ± 0.2	9.6 ± 0.7
3	800	11	2	++	9.9 ± 0.3	9.6 ± 0.7
4	1600	9	0	++	9.6 ± 0.4	11.3 ± 0.4

Table 15. The effect of cadmium chloride on the induction of autoimmune aspermatogenesis in the immature guinea-pig testis.

Cadmium Chloride dose mgm/kgm	Hrs between injection of $CdCl_2$ and sacrifice.	Guinea-pigs isoimmunized with testis 20 days previously				Guinea-pigs injected with saline.	
		No. of animals	No. of right testes damaged immunologically prior to $CdCl_2$	No. of left testes damaged ischaemically after $CdCl_2$	No. of left testes also damaged immunologically at sites of ischaemia.	No. of animals.	No. of testes with ischaemic damage.
6	24	5	1*	0	1*	2	0
	48	5	0	2+	2+	2	1
7.5	24	5	0	0	0	2	0
	48	5	0	0	0	2	1
10	24	5	1	4⊛	4⊛	2	2
	48	5	1	4⊛	4⊛	2	2

* Same animal - damaged at and around rete.

\+ Same testes.

⊛ Same testes and including one from animal with damaged right testis.

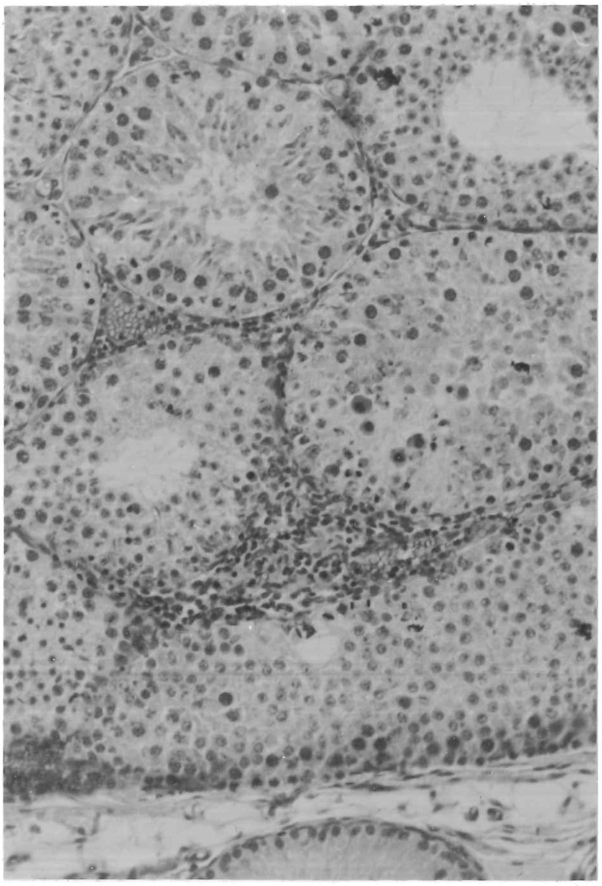
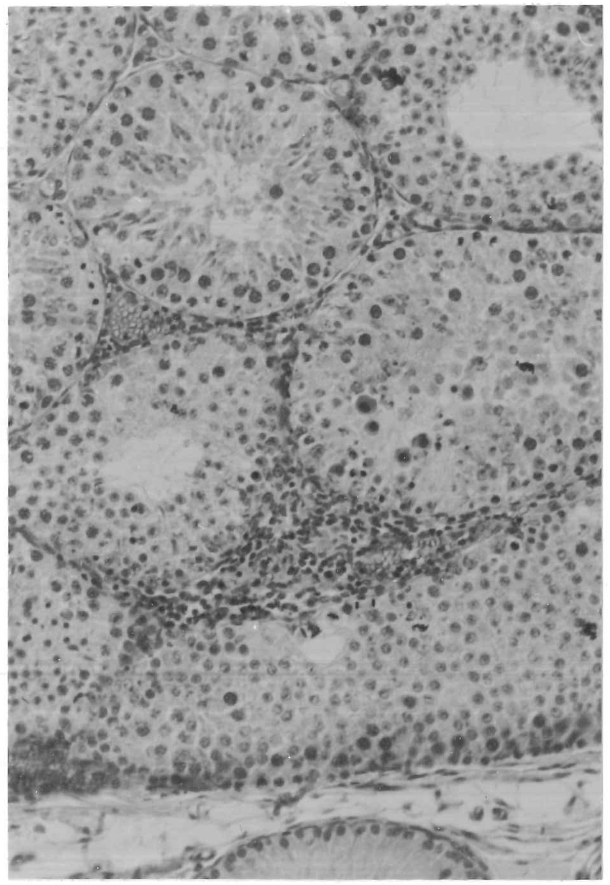


Figure 68.

Testis from maturing guinea-pig isoimmunized with spermatozoa and injected with $CdCl_2$. Note inflammatory cells at site of focal ischaemic damage (compare figure 59). X480

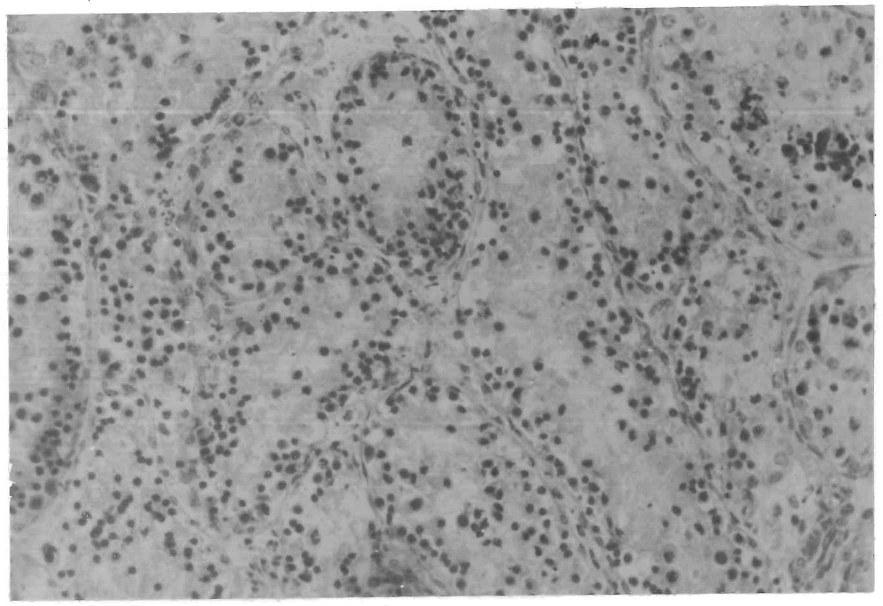


Figures 69 and 70.

guinea-pig testes 24 hours after traumatization with a sterile needle.

69) from a nonimmunized male. X120

70) from a male isoimmunized with testis twenty days previously. Note accumulation of inflammatory cells.



