THE INFLUENCE OF THE TESTIS ON THE SURVIVAL OF SPERMATOZOA IN THE EPIDIDYMIS OF THE GOLDEN HAMSTER, *MESOCRICETUS AURATUS*

C. M. LUBICZ-NAWROCKI* AND T. D. GLOVER

*Unit of Reproductive Biology, University of Liverpool,
Life Sciences Building, P.O. Box 147, Liverpool L69 3BX*

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Summary. If both testes are removed and both epididymides are allowed to remain *in situ* in the golden hamster, the period of sperm survival in the tail of the epididymis is reduced by about half, since the spermatozoa lose their capacity to fertilize in 10 to 12 days. This contrasts with the 20- to 24-day survival of epididymal spermatozoa when one or both testes are present. It appears that only one testis is needed to ensure the normal survival of spermatozoa in both epididymides. Replacement of testosterone by a subcutaneous implant in bilaterally gonadectomized males resulted in normal sperm survival.

Assessment of the ratio of live to dead spermatozoa showed that the ultimate loss of fertilizing capacity by the spermatozoa in both intact and gonadectomized males was not at first due to an increased incidence of dead spermatozoa in the epididymis, though progressively more dead and degenerated spermatozoa were gradually to be seen in the samples.

The fertilizing capacity of the spermatozoa was lost more rapidly (within 8 days) in gonadectomized animals if they were also hypophysectomized. Administration of HCG to these animals failed to maintain normal viability in the spermatozoa.

A gradual decrease in the occurrence of 'light' cells in the lining tissue of the duct is reported, following gonadectomy.

It is concluded that circulating androgen is unquestionably required for the normal survival of spermatozoa in the tail of the hamster epididymis and that it probably achieves its effect through its influence on the epididymal epithelium. The findings are discussed in relation to the general hormonal control of sperm survival in the epididymis.

INTRODUCTION

It is well known that the epididymis serves to convey maturing spermatozoa from the testis to the ductus deferens, and to store the matured forms in the cauda epididymidis. The spermatozoa cannot be stored here indefinitely, however, and if they are not ejaculated, they gradually lose their capacity to fertilize

* Present address: Worcester Foundation for Experimental Biology, 222 Maple Avenue, Shrewsbury, Massachusetts 01545, U.S.A.

or to produce viable embryos (Hammond & Asdell, 1926; Tesh & Glover, 1969). Temporally, this loss of fertilizing capacity varies between species, although viability is retained for quite some time: in the guinea-pig, for 20 to 35 days (Young, 1929); in the rabbit, for 40 to 50 days (Hammond & Asdell, 1926; Tesh & Glover, 1969); in the mouse, for 10 to 14 days (Snell, 1933); in the rat, for 21 days (White, 1933). By contrast, spermatozoa only survive for a short period in the female tract, and in the rabbit, for example, their fertilizing capacity is lost after about 3 hr (Tesh, 1969).

The special physiological conditions that allow prolonged survival of spermatozoa in the male tract are not precisely known although some factors, such as temperature, are recognized as being involved (Glover, 1960; Cummins & Glover, 1971). The endocrine control of sperm survival is certainly not clear although it is known that the epididymis requires male sex hormone for its growth, structure and functional maintenance (Benoit, 1926; Risley, 1963; Price & Ortiz, 1965). The motility of epididymal spermatozoa *in vitro* is known to be impaired when the testes are removed (Moore, 1928) and it would seem likely that the preservation of fertilizing capacity of the spermatozoa is also androgen-dependent.

Apart from circulating testicular androgen, epididymal spermatozoa have access to two other potential sources of male sex hormone. First, androgens are present in the testicular fluid entering the epididymis and, secondly, the epididymis might itself be capable of androgenesis (Inano, Machino & Tamaoki, 1969; Hamilton, Jones & Fawcett, 1969; Hamilton, 1971), even though species differences in the degree of such activity appear to exist (Frankel & Eik-Nes, 1968, 1970).

The relative importance of these possible sources of androgen in preserving the spermatozoa is unknown and, in the present work, an attempt is made to establish the part played by circulating steroid. The paper first reports on the effects of bilateral gonadectomy and testosterone replacement on the survival of mature spermatozoa in the epididymis of the golden hamster. An initial enquiry has also been made into the rôle of the pituitary in this context.

MATERIALS AND METHODS

Adult, 10-week-old, male and female golden hamsters were kept