PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

The Reproductive Processes of Certain Mammals. Part V. Changes in the Reproductive Organs of the Male Grey Squirrel (Sciurus carolinensis)

Marjorie Allanson

Phil. Trans. R. Soc. Lond. B 1932 **222**, 79-96
doi: 10.1098/rstb.1932.0012

Part V. *Changes in the Reproductive Organs of the Male Grey Squirrel* (Sciurus carolinensis).

By Marjorie Allanson, *Department of Zoology, King's College, London.*

(*Communicated by Sir* Henry Dale, *Sec. R.S.*)

(Received August 25, 1932.—Read January 26, 1933.)

[Plate 15.]

Contents.

I. *Introduction.*

Both sexes of the grey squirrel show diminished general activity during the winter months and, while there is no true hibernation, squirrels probably sleep during severe weather (Middleton, 1930). In Part IV, Deanesly and Parkes show that sexual activity in the female is limited to the spring and summer, the reproductive organs being quiescent throughout the rest of the year. In view of these facts it seemed probable that the male squirrel would also show seasonal reproductive activity. The present paper deals with an investigation into this question and also with a study of the correlation between the size of the testes and that of the accessory reproductive organs.

From the material it is evident that there is no regular seasonal cycle in the reproductive organs of the male squirrel, which considered as a species, therefore, has no "anœstrus." The possibility that individual males may experience temporary periods of inactivity cannot be excluded, but there is no doubt that the great majority of inactive animals are prepubertal. Therefore size changes in the testis, in its constituent parts, and in the accessory organs, are considered to represent initial growth.

II. *Material and Technique.*

(*a*) *Source of Material.*—The material consists of the males of the grey squirrel collection described in Part IV. Most of these taken alive were killed and dissected immediately, or within a few days of trapping. Animals kept in the laboratory for longer periods are not included, so that this account is of the animal in the wild state.

(*b*) *Method of Weighing.*—After fixation the reproductive organs were upgraded to 70 per cent. alcohol, dissected free of fat and connective tissue and then weighed. No difficulty was experienced in attaining a reasonably constant degree of dissection except in the case of the penis, for which the weights are therefore less valid. For the smaller organs a torsion balance was used having an accuracy of $\pm 0 \cdot 5$ mg.; the larger material was weighed to within 10 mg. on a chemical balance. The full data for all the 112 animals used are given in Table I. In the case of paired organs the values given represent the combined weights of the two.

(*c*) *Histological Treatment.*—The reproductive organs of all animals considered were fixed within 30 minutes of killing when possible, mostly in Bouin's fluid (alcoholic), but a few were fixed in formalin. Many of the animals, however, were obtained a considerable time *post-mortem*, or the organs were badly fixed for other reasons, and histological examination was restricted to 76 of the more suitable animals. Sections of the testis were cut at 7 μ, those of the epididymis at 10 μ. In 9 animals a fragment of one testis was fixed in Flemming's strong fluid. The Bouin material was stained with Ehrlich's hæmatoxylin and eosin; the Flemming material was mounted, unstained, in Farrant's glycerine medium to preserve the fat content of the interstitial cells.

(*d*) *Measurement of Tissues.*—Only Bouin-fixed material has been used in the measurement of constituent parts of testis and epididymis. The diameter of the testis tubules and of the epididymis tube, and the area of the interstitial cells given in Table I are the average of ten measurements in each case. The diameters of the seminiferous tubules were obtained from camera lucida drawings, at a magnification of 100 diameters, made from approximately median transverse sections. The least diameters of tubules from the periphery of the section only were measured. In the epididymis, measurements were all taken in the body region; the diameters of the tube include the surrounding muscle layers, and the area of interstitial cells was calculated from camera lucida drawings at a magnification of 1500 diameters.

(*e*) *Statistical Treatment.*—The statistical treatment of the material has been in accordance with the advice of Dr. R. A. FISHER. The weights of the accessory organs (except

TABLE I.

Number of squirrel.	Date.	Body weight.	Testis. Weight (pair).	Testis. Mean diameter of tubules.	Testis. Mean area of interstitial cells.	Epididymis. Weight (pair).	Epididymis. Mean diameter of tube.	Weight of prostate.	Weight of Cowper's glands (pair).	Weight of seminal vesicles (pair).	Weight of Penis.
		gm.	gm.	μ	sq. μ	gm.	μ.	gm.	gm.	gm.	gm.
97	2.7.31	—	0·062	—	—	0·066	—	0·053	0·032	0·010	0·287
192	19.12.31	—	0·074	—	—	0·064	—	0·034	—	0·004	—
206	22.12.31	—	0·092	—	—	0·052	—	0·036	0·032	0·008	0·384
191	19.12.31	—	0·098	—	—	0·064	—	0·042	0·040	0·004	0·642
188	19.12.31	—	0·098	—	—	0·076	—	0·044	0·004	0·004	0·524
101	16.7.31	300	0·106	64	54	0·072	63	0·037	—	0·010	0·269
89	1.7.31	240	0·120	66	48	0·100	58	0·062	—	0·004	0·478
71	–.11.30	580	0·120	63	60	0·098	—	0·037	—	0·002	—
157	28.11.31	—	0·123	—	69	0·088	—	0·045	0·028	—	0·602
207	23.12.31	—	0·128	—	—	0·074	—	0·036	—	0·004	0·448
201	3.1.32	—	0·142	—	—	0·088	—	0·034	0·032	0·012	0·615
181	22.12.31	425	0·143	61	59	0·078	51	0·052	0·008	0·008	0·548
186	19.12.31	—	0·148	—	—	0·066	—	0·044	0·038	0·008	—
142	7.12.31	420	0·166	69	63	0·070	48	0·056	—	0·006	0·950
130	23.11.31	410	0·188	75	69	0·078	61	0·062	—	—	0·570
45	16.2.31	450	0·192	62	66	0·138	—	0·074	—	—	0·504
17	11.11.30	520	0·244	67	62	0·140	—	—	—	—	0·427
100	9.7.31	—	0·298	—	—	0·160	—	—	0·060	—	0·890
18	17.11.30	550	0·358	—	—	0·242	—	—	—	—	0·586
234	23.3.32	480	0·366	80	103	0·251	93	0·160	0·078	0·038	0·890
220	14.3.32	320	0·374	96	89	0·136	77	0·051	0·022	0·012	0·639
5	7.10.30	565	0·484	81	66	0·410	—	—	—	—	—
116	28.8.31	—	0·542	110	103	0·258	—	—	0·182	—	1·580
114	28.8.31	—	0·612	81	88	0·338	—	—	0·256	—	2·220
109	26.8.31	550	0·628	—	—	0·360	—	0·198	—	0·038	2·320
135	9.9.31	—	0·634	—	—	0·300	—	—	—	—	—
30	6.1.31	550	0·636	77	76	0·370	—	—	—	—	2·352
104	25.7.31	440	0·648	103	—	0·488	90	0·223	0·458	0·030	1·850

TABLE I—*continued.*

Number of squirrel.	Date.	Body weight.	Testis.			Epididymis.		Weight of prostate.	Weight of Cowper's glands (pair).	Weight of seminal vesicles (pair).	Weight of Penis.
			Weight (pair).	Mean diameter of tubules.	Mean area of interstitial cells.	Weight (pair).	Mean diameter of tube.				
		gm.	gm.	μ	sq. μ	gm.	μ.	gm.	gm.	gm.	gm.
111	26.8.31	480	0·692	—	—	0·368	—	0·184	—	0·032	2·080
39	27.1.31	540	0·736	—	—	0·322	—	0·136	—	—	1·460
141	9.9.31	—	0·746	—	—	0·326	—	—	—	—	1·000
235	23.3.32	410	0·758	124	134	0·260	100	0·216	0·040	0·036	1·150
98	2.7.31	—	0·794	—	—	0·458	—	—	0·778	—	1·800
105	25.7.31	500	0·798	100	—	0·420	88	0·248	—	0·038	1·970
96	2.7.31	—	0·798	—	—	0·412	—	—	—	—	1·920
6	8.10.30	586	0·808	94	93	0·474	—	—	—	—	—
33	15.2.31	—	0·816	—	—	0·394	—	—	—	—	1·620
108	29.7.31	—	0·846	—	—	0·506	—	—	0·846	—	1·990
21	12.12.30	470	0·856	99	85	0·338	77	—	—	—	1·216
241	23.3.32	—	0·900	—	—	0·180	—	0·086	—	0·012	0·688
136	9.9.31	—	0·906	—	—	0·432	—	—	—	—	—
28	24.11.30	500	0·942	106	106	0·582	—	—	—	—	1·804
166	15.12.31	360	0·956	68	59	0·134	53	0·058	0·034	0·004	0·624
106	25.7.31	—	0·956	108	—	0·536	87	0·235	0·610	0·032	1·840
232	23.3.32	500	1·050	118	143	0·360	100	0·117	0·118	0·036	1·400
140	9.9.31	—	1·058	—	—	0·440	—	—	—	—	—
134	9.9.31	—	1·124	—	—	0·432	—	—	—	—	—
115	28.8.31	—	1·158	—	109	0·450	—	—	—	—	1·710
132	20.10.31	—	1·172	141	125	0·202	—	0·112	0·072	—	1·330
222	18.3.32	360	1·224	130	140	0·346	88	0·108	0·100	0·012	1·550
217	16.3.32	580	1·230	123	179	0·514	108	0·275	0·358	0·036	2·120
138	9.9.31	—	1·248	—	100	0·402	—	—	—	—	1·500
13	3.11.30	700	1·328	120	119	0·550	—	—	—	—	—
139	9.9.31	—	1·352	—	—	0·550	—	—	—	—	—
15	5.11.30	500	1·386	129	117	0·608	85	—	—	—	—
231	19.3.32	600	1·460	103	134	0·564	128	0·250	0·770	0·041	1·700

223	18.3.32	525	1·530	117	149	0·636	108	0·248	0·642	0·056	2·070
126	27.10.31	—	1·531	—	—	0·574	—	0·150	—	0·026	2·025
120	10.10.31	—	1·536	134	135	0·458	—	0·138	0·236	0·024	1·650
125	11.11.31	525	1·542	147	151	0·456	107	0·081	0·074	0·010	1·250
137	9.9.31	—	1·582	153	124	0·506	—	—	—	—	—
99	2.7.31	—	1·582	130	—	0·506	—	0·249	0·594	0·046	2·000
41	1.7.31	420	1·740	—	—	0·620	—	0·304	0·540	0·036	2·295
91	1.7.31	400	2·000	—	—	1·390	159	0·720	2·020	0·130	2·030
34	19.2.31	—	2·100	—	—	0·458	—	0·238	—	—	2·280
224	18.3.32	550	2·330	154	—	0·640	137	0·504	0·756	0·047	2·250
60	27.4.31	—	2·450	—	—	0·816	130	0·492	—	0·060	1·210
59	27.4.31	—	2·460	—	—	0·732	—	0·616	—	0·072	1·465
156	6.12.31	—	2·588	175	—	0·850	—	0·218	—	0·036	1·340
128	14.5.31	—	2·594	—	—	0·827	—	0·614	0·532	—	1·400
20	17.11.30	600	2·764	—	165	0·976	—	—	—	—	1·590
76	17.6.31	—	2·880	160	149	0·800	—	0·520	—	—	1·280
79	22.6.31	380	3·026	190	—	1·144	175	0·703	1·034	0·054	1·610
42	11.3.31	540	3·304	—	—	0·566	—	0·500	—	—	1·450
127	8.6.31	—	3·368	—	—	1·240	—	—	2·480	—	1·690
55	30.4.31	705	3·516	205	—	1·620	202	—	—	—	—
161	12.12.31	480	3·600	211	256	1·340	160	0·904	1·320	0·076	2·220
147	11.12.31	520	3·930	209	167	0·834	160	0·113	0·176	0·028	1·820
67	10.6.31	400	3·950	198	—	1·002	168	1·100	3·140	—	1·420
83	27.6.31	—	4·100	—	—	1·610	—	0·674	2·640	0·066	2·140
211	14.9.31	—	4·100	—	—	1·650	—	1·000	1·670	0·100	1·940
123	10.10.31	—	4·130	—	199	0·942	—	0·274	0·218	0·062	2·020
75	17.6.31	—	4·280	—	—	1·210	—	0·970	—	0·060	1·700
81	27.6.31	350	4·330	—	—	1·310	—	0·933	2·690	0·052	2·050
70	16.6.31	470	4·440	—	—	1·550	—	1·290	3·660	0·078	2·000
35	15.1.31	—	4·496	—	—	—	—	—	—	—	1·670
74	17.6.31	500	4·700	—	—	1·270	—	0·720	—	0·080	1·290
50	1.7.31	460	4·800	—	—	1·330	—	1·032	1·450	0·068	1·780
90	1.7.31	420	4·840	197	—	1·380	157	0·900	2·230	—	1·650
52	24.4.31	520	4·850	—	—	1·450	146	0·750	—	—	1·700
189	19.12.31	—	4·850	—	—	1·570	—	—	0·860	—	1·870
182	22 12.31	500	4·920	—	—	—	—	—	—	—	—
163	15.12.31	430	4·926	219	167	1·346	159	0·780	0·820	0·060	2·020
218	16.3.32	640	5·058	196	192	1·346	177	0·675	1·148	0·056	2·220
187	19.12.31	—	5·170	—	—	1·570	—	0·818	1·266	0·062	1·730
202	22.12.31	—	5·200	—	—	2·120	—	1·378	3·850	0·122	1·420
190	19.12.31	—	5·250	—	—	1·550	—	0·818	0·950	0·072	2·370

TABLE I.—*continued.*

Number of squirrel.	Date.	Body weight.	Testis.			Epididymis.		Weight of prostate.	Weight of Cowper's glands (pair).	Weight of seminal vesicles (pair).	Weight of Penis.
			Weight (pair).	Mean diameter of tubules.	Mean area of instertitial cells.	Weight (pair).	Mean diameter of tube.				
		gm.	gm.	μ	sq. μ	gm.	μ	gm.	gm.	gm.	gm.
183	22.12.31	520	5·282	218	191	1·550	163	1·125	2·090	0·070	2·080
58	28.4.31	—	5·300	—	—	1·970	—	1·104	—	0·106	1·585
77	17.6.31	—	5·380	—	—	1·600	—	1·300	—	—	1·700
205	4.1.31	—	5·500	—	—	1·600	—	1·388	1·750	0·082	2·800
37	16.1.31	550	5·504	208	168	1·586	152	1·340	—	—	1·852
204	3.1.31	—	5·520	—	—	1·742	—	1·136	2·830	0·134	1·920
145	7.12.31	500	5·540	210	236	1·466	151	0·956	1·084	0·064	2·370
40	8.4.31	560	5·548	—	—	1·284	—	0·890	—	—	1·500
150	11.12.31	600	5·640	188	238	1·404	183	0·821	0·850	0·058	2·540
64	26.5.31	460	5·700	208	205	1·650	192	1·070	2·350	0·056	2·860
194	29.12.31	460	5·710	—	217	1·600	155	0·750	1·840	0·060	2·400
170	17.12.31	600	5·936	208	238	1·914	196	1·302	3·580	0·088	1·490
162	15.12.31	480	5·960	218	204	1·464	170	0·780	0·954	0·080	1·900
149	11.12.31	600	5·990	—	259	1·764	178	1·342	1·730	0·108	2·420
203	22.12.31	—	7·400	—	—	1·890	—	1·276	3·000	0·124	2·880

penis) plotted against the weight of the testes showed obvious linear correlations, and linear regression formulæ were therefore calculated for these relationships. In other cases, since the relative abundance of information varies in different parts of the range, and since the variates represent simultaneous states of reaction to the same general physiological condition, graphical representation is relied upon rather than algebraic analysis.

III. *Breeding Season.*

In the grey squirrel, litters are born in early spring (February–March) and summer (June–July). Animals having litters in the first group are largely parous females, with probably a few of the early young from the previous year. The summer litters are from the remaining young of the previous year and the second mating of the parous does. It is extremely improbable that females born in February ever breed in the same year.

Males trapped or shot in the first half of the year will therefore be from 6–15 months of age or else old males. The current young of March–April will begin to be obtained as partly grown animals from June onwards. Later in the second half of the year young from both batches of litters will be obtained, together with old males.

IV. *General Morphology of the Reproductive Organs.*

The reproductive organs of the male grey squirrel have been described by Owen (1868) and of the red squirrel by Grosz (1905). In the adult the testes lie in scrotal sacs on either side of the base of the penis. The epididymis and spermatic cord are heavily invested with fat. The prostate is a single, elongated, compact body attached to the ventral surface of the muscular part of the urethra. Its anterior end is prolonged forward in two blunt outgrowths which may indicate its paired origin. The seminal vesicles are small and adhere closely to the prostate gland. The main part of each seminal vesicle is bent back upon the ventral surface of the prostate, and a long duct joins the vasa efferentia on either side just before they open to the urethra, Fig. 1.

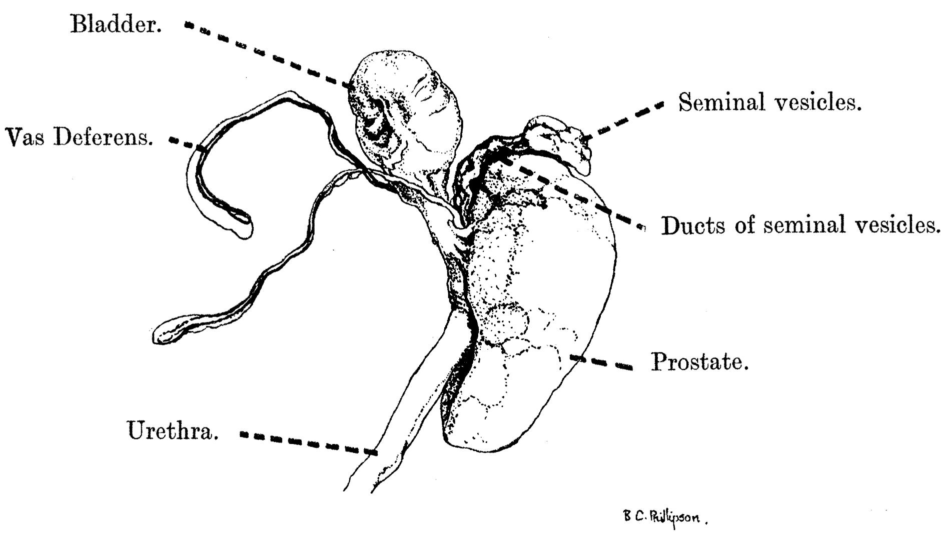


Fig. 1.—Dissection of the anterior end of urethra to show relationships of the prostate and seminal vesicles.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Grosz maintains that the unpaired organ should be called a "vesicular gland" while the paired glands should be "ampullary glands." Since the histological structure of these organs resemble that of prostate and seminal vesicles respectively, the usual terms have been employed in this description.

A pair of large, spirally wound Cowper's glands are situated at the sides of the rectum and lie embedded in the fascia of the thigh. A long thick duct passes from each to open into the bulb of the urethra. The penis is sharply bent backwards at its distal end. See Plate 15.

In the immature animal the testes lie subcutaneously on each side of the penis, close to where the spermatic cord passes through the abdominal muscle; the accessory organs are small and the seminal vesicles and Cowper's glands difficult to distinguish.

V. *Seasonal Changes in the Testis.*

The essential criterion of the occurrence of an anœstrous period in a species is that at some season of the year all individuals obtained shall have quiescent reproductive organs. It will be seen from Table II and fig. 2 that animals with fully functional testes were obtained in all months except two. In this material there are no animals with fully active testes in February and August, but Mr. A. D. Middleton of Oxford, in collecting squirrels for an ecological study, has found animals with heavy testes and an abundance of sperms in the epididymis in both these months. Judged both by the size and the condition of the testes, therefore, the male grey squirrel, considered as a species, has no period of quiescence.

While there is no seasonal periodicity in the activity of the adults, the young males differ considerably in the state of development of their reproductive organs at different seasons. From April to the end of June in all animals observed the testes weighed more than 2 gm. and contained spermatozoa; therefore, by the beginning of April, all young from the previous year have reached puberty. In all other months there are animals with small aspermatic testes; and in March such animals cannot be less than 9 months old. Hence it is evident that in the male squirrel the period of immaturity may be not less than 9 months, but is certainly not more than 13 months.

It is possible that males born in early spring may develop functional testes during the same year, but it is improbable that they develop rapidly enough to be ready for breeding in June and July. The occurrence late in the year of large, fully-grown males with small testes shows that the growth of the testes in relation to body weight is often retarded during the first year, *e.g.* Nos. 5, 17, 71. Most of the males born in the summer probably remain in a state of sexual immaturity throughout the winter and begin breeding in the following spring.

This variation in the rate of development in the males of the spring and summer litters is shown by the absence of any constant relation between testes weight and body weight such as has been found in rats (Donaldson, 1924). The conditions in which

TABLE II.

Month.	Number of squirrel.	Body weight.	Weight of testes (pair).	Sperm-atocytes.	Sperm-atids.	Sperm-atozoa.
		gm.	gm.			
January 6	30	550	0·636	+	—	—
,, 15	35	—	4·496	+	+	+
,, 16	37	550	5·504	+	+	+
February 16	45	450	0·192	+	—	—
March 16	217	580	1·230	+	—	—
,, 16	218	640	5·058	+	+	+
,, 14	220	320	0·374	+	—	—
,, 18	222	360	1·224	+	+	—
,, 18	223	525	1·530	+	—	—
,, 18	224	550	2·330	+	+	+
,, 19	231	600	1·460	+	+	—
,, 23	234	480	0·366	+	—	—
,, 23	232	500	1·050	+	+	—
,, 23	235	410	0·758	+	—	—
April 24	52	520	4·850	+	+	—
,, 27	59	—	2·460	+	+	+
,, 27	60	—	2·450	+	+	+
,, 30	55	705	3·516	+	+	+
May 5	128	—	2·594	+	+	+
,, 26	64	460	5·700	+	+	+
June 10	67	400	3·950	+	+	+
,, 17	76	—	2·880	+	+	+
,, 22	79	380	3·026	+	+	+
,, 27	81	350	4·330	+	+	+
July 1	41	420	1·740	+	+	—
,, 1	89	240	0·120	+	—	—
,, 1	90	420	4·840	+	+	+
,, 1	91	400	2·000	+	+	+
,, 2	99	—	1·582	+	+	—
,, 16	101	300	0·106	+	—	—
,, 25	104	440	0·648	+	—	—
,, 25	105	500	0·798	+	—	—
,, 25	106	—	0·956	+	—	—
August 28	114	—	0·612	+	—	—
,, 28	115	—	1·158	+	—	—
,, 28	116	—	0·542	+	—	—
September 9	134	—	1·124	+	—	—
,, 9	135	—	0·634	+	—	—
,, 9	136	—	0·906	+	—	—
,, 9	137	—	1·582	+	+	—
,, 9	138	—	1·248	+	—	—
,, 9	139	—	1·352	+	—	—

TABLE II—*continued.*

Month.	Number of squirrel.	Body weight.	Weight of testes. (pair)	Sperm-atocytes.	Sperm-atids.	Sperm-atozoa.
		gm.	gm.			
September 9	140	—	1·058	+	—	—
,, 9	141	—	0·746	+	—	—
,, 14	211	—	4·100	+	+	+
October 7	5	565	0·484	+	—	—
,, 8	6	586	0·808	+	—	—
,, 10	120	—	1·536	+	+	+
,, 10	123	—	4·130	+	+	+
,, 20	132	—	1·172	+	+	—
,, 27	126	—	1·531	+	+	+
November —	71	580	0·120	+	—	—
,, 3	13	700	1·328	+	+	—
,, 5	15	500	1·386	+		—
,, 11	17	520	0·244	+	—	—
,, 11	125	525	1·542	+	+	—
,, 17	20	600	2·764	+	+	+
,, 17	18	550	0·358	+	—	—
,, 23	130	410	0·188	+	—	—
,, 24	28	500	0·942	+	+	—
,, 28	157	—	0·123	+	—	—
December 6	156	—	2·588	+	+	+
,, 7	142	420	0·166	+	—	—
,, 7	145	500	5·540	+	+	+
,, 11	147	520	3·930	+	+	+
,, 11	149	600	5·990	+	+	+
,, 11	150	600	5·640	+	+	+
,, 12	21	470	0·856	+	—	—
,, 12	161	480	3·600	+	+	+
,, 15	162	480	5·960	+	—	—
,, 15	163	430	4·926	+	+	+
,, 15	166	360	0·956	+	—	—
,, 17	170	600	5·936	+	+	+
,, 22	181	425	0·143	+	—	—
,, 22	182	500	4·920	+	+	+
,, 22	183	520	5·282	+	+	+
,, 29	194	460	5·710	+	+	+

most of the animals were killed made it impossible to obtain the cleaned body weight, but the variation in body weight of animals with testes of comparable size is too great to be accounted for by the inclusion of the weight of the alimentary canal. The lack of any constant relation between testes weight and body weight may also be due to individual fluctuations in reproductive activity in the adult males. Animals kept under observation in the laboratory showed irregular changes in size and position of the testes although diet and other conditions were stable.

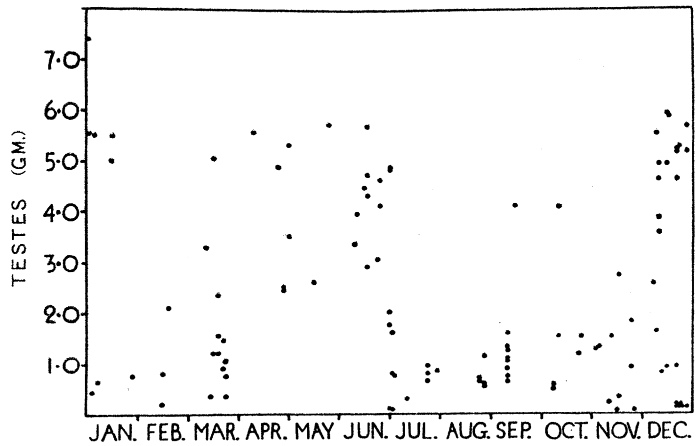


FIG. 2.—Variation in the weight of the testes.

VI. *Histology of the Testis.*

(*a*) *Seminiferous Tubules.*—The material includes all developmental stages from the prepubertal condition to full activity. In testes weighing 0·30 gm. and under, the seminiferous tubules measure 60–90 μ in diameter, and are in a quiescent condition. Their walls are lined by crowded Sertoli nuclei, spermatogonia are numerous and lie among the Sertoli nuclei or towards the centre of the tubules, while primary spermatocytes are present in relatively few tubules in a cross-section and often show signs of degeneration. Between 0·30 gm. and 1·0 gm. weight, the testes show the commencement of activity. The tubules increase to approximately 120 μ in diameter, and a lumen is apparent. Spermatocytes are present in large numbers in all tubules in a section. In testes weighing above 1·0 gm. spermatids are usually present, and above 2·0 gm. spermatozoa are being produced. Variation is found in the degree of activity above 1·0 gm., but without exception testes of more than 2·0 gm. were fully functional.

The diameter of the seminiferous tubules increases only to 200–220 μ in testes

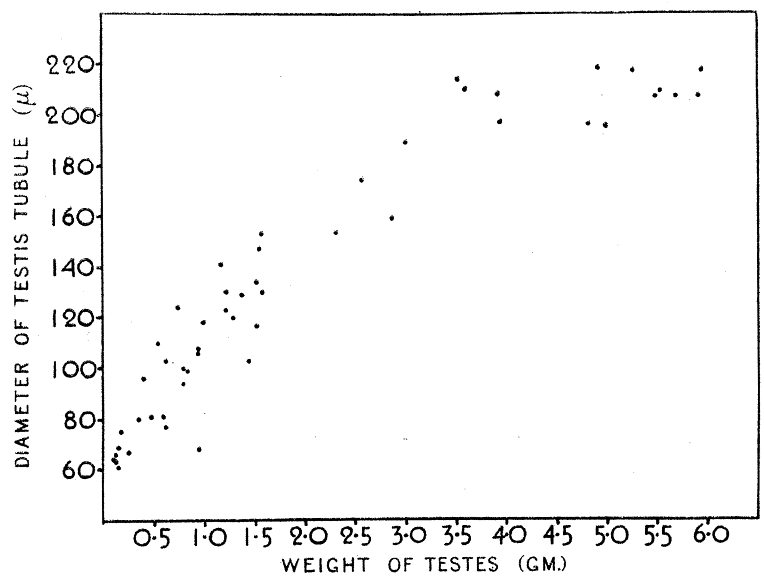


FIG. 3.—Diameter of seminiferous tubules plotted against weight of testes.

of 3·5 gm. and over. Unless, therefore, other constituents of the testes undergo relative increase there must be some lengthening of the tubules in the larger testes, fig. 3.

(*b*) *Interstitial Tissue.*—The interstitial cells increase from an area of 60–70 sq. μ in the smaller testes to 180–260 sq. μ in testes weighing more than 3·5 gm. In fig. 4 the mean area of the interstitial cells is plotted against the weight of the testes and it is

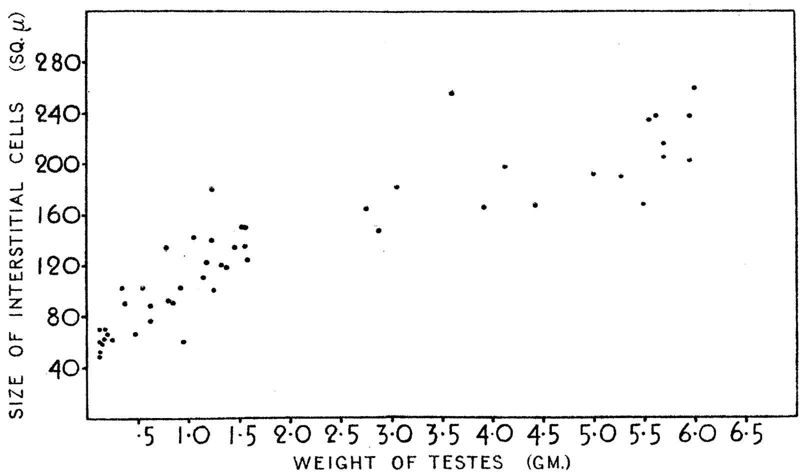


FIG. 4.—Area of interstitial cells plotted against weight of testes.

seen that growth of the interstitial cells is rapid until the testes reach approximately 2·0 gm., while in the larger testes the rate of growth is relatively slower. When fully grown the cells have a coarsely granular cytoplasm which stains strongly with eosin. In the smaller cells the nucleus is large in proportion to the amount of cytoplasm, but this condition is reversed as the cells become more active.

Fat.—Flemming-fixed material was obtained from animals with testes weighing from 0·244 gm. to 5·98 gm. Fine fat droplets are present in the interstitial cells of testes weighing more than 1·4 gm., and in the heavier organs this fat is in coarser droplets. In no case is there the large accumulation of fat characteristic of the interstitial cells of animals with seasonal activity, *e.g.* ferret (ALLANSON, 1932), woodchuck (RASMUSSEN, 1917), where towards the end of the reproductive period the cells become heavily charged with fat. The absence of any fat accumulation in the interstitial cells of the squirrel may be connected with the absence of any regular cyclic decline in secretory activity. Since the fat content of the cells shows little variation, cell size has been used as an index of activity.

Pigment.—WHITEHEAD (1908), in a study of the histology of interstitial cells in various forms, failed to find pigment in the grey squirrel. Pigment is present in 39 of the 76 testes examined in this investigation, either as fine granules or in coarse masses. Pigment is not present in the human testis until the adult state is reached, and many workers, from study of other forms, consider it to be a degeneration product of the interstitial cells (see RASMUSSEN, 1928). This view is supported by the present squirrel material: pigment is not found in the testes of the younger animals (*i.e.* below 400 gm. body weight) although they may be functionally active, *e.g.* No. 90. Apart from this, occurrence of pigment is irregular, and has no connection with the state of the seminiferous tubules since it may be present in quiescent testes, No. 71, and be absent from testes in full activity, No. 149.

VII. *Histology of the Epididymis.*

The weight of the epididymides ranges from 0·066 gm. to 2·12 gm., and the diameter of the tube increases from 40 μ to 200 μ. Fig. 5 shows that the maximum diameter of the tube is not reached until the organ has attained full size. On the evidence, therefore, there is not necessarily any increase in length of the epididymal tube, and comparison of the increase in the sectional area of the tube and of the weight of the epididymis suggests that the growth of the latter is largely accounted for by increase in the diameter of the tube.

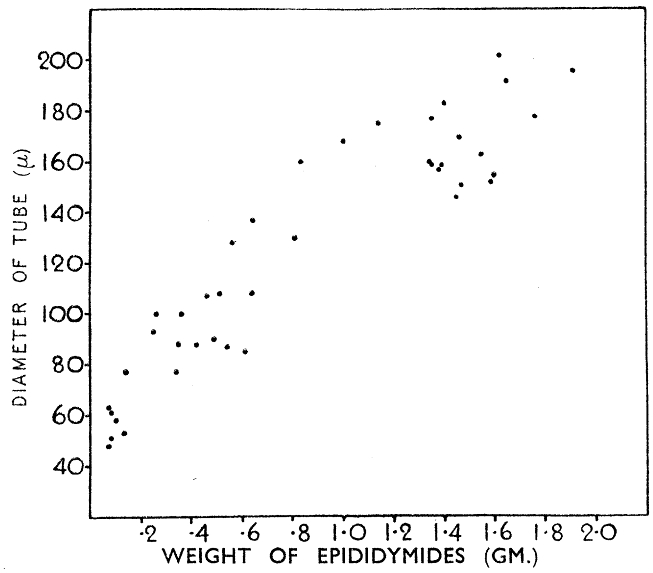


Fig. 5.—Diameter of epididymal tube plotted against weight of epididymides.

The epithelial cells lining the tube increase in height as the organ attains the active condition. In epididymides weighing not more than 0·1 gm. the epithelial cells measure 15–25 μ. The nuclei are densely crowded and the non-vibratile threads characteristic of the active epididymis are absent. When the organs have reached approximately 1·0 gm. and contain spermatozoa, the epithelium measures 30–40 μ, and the cells are stretched apart so that the nuclei now lie in a single row at the base of the cells. The cells show no further growth in the heavier epididymides.

The non-vibratile threads begin to appear in epididymides above 0·1 gm. At first they measure less than 1 μ, but they attain a length of 12–13 μ by the time the epididymides weigh 0·5 gm., although there are still no spermatozoa. Once spermatozoa are present in the tube these threads are difficult to measure since they may be pressed against the epithelium by the contained mass, but they appear to remain at between 12 and 15 μ.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

The muscular coat surrounding the tube is 4–5 fibres thick in the smaller epididymides, where the diameter of the tube is small, and measures 8–10 μ in width. As the tube increases in girth the muscles are stretched apart, so that when the diameter of the tube is at its maximum the fibres are reduced to 2–3 deep and measure 2–4 μ in width. Apparently, therefore, no new elements are added to the muscular coat during this phase of the growth of the organ.

VIII. *Growth of the Accessory Sexual Organs.*

(*a*) *Relation with growth of testes.*—The weight of the accessory sexual organs, like that of the testes, bears no regular relation to body weight. Their growth, with the exception of the penis, shows, however, a close correlation with the growth of the testes. In figs. 6–10 the weights of the accessory organs are plotted against the weight of the testes. Inspection of these graphs shows that the points fall about a close

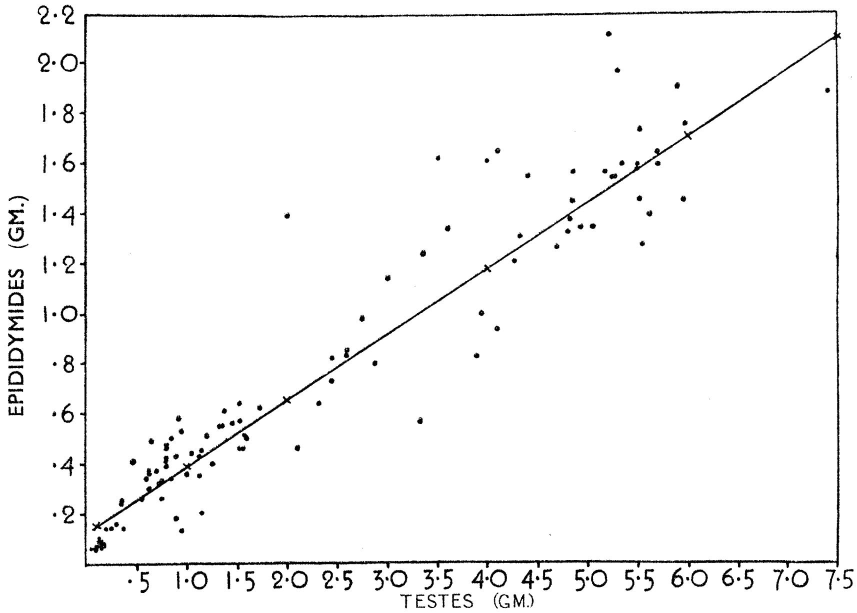


FIG. 6.—Weight of testes and weight of epididymides.

approximation to a straight line, except for the penis. Regression formulæ have therefore been calculated for epididymides, prostate, seminal vesicles and Cowper's glands, in terms of testes weight.

Equations for the Weight of the Accessory Organs (y) *in relation to Weight of Testes* (x).

Epididymides	$y = 0{\cdot}1262 + 0{\cdot}2641\ x$
Prostate	$y = 0{\cdot}005 + 0{\cdot}1857\ x$
Seminal vesicles	$y = 0{\cdot}0115 + 0{\cdot}0138\ x$
Cowper's glands	$y = 0{\cdot}1339 + 0{\cdot}3336\ x$

As explained on p. 80, one is justified in supposing that these relationships largely represent those existing during initial growth. Nevertheless, assuming that the accessory organs have a lag in responding to the testes, the possibility cannot be over-

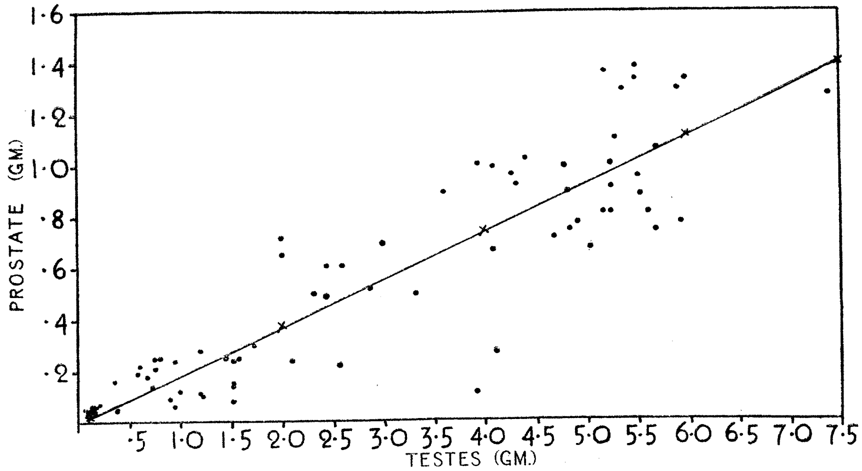


FIG. 7.—Weight of testes and weight of prostate.

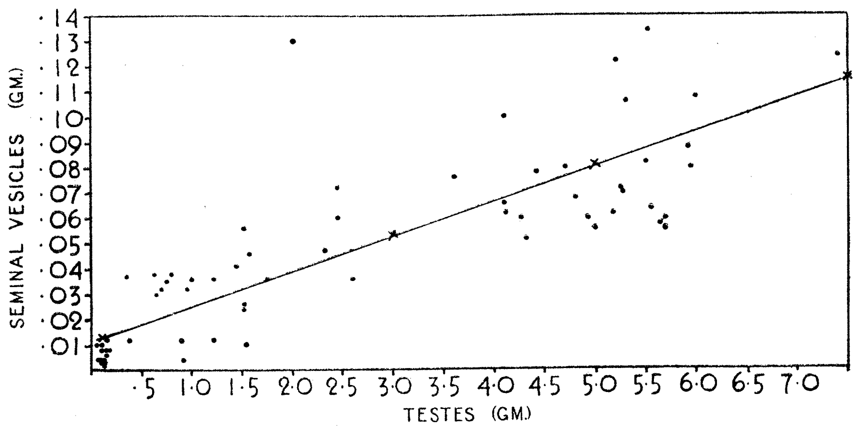


FIG. 8.—Weight of testes and weight of seminal vesicles.

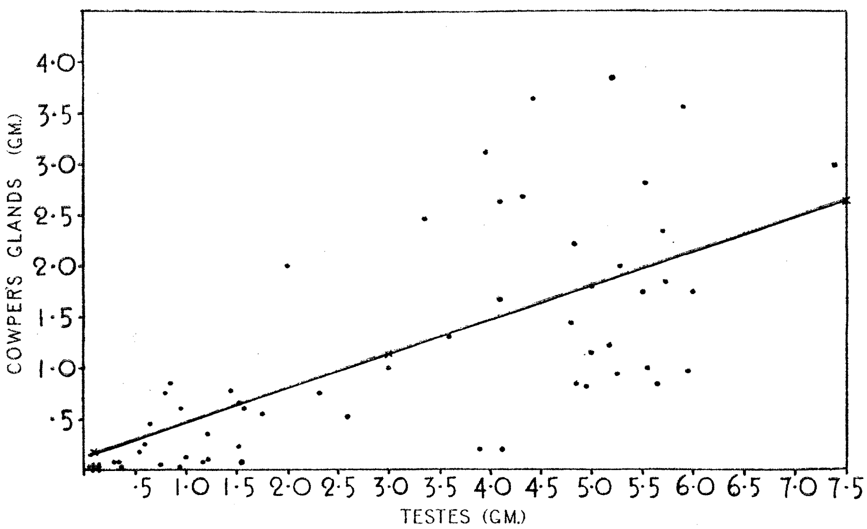


FIG. 9.—Weight of testes and weight of Cowper's glands.

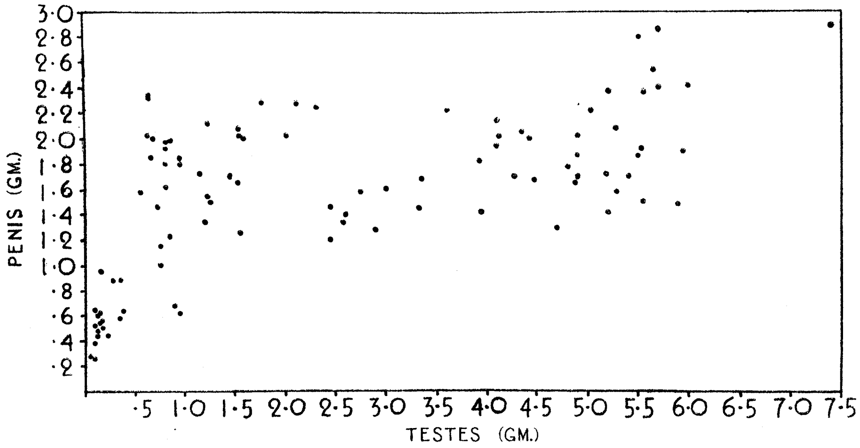


FIG. 10.—Weight of testes and weight of penis.

looked that the "fanning out" of the points, particularly for Cowper's glands, is caused by the inclusion of some animals in which the organs are undergoing regression.

The growth of the penis would appear to follow a rather different course, fig. 10. The weights rise rapidly until the testes weigh about 1·0 gm. and then remain fairly constant. It seems evident, however, that the penis would respond to the testes more slowly than the other accessory organs, and the number of animals with small testes and large penes may be increased by the inclusion of animals with regressing testes.

(*b*) *Relation with interstitial tissue.*—As there is evidence that the development of the accessory organs is connected with the secretory activity of the interstitial tissue the growth of these organs in the squirrel has been studied in relation to the size of the interstitial cells. In view of the difficulty of estimating the volume of the interstitial cells, the weights of the accessory organs have been considered against sectional area. The points tend to fall on an ascending curve, especially in the case of the prostate gland, fig. 11. Plotted against the more comparable index of volume, however, the relationships would become approximately linear.

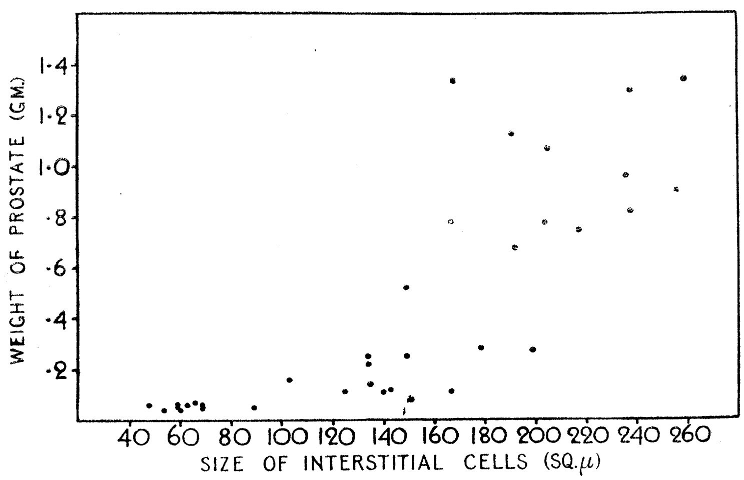


FIG. 11.—Weight of prostate plotted against area of interstitial cells.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

IX. *Discussion.*

In mammals having a limited sexual season the period of male reproductive activity as a general rule roughly corresponds to that of the female. But this is not invariably the case. The female camel is said to experience a continuous series of reproductive cycles and be capable of conception throughout the year, while the male experiences only a short season of rut (see ZUCKERMAN, 1932). Again, in the sea lion, while the reproductive organs of the male have a period of quiescence, those of the female are continuously active since pregnancy lasts eleven months. But although the female does not have an anœstrus it still experiences a cyclical mating season ; ripe ova and spermatozoa are produced only at fixed times of the year. HEAPE (1900) gives examples of mammalian species in which the condition is reversed, the males remaining potent throughout the year even though the females experience breeding seasons and periods of complete anœstrus. In the dog, males are able to propagate throughout the year while the female is limited to two sexual seasons. But though spring and autumn are the usual times when the sexual seasons occur, the active periods of each bitch have a more or less exact periodicity peculiar to herself and the extent of the anœstrus may vary from four to eleven months in different individuals. The condition in the squirrel is similar except that there is only one sexual season in the female and this occurs at approximately the same time of the year for all members of the species.

HEAPE suggested that when rutting exists it is probably excited by similar influences to those which induce the advent of œstrus in the female ; while when the sexual season of a male is permanent either all the females of that species have a continuous sexual season or individual females have different times for their sexual seasons. The condition in the squirrel shows that males of a species may be in more or less continuous reproductive activity while all the females are in anœstrus for six months of the year.

I wish to express my gratitude to Dr. A. S. PARKES for his advice and help during the progress of this investigation, and for reading the paper.

My thanks are also due to Mr. A. D. MIDDLETON who has generously put his records of the grey squirrel at my disposal ; and to Dr. R. A. FISHER, F.R.S., for his advice as to the statistical interpretation of the data.

The expenses of the work were defrayed by a grant from the Medical Research Council, for which I am grateful.

The photograph, Plate 15, is by Mr. D. KEMPSON and figure 1 is by Miss B. C. PHILLIPSON.

X. *Summary.*

1. One hundred and twelve male squirrels with body weights ranging from 240 gm. to 705 gm. have been examined and the condition of the testes and accessory reproductive organs studied, to find whether there is a seasonal periodicity in reproductive activity similar to that in the female squirrel.

2. Animals with fully functional testes were found throughout the year and there is thus no regular period of quiescence in the male of this species. It is possible, however, that individual males do not remain continuously in reproductive activity.

3. The weight of the two testes varies from 0·062 gm. to 7·4 gm. Spermatozoa are always present in testes weighing more than 2 gm. the pair. The diameter of the seminiferous tubules increases with the growth of the testes until the latter have reached approximately 3·0 gm. (pair): after this the tubules remain at approximately 220 μ in diameter.

4. The epididymides weigh from 0·066 gm. to 2·12 gm. (pair), and the diameter of the tube increases from 40 μ to 200 μ. The maximum diameter is not reached until the organ has attained full size. Changes in the epithelial cells and muscular coat of the epididymal tube are described.

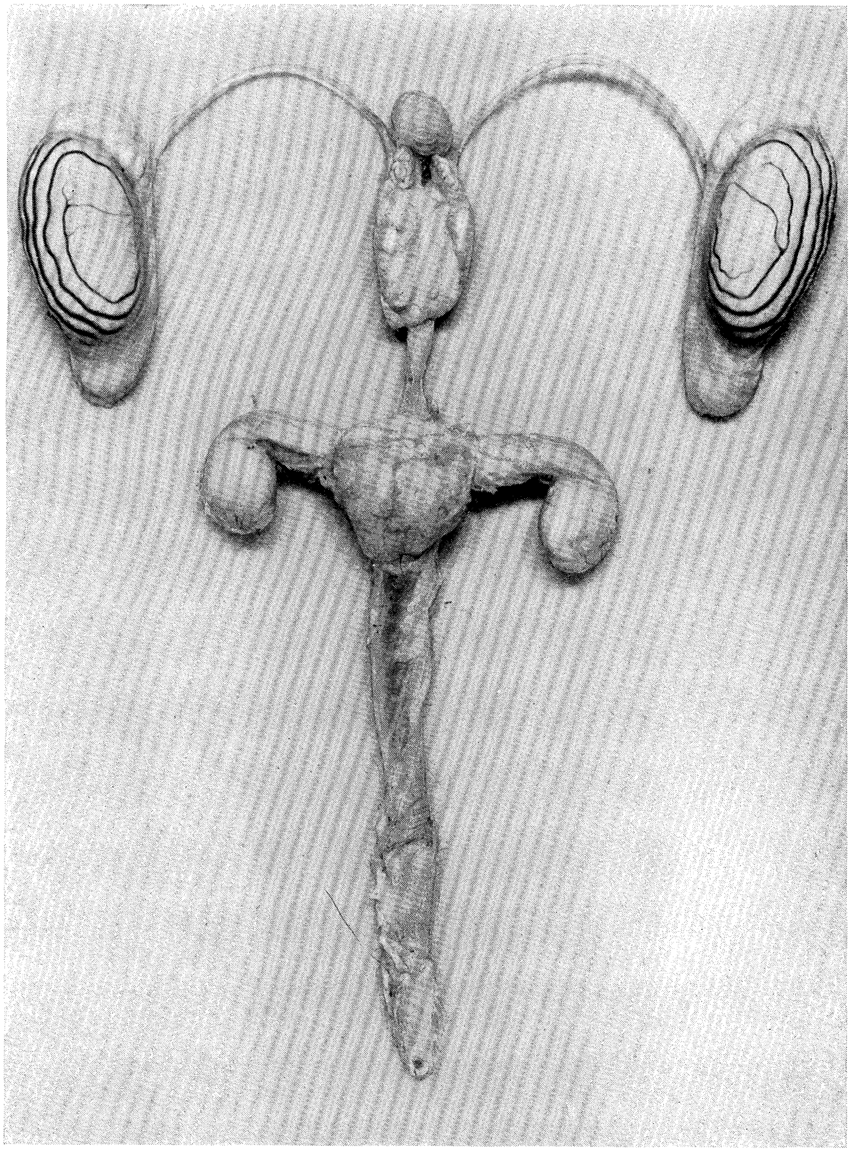
5. The weight of the testes and accessory sexual organs bears no regular relation to body weight. It is suggested that this is due to differences in the rate of sexual development in the young male according to whether birth occurred in early spring or in summer, as well as to fluctuation in reproductive activity in the adults.

6. Growth of the accessory sexual organs is correlated with growth of the testes, but the penis does not show as close a correlation as do the other accessory organs.

7. The area of the interstitial cells increases from approximately 60 sq. μ in the smaller testes to a maximum of 240 sq. μ. Growth of these cells is rapid until the testes reach 2·0 gm. (pair), but is relatively slower in the larger testes. There is correlation between growth of the interstitial cells and of the accessory sexual organs.

REFERENCES.

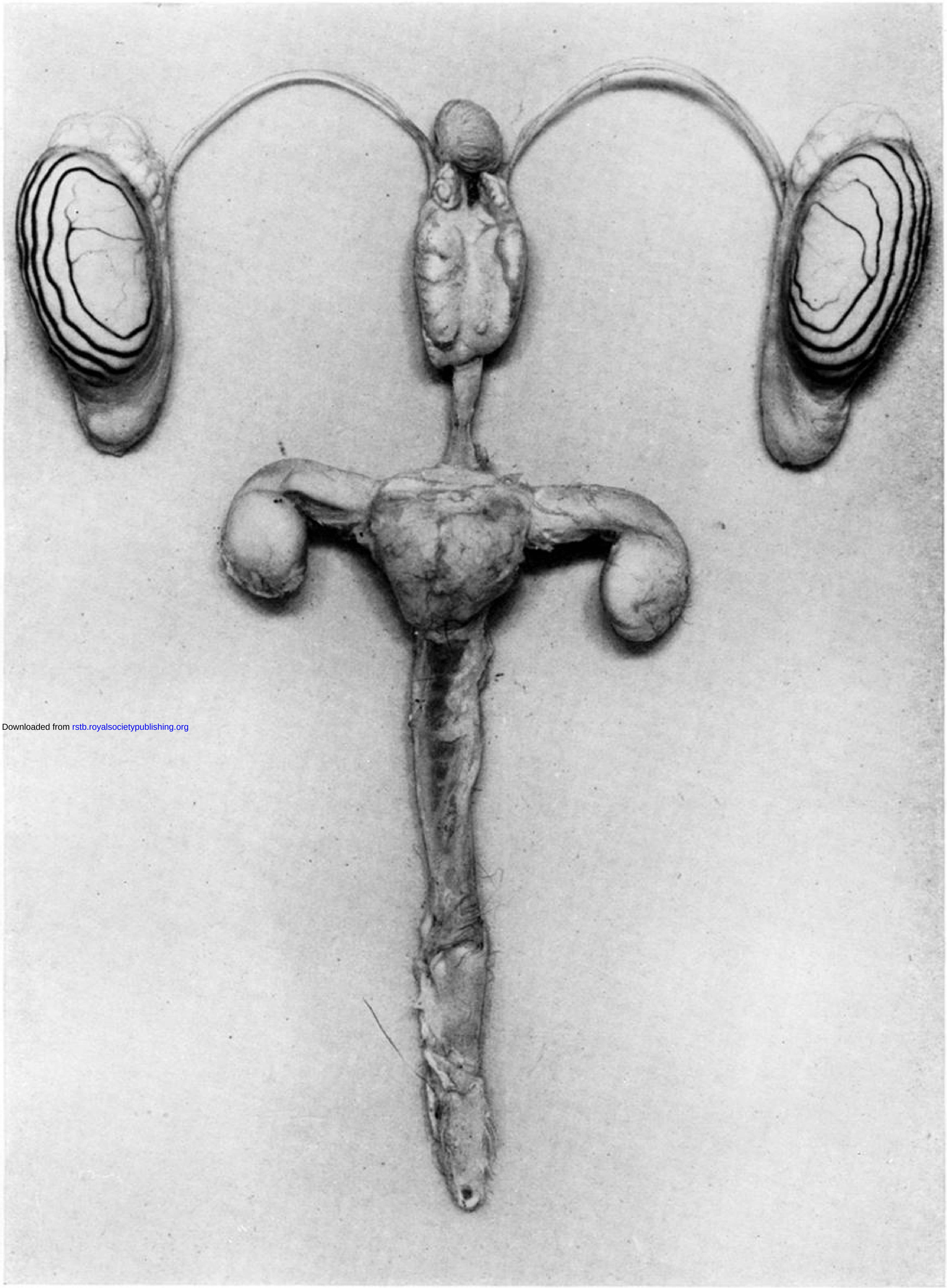
ALLANSON (1932). 'Proc. Roy. Soc.,' B, vol. **110**, p. 295.
DONALDSON (1924). "Mem. Wistar Inst.," No. 6.
GROSZ (1905). 'Arch. Mikr. Anat.,' vol. **66**, p. 567.
HEAPE (1900). 'Quart. J. Micr. Sci.,' vol. **44**, p. 1.
OWEN (1868). "The Anatomy of Vertebrates," Longmans, Green and Co., London.
RASMUSSEN (1917). 'Amer. J. Anat.,' vol. **22**, p. 475. (1928). "Special Cytology," Paul B. Hoeber, Inc., New York.
MIDDLETON (1930). 'Proc. Zool. Soc.,' p. 809.
WHITEHEAD (1908). 'Anat. Rec.,' vol. **1**, p. 213.
ZUCKERMAN (1932). "The Social Life of Monkeys and Apes," Kegan Paul, Trench, Trubner and Co., Ltd., London.



Description of Plate.

Photograph of reproductive tract of adult male grey squirrel after removal of spermatic cord and surplus fat and connective tissue. The organs are in their normal relationships except for the penis which has been stretched out. The paired seminal vesicles are seen immediately below the bladder, lying on the large prostate. Cowper's glands are situated at each side of the base of the penis.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES



PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Description of Plate.

Photograph of reproductive tract of adult male grey squirrel after removal of spermatic cord and surplus fat and connective tissue. The organs are in their normal relationships except for the penis which has been stretched out. The paired seminal vesicles are seen immediately below the bladder, lying on the large prostate. Cowper's glands are situated at each side of the base of the penis.