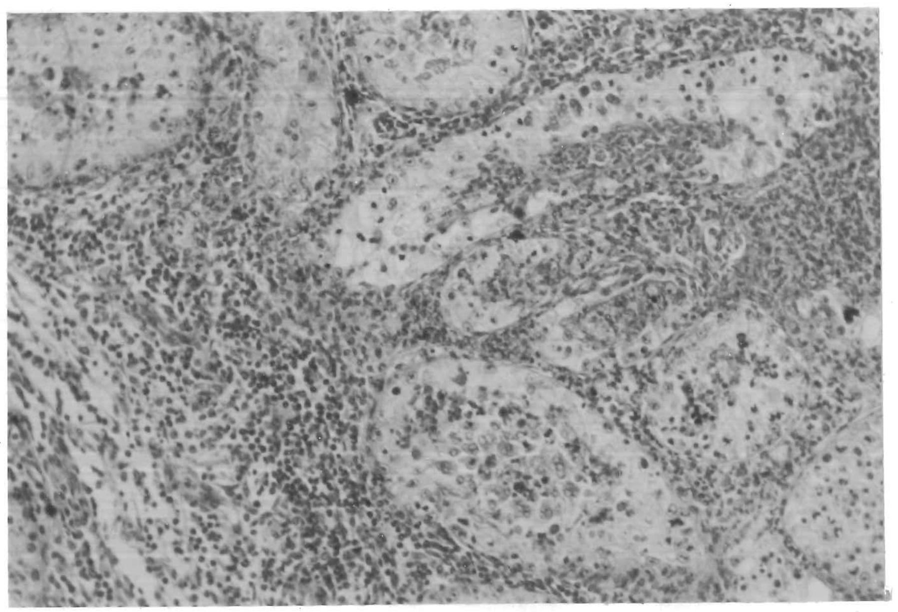


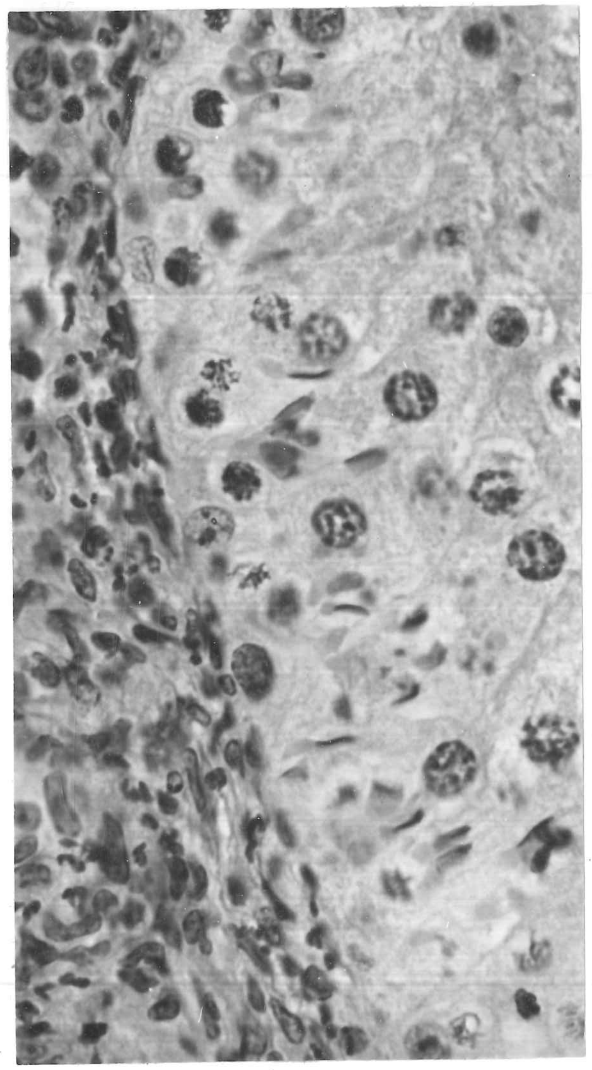
Figure 71, 72, and 73.

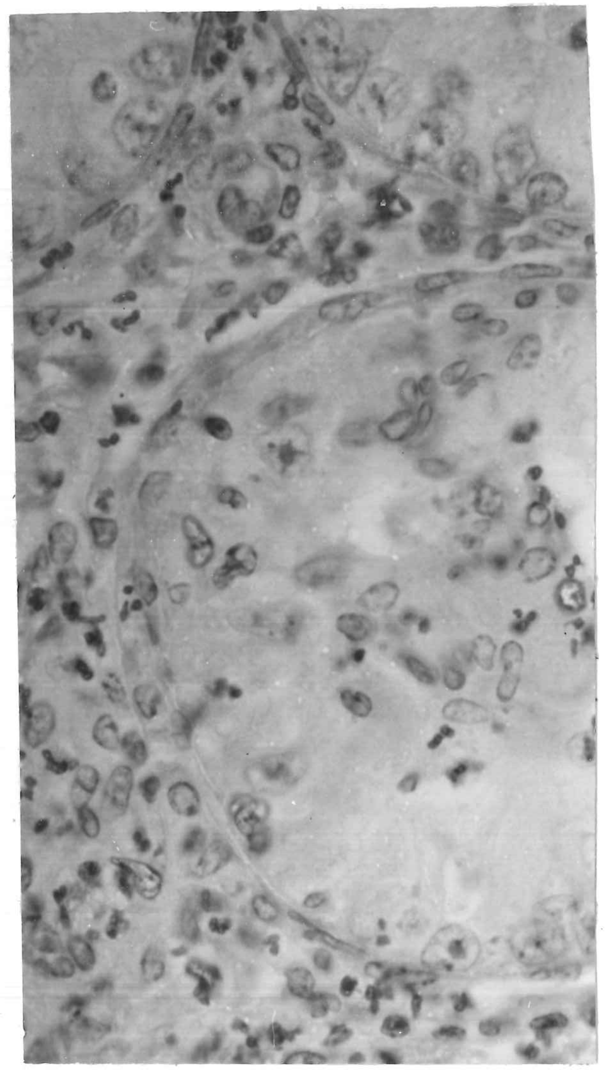
Testes, injected with Freund's complete adjuvant, from animals immunized 20 days previously with

71) complete adjuvant only. view 24 hours after intratesticular injection. Interstitial inflammation. X720

72) Complete adjuvant + isologous testis. View 24 hours after intratesticular injection. Inflammation within tubules also. X720

73) As for (71) but view 78 hours after intratesticular injection. Tubules now inflamed. X720





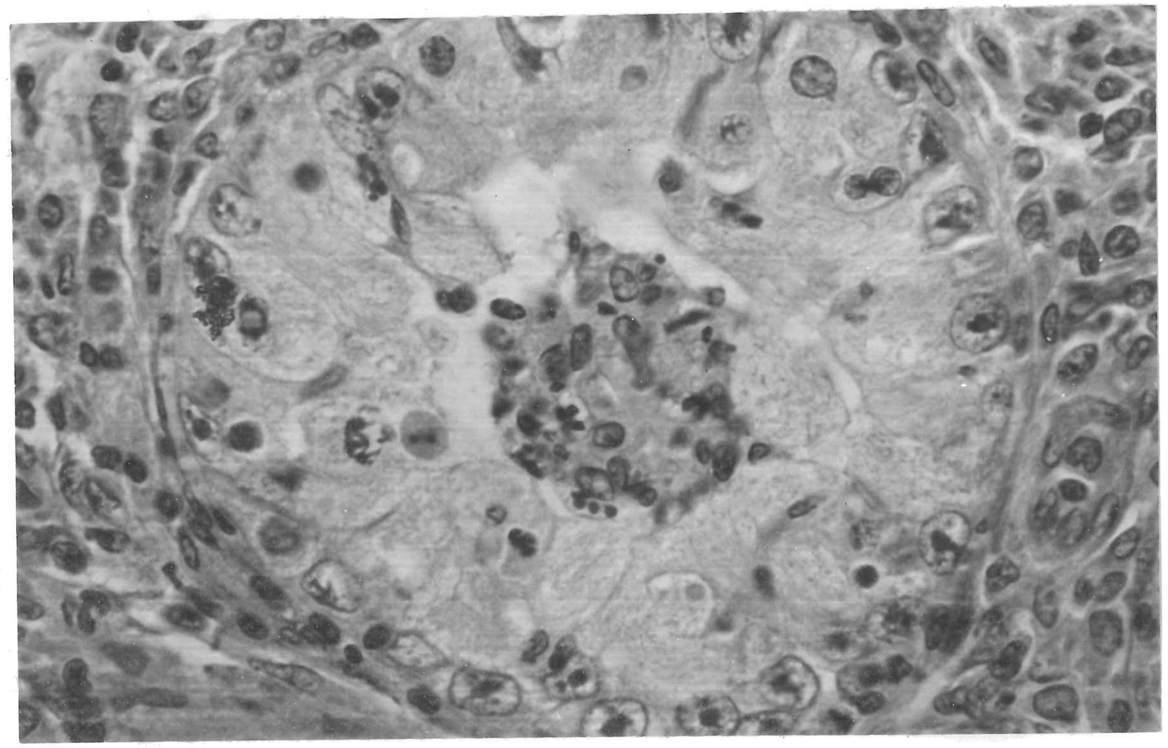
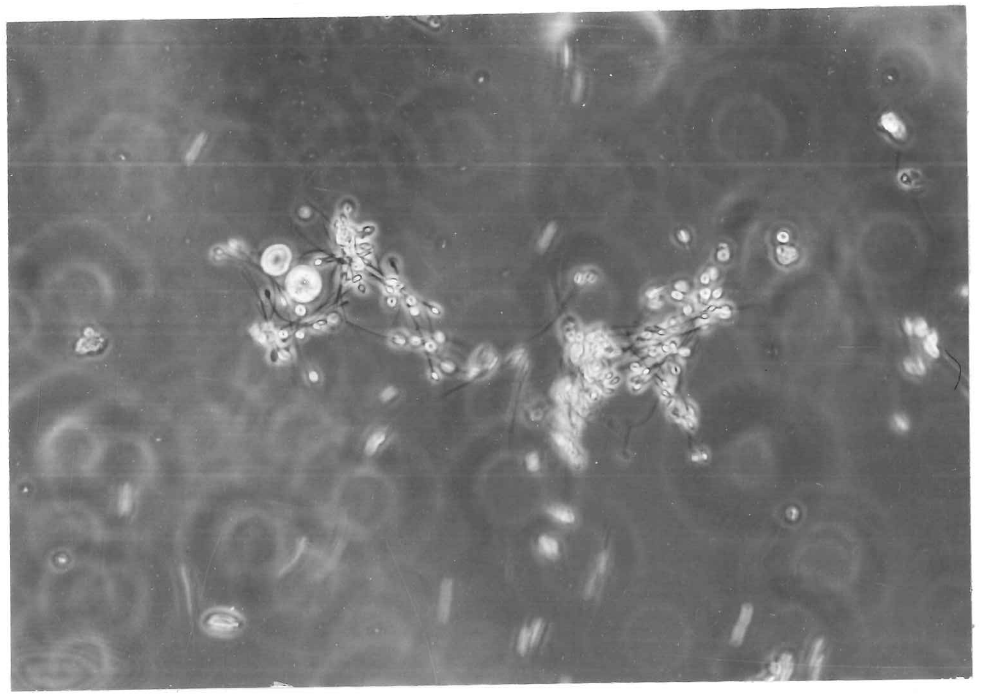


Figure 74.

Human spermatozoa after incubation with serum R for 1 hour. Note agglutination tail-to-tail.

Upper X180

Lower X720



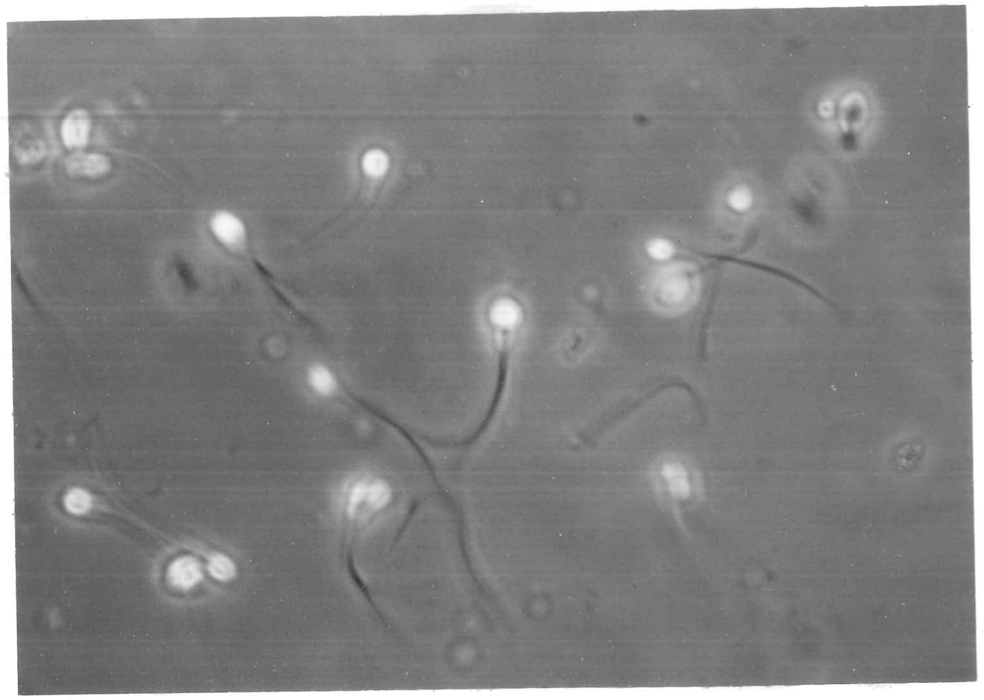


Figure 75.

Immunoelectrophoretic analysis of Sephadex G-200 fractions of Serum R.

A = Serum R

B = Peak 3

C = Peak 2

D = Peak 1

E = Antiserum to human serum (rabbit).

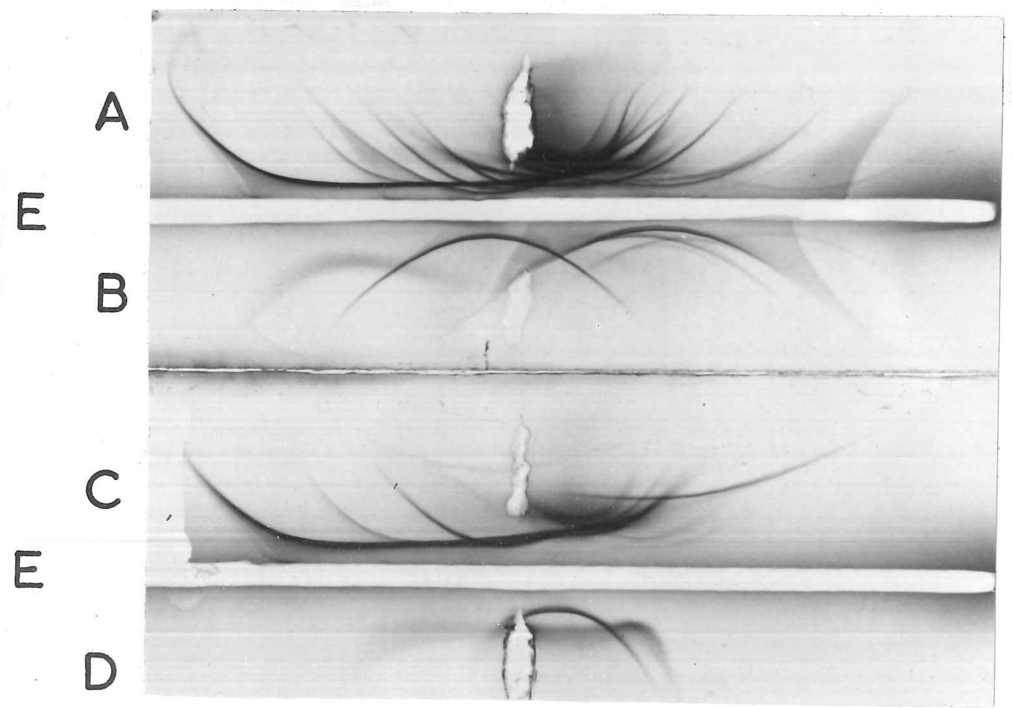


Figure 76.

Immunoelectrophoretic analysis of

DEAE - Cellulose fractions of Serum R.

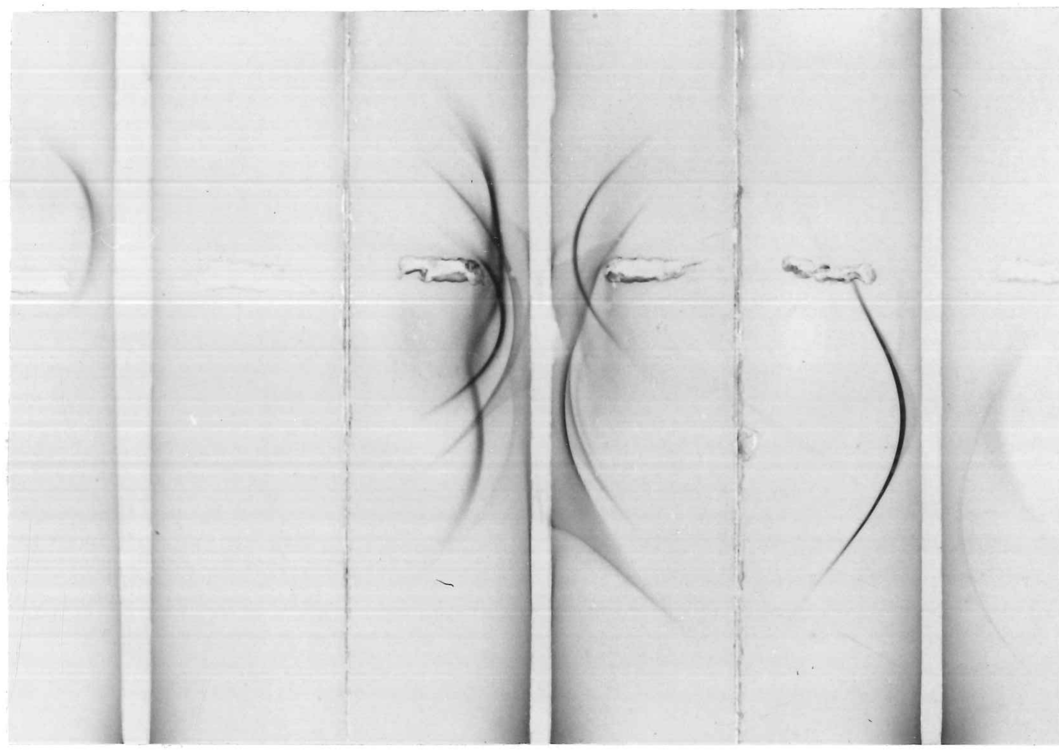
Wells (from top left to bottom right)

Serum R

Fractions as eluted

Troughs

Antiserum to human serum (rabbit)



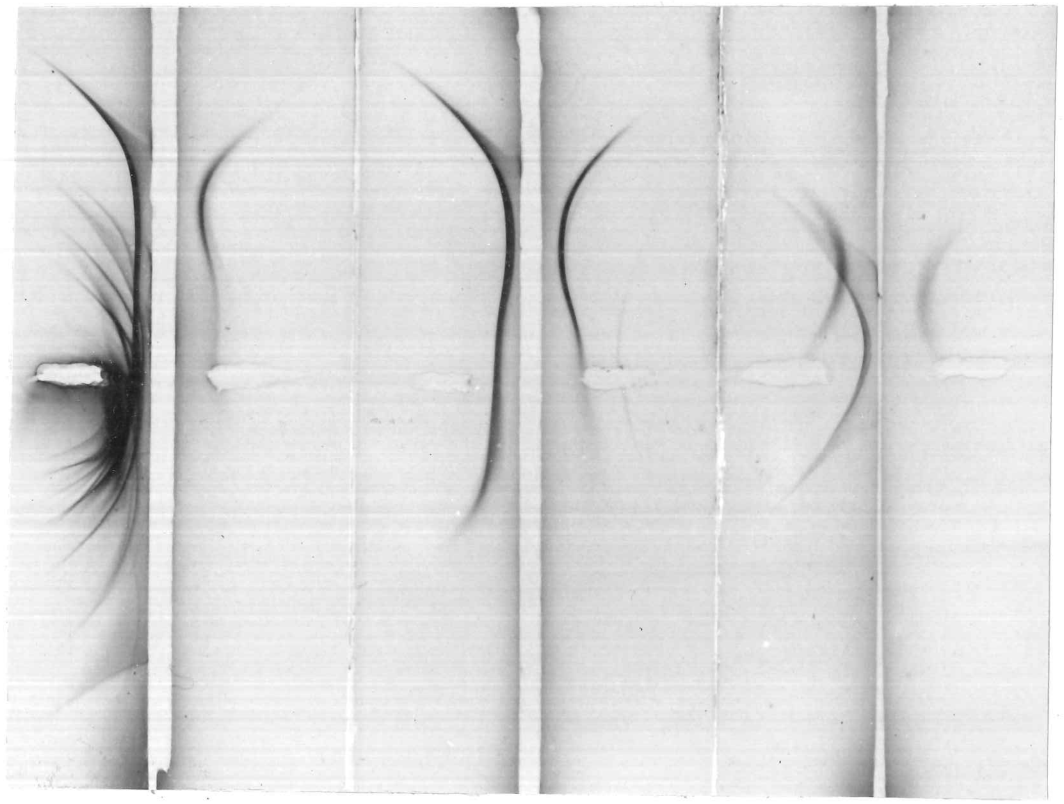


Figure 77.

Immunoelectrophoretic analysis.

A = Human seminal plasma

B = Antiserum to human serum

C = Human serum

Figure 78.

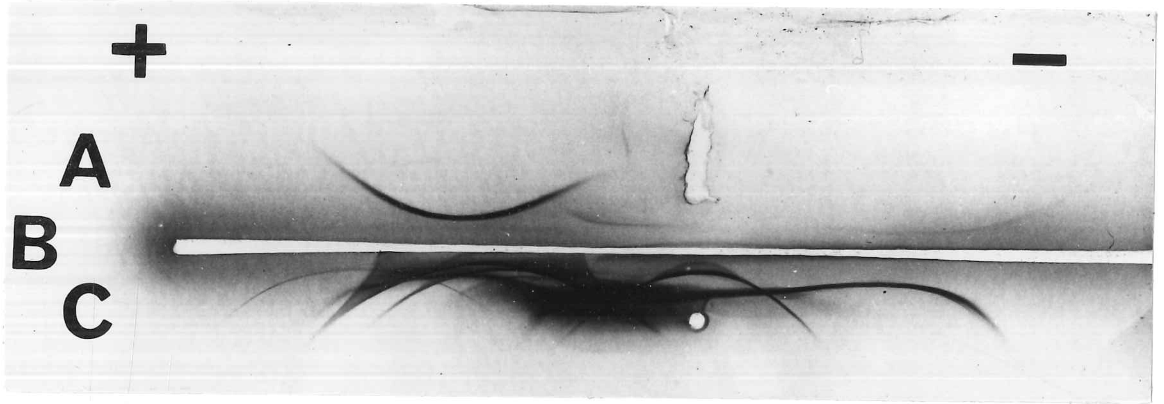
Immunoelectrophoretic analysis

A = Human seminal plasma

C = Antiserum to human serum (rabbit)

B = Human serum

Gamma -globulin arc of serum fuses with that of seminal plasma



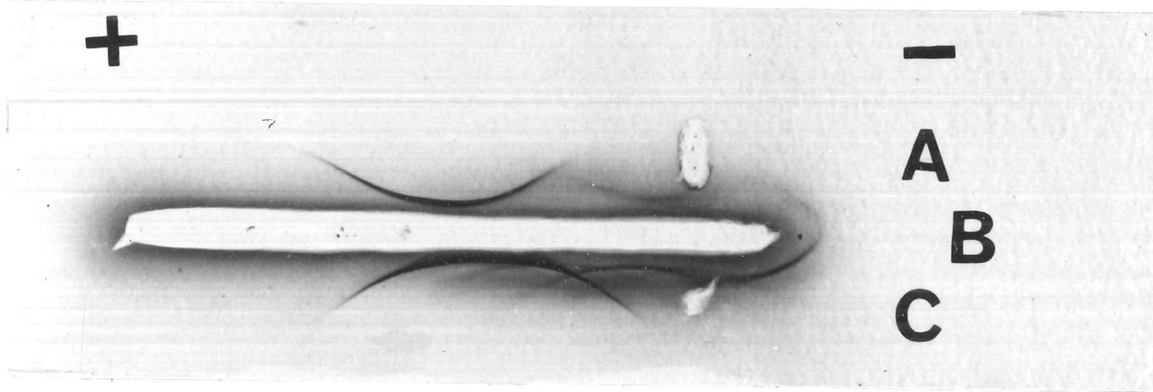


Figure 79.

A = Human seminal plasma

B = Human serum

C = Antiserum to human serum (rabbit)

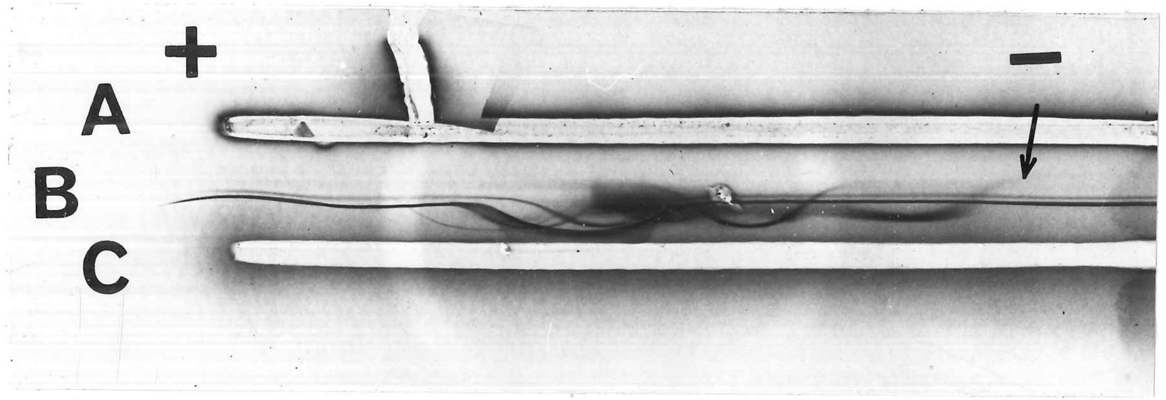
Note fusion of gamma-globulin arc with longitudinal line (arrowed)

Figure 80.

A = Human serum

B = Antiserum to human seminal plasma (rabbit)

C = Human seminal plasma



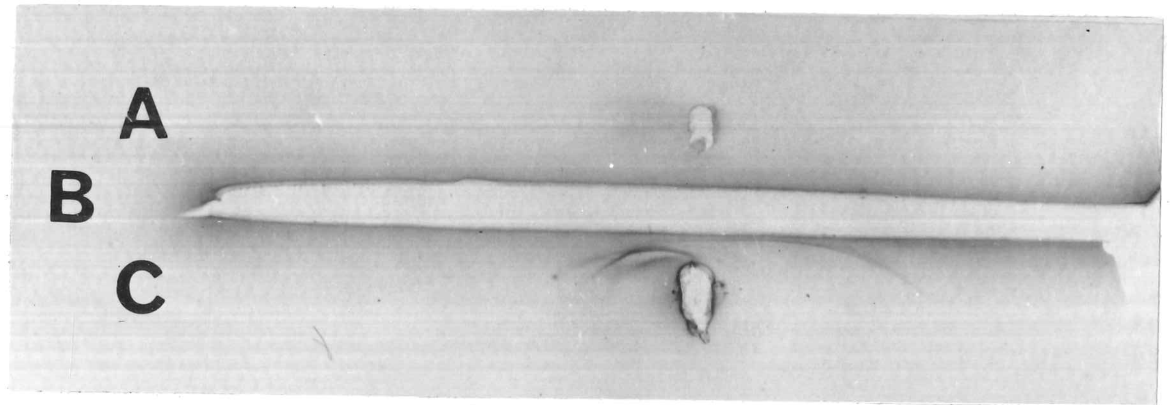


Figure 81.

Immunoelectrophoresis

A = human seminal plasma

C = human serum

B = antiserum to human serum

B'= antiserum to human serum absorbed 3X with human seminal plasma. Antibodies to IgG, IgM and IgA not removed.

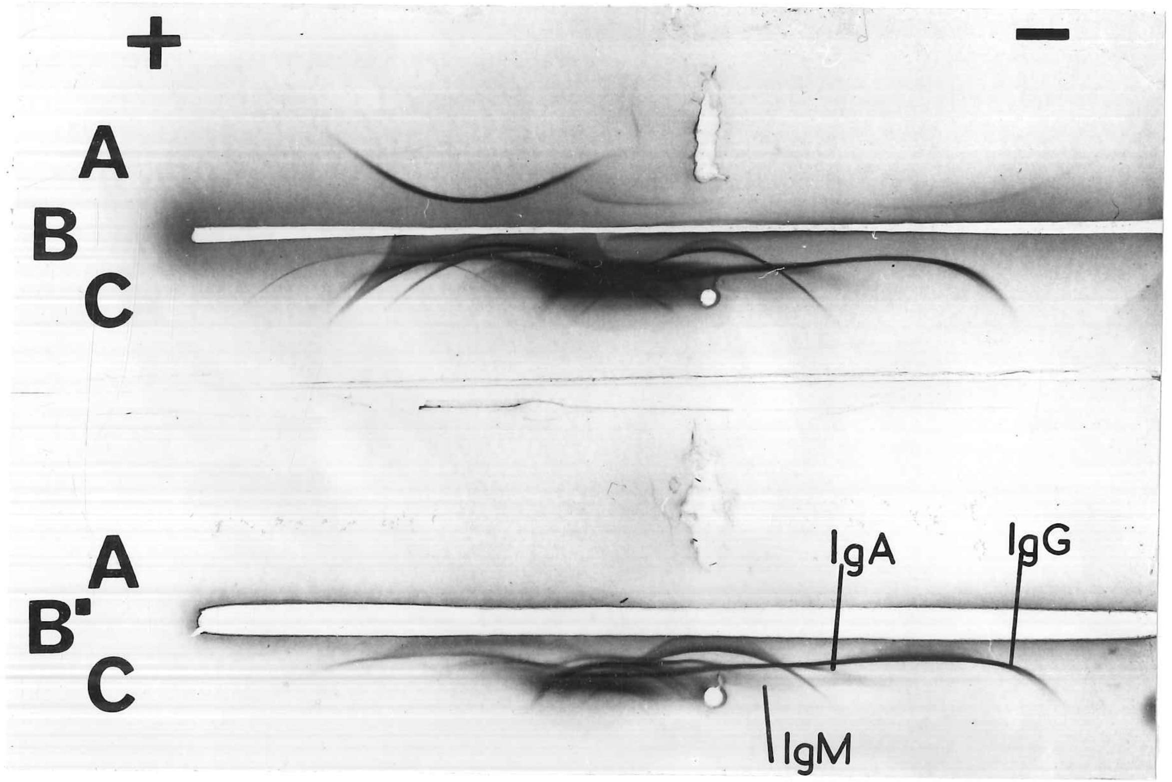


Figure 82.

A = human seminal plasma

B = human serum

C = antiserum to human IgG

D = antiserum to human IgA

E = antiserum to human IgM

Note faint IgG line in seminal plasma (arrowed)

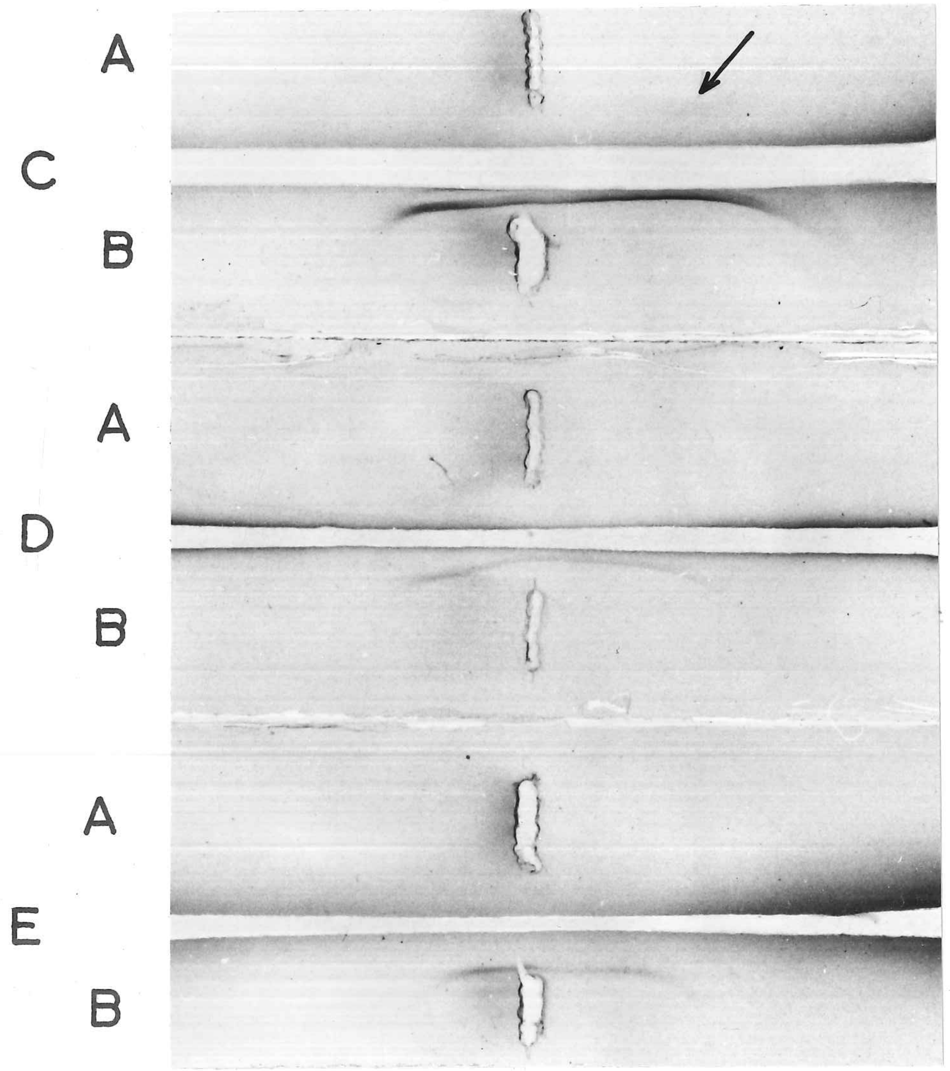


Figure 83.

Immunoelectrophoresis.

A Bovine seminal plasma

B Bovine serum

C Antiserum to bovine gamma-globulin.

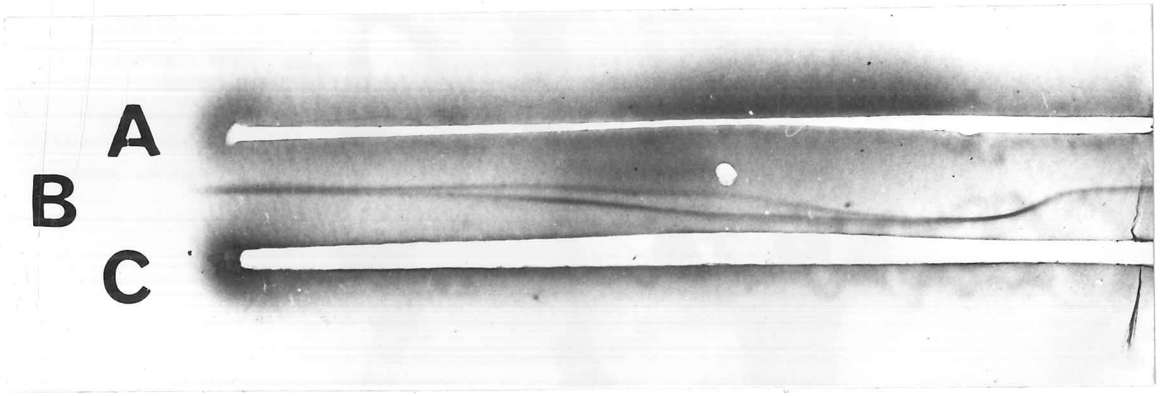


Figure 84.

Uterine diffusion chamber before and after adding the cellulose ester membrane.

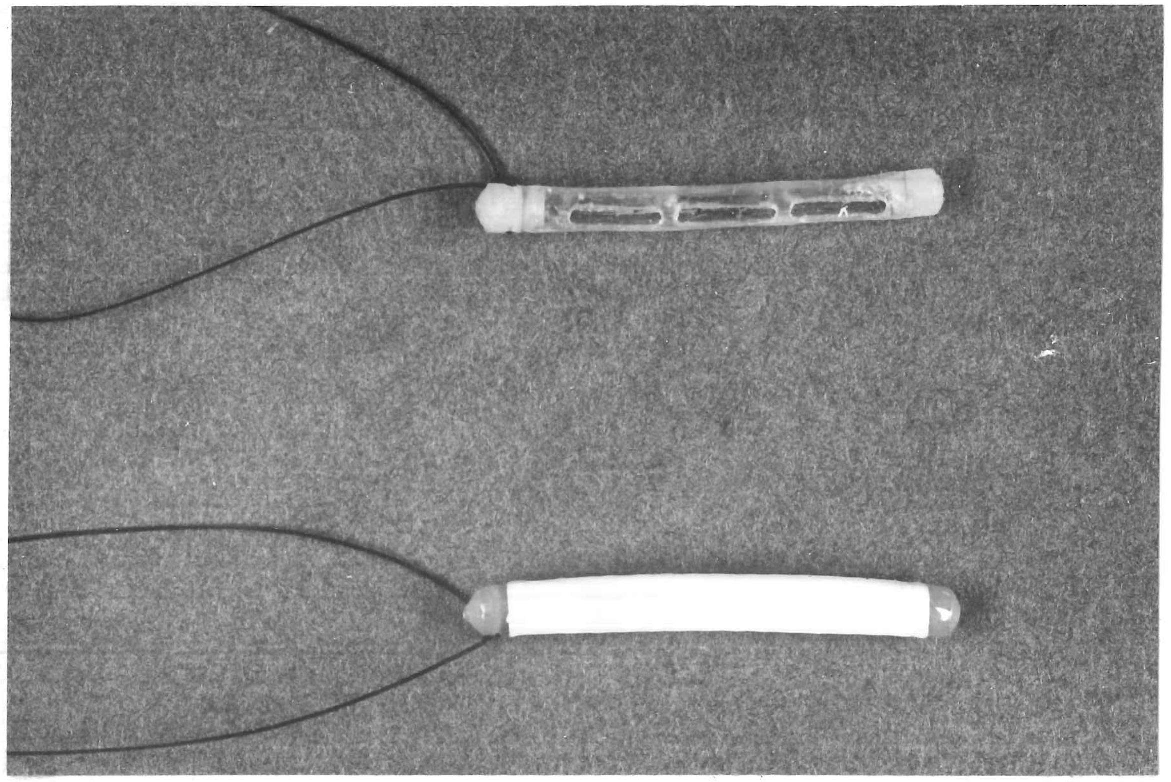
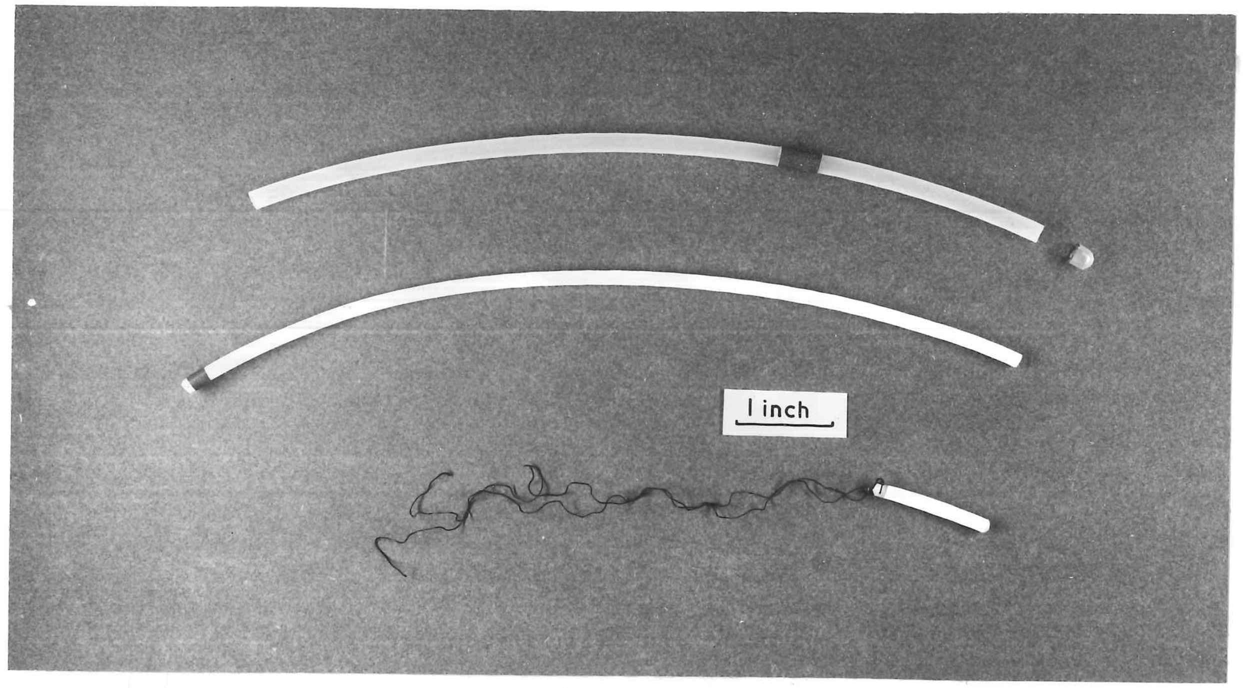


Figure 85.

a) Inserter tube plus cap which protects contents on entry but melts at body temperature.

Inserter rod.

Chamber.



b) Inserter tube and rod for Saf-T-Coil for comparison.

Inserter tube containing rod and chamber, and with cap over tip.

Bag in which assembled unit is sealed and sterilized.

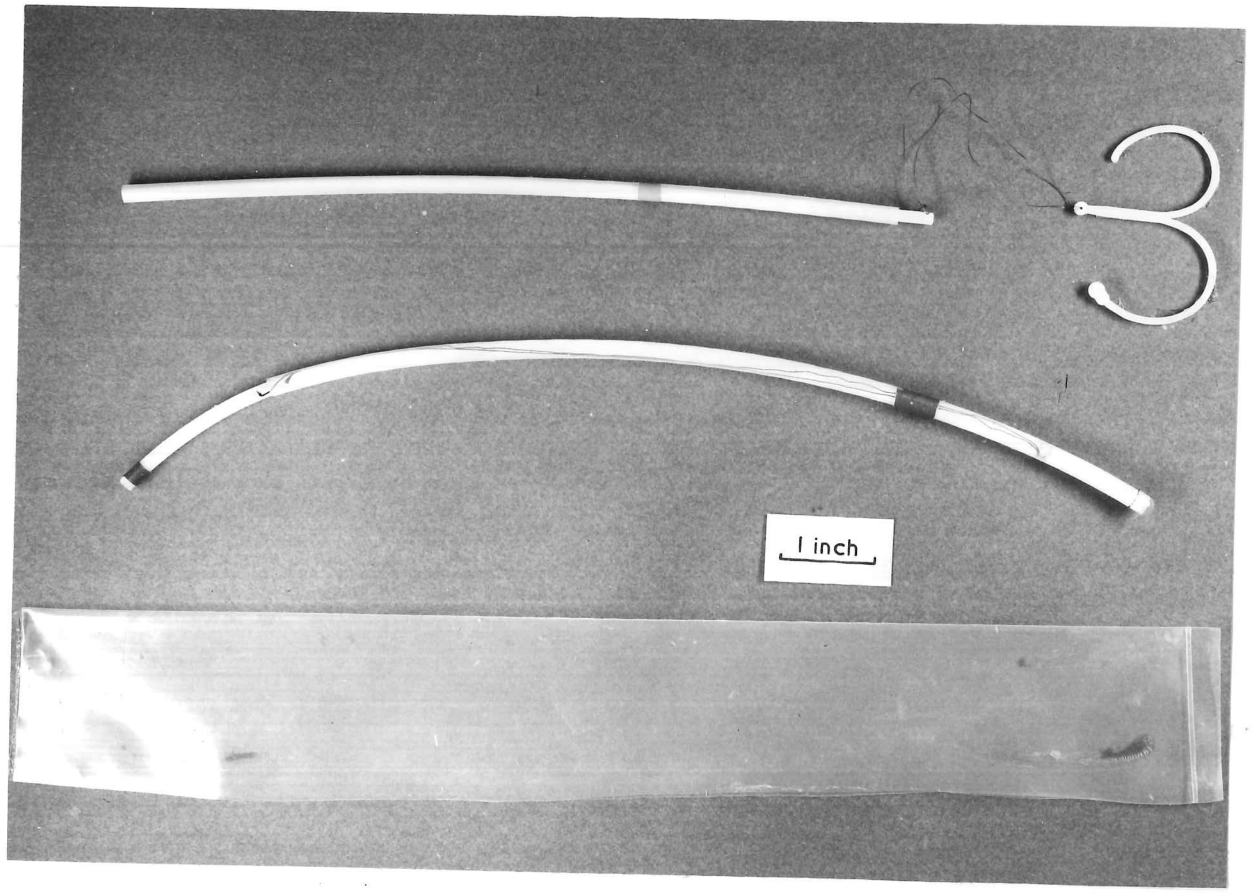


Figure 86.

Cervical os of rabbit exposed and gently held ready for insertion of blunt pipette.

Figure 87.

Pipette containing flushing saline inserted.

Table 16. Composition of the fluid in uterine chambers removed from women 12 hours after insertion.

Component	Chamber 1ø (pore size 1.2μ)	Chamber 2 (pore size 3.0μ)	Chamber 3 (pore size 1.2μ)
Na^{+} (mEq/L.)	128 (133)⌶	143 (133)	158 (146)
K^{+} (mEq/L.)	6.1 (4.4)	7.1 (4.4)	9.2 (5.3)
Cl^{-} (MEq/L.)	112 (100)	108 (100)	N.D.ꝗ
Total protein (Gm%)	5.2 (6.6)	5.0 (6.6)	4.8 (7.0)

ø A few erythrocytes were found in this chamber.

⌶ Values in brackets are for serum taken from the woman at the time of insertion of the chamber

ꝗ Not done.

Table 17. Analysis of proteins in uterine fluid by cellulose acetate electrophoresis and microdensitometry (values for serum in parenthesis)

Component	Percentage of total protein		
	Chamber 1* (pore size 1.2μ)	Chamber 2 (pore size 3.0μ)	Chamber 3 (pore size 1.2μ)
Albumin	53.0 (53.1)	52.7 (53.1)	57 (54)
α_1	5.2 (4.1)	6.0 (4.1)	5.5 (3)
α_2	9.5 (13.3)	14.9 (13.3)	23.0 ⌶ (16) ⌶
β	10.9 (10.2)	11.9 (10.2)	5.5 (11)
γ	19.6 (19.4)	14.0 (19.4)	8.5 (16)
Extra peak ø	1.8 (0)	0.5 (0)	(0) (0)

* Affew erythrocytes were found in this chamber.

⌶ Point of application in α_2 position in these samples. Inert material included in the α_2 reading may correspond to the extra peak found in Chambers 1 and 2.

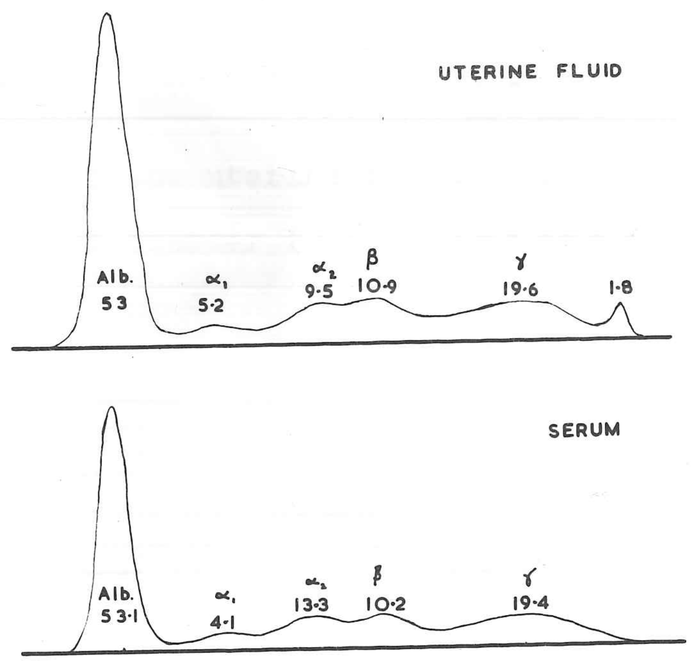
ø Extra peak was material left very close to the point of application.

Figure 88.

Cellulose acetate traces of fluid from human uterus collected by diffusion chamber and of human serum.

Figure 89.

Immunoelectrophoresis of fluid from human uterus collected by diffusion chamber and of human serum.



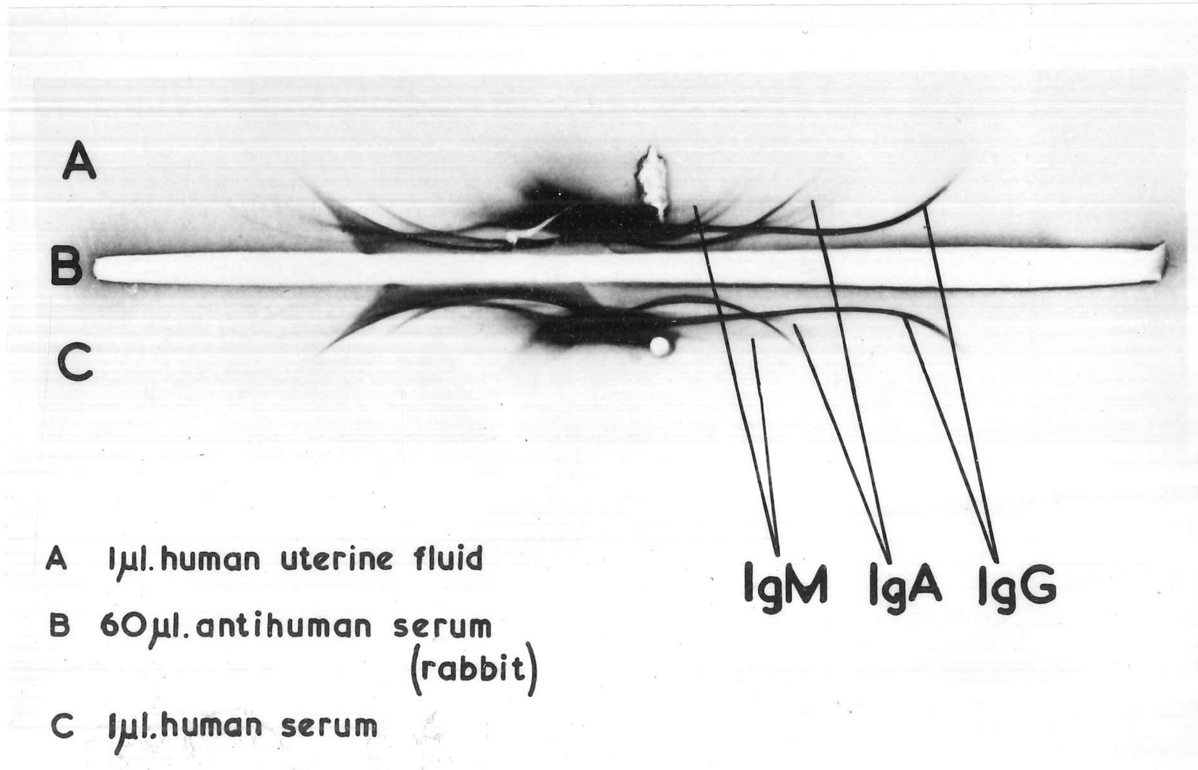


Table 18.

Analysis of cow uterine fluid 12 hours after insertion of chamber (Data from 8 cows).

Components	Uterine fluid	Serum
Na^+ (mEq/L)	158.8	137.8
K^+ (")	11.9	8.4
Total protein (gm%)	2.8	8.7
Na^+/K^+ ratio	14.0	13.4

Figure 90.

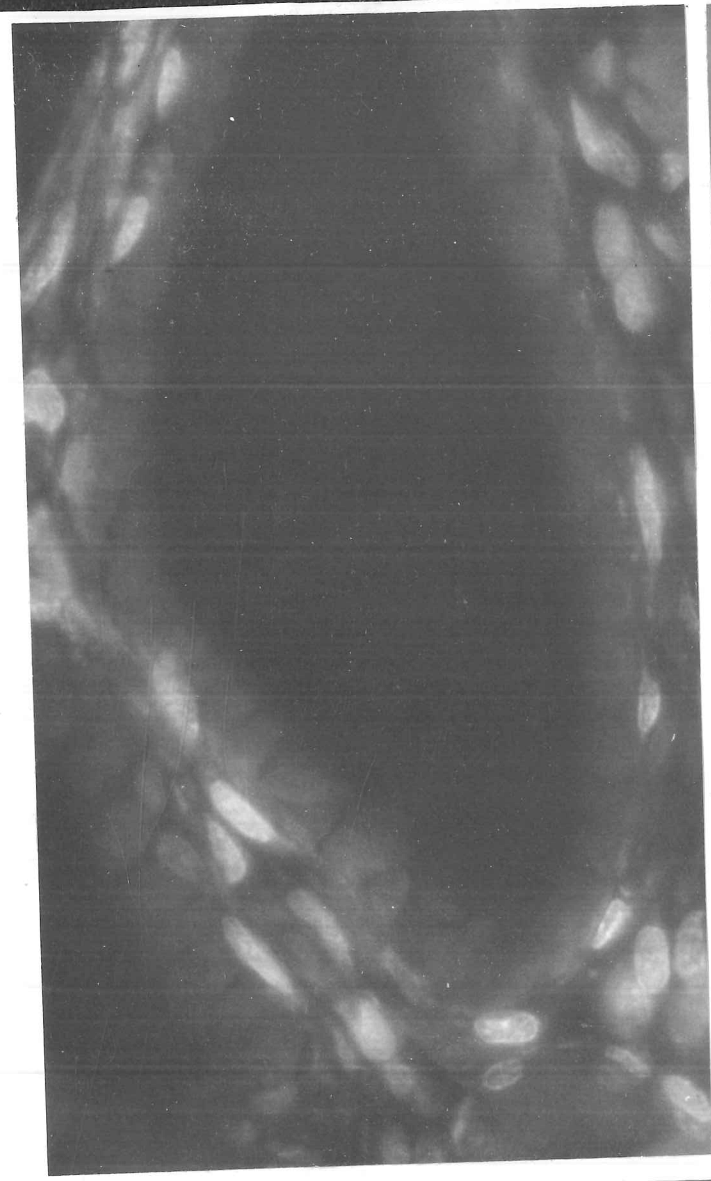
Section of oestrous uterus of rabbit injected intravenously with acriflavine 30 minutes previously. Note paleness of surface epithelium fluorescence. X 720

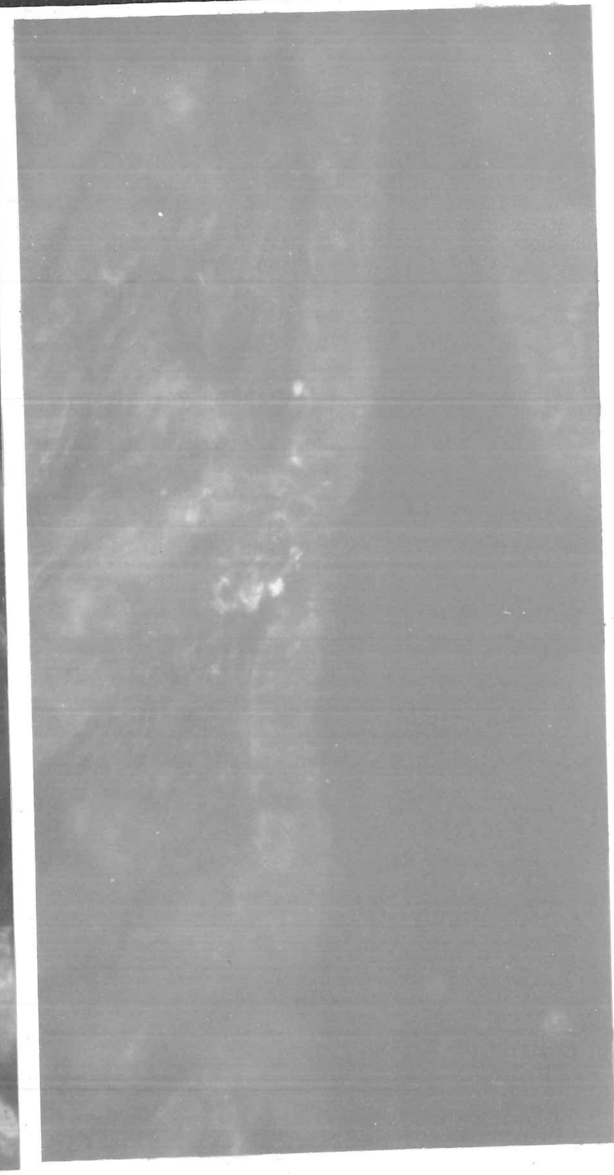
Figure 91.

Similar section from an animal not injected with acriflavine. X 180

Figure 92.

Similar section from animal into the uterus of which a solution of acriflavine had been injected 30 minutes previously. Note that only surface epithelium stains X 180





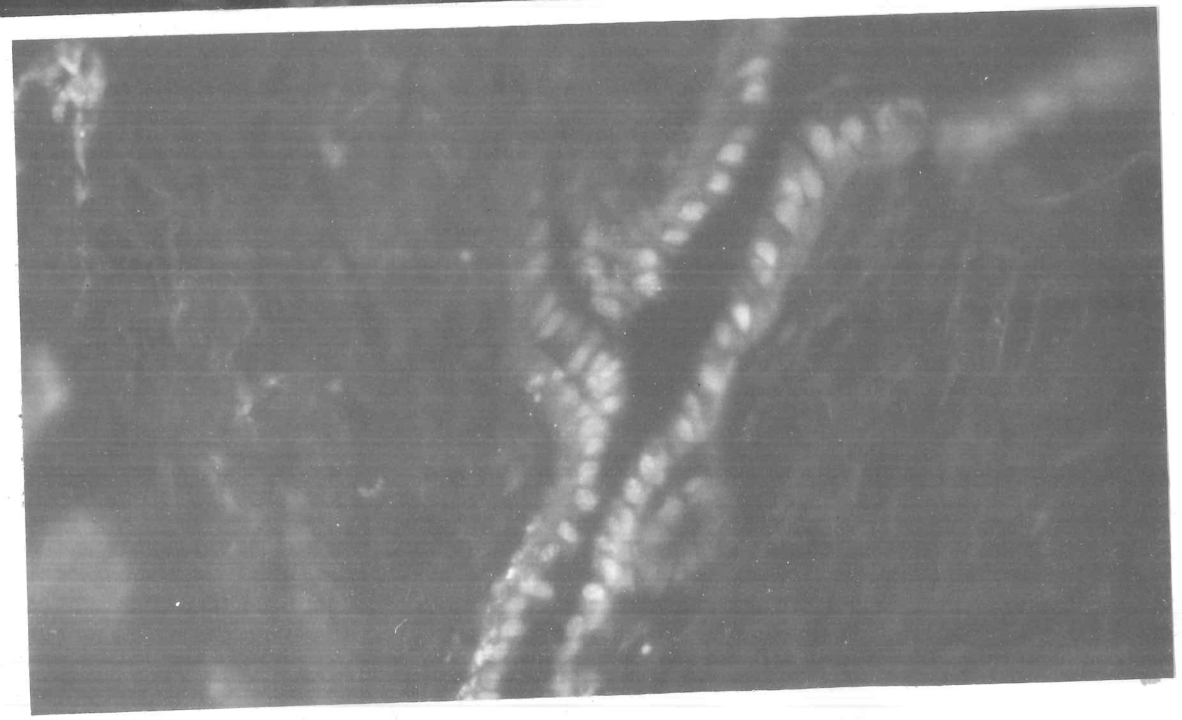


Figure 93.

Immunoelectrophoresis.

A = Human serum

B = Human follicular fluid

C = Antiserum to human serum (rabbit)

D = Antiserum to human IgG (rabbit)

E = Antiserum to human IgA (rabbit)

F = Antiserum to human IgM (rabbit)

Note absence of IgM from follicular fluid.

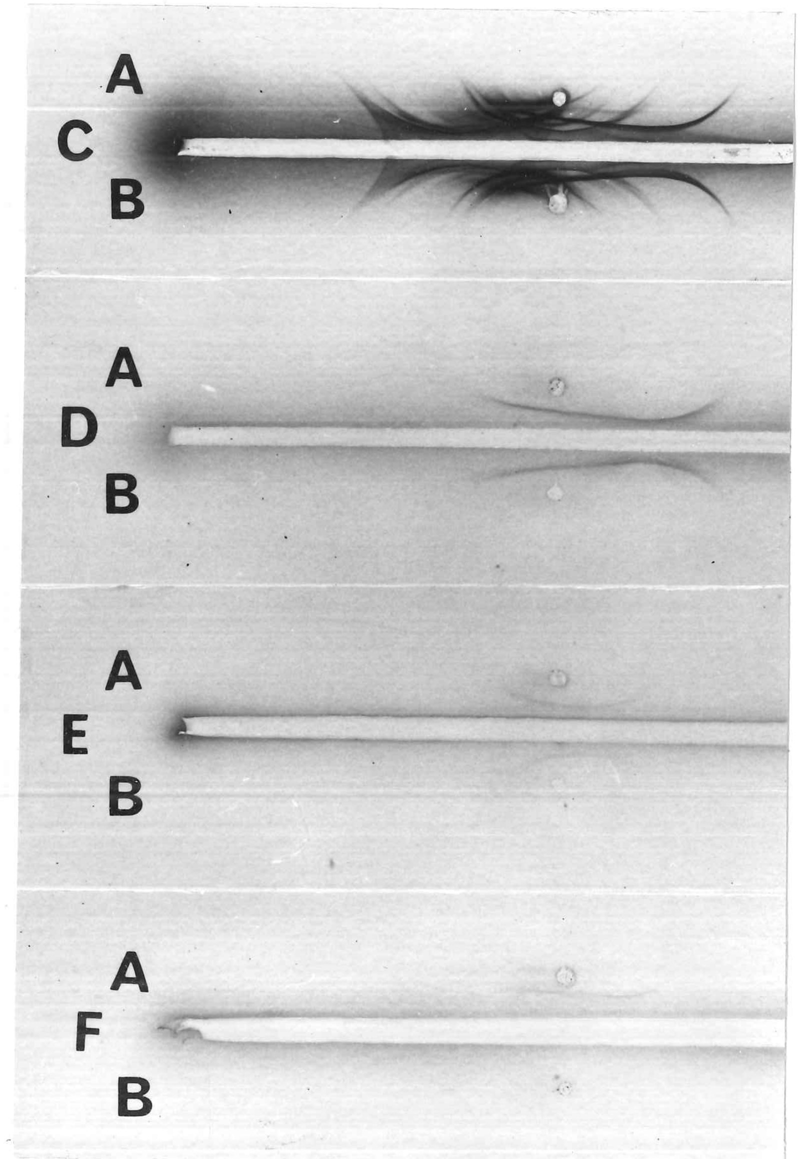


Table 19. Concentration of some serum proteins in human follicular fluid.

Protein	Concentration mgm% Follicular fluid ± S.E. ⊛	Concentration mgm% Serum*	Follicular fluid as %age of serum.
Albumin	2560 ± 499	4400	58
α_1-glycoprotein	45 ± 7	90	50
β_1A-globulin	45 ± 7	100	45
Transferrin	149 ± 24	320	41
Haptoglobin	65 ± 23	160	41
α_2-Macroglobulin	28 ± 6	240	12
IgM	17 ± 2	140	12
IgA	75 ± 12	210	36
IgG	580 ± 90	1300	45

⊛ Variations between fluids could be due to sampling of follicles of variable maturity.

* taken from Storiko (1969)

Figure 94.

Immunoelectrophoresis

A = hamster serum

B = antiserum to hamster serum

C = fluid extracted from egg and cumulus cell mass recovered from hamster oviduct.

Note gamma-globulin line (arrowed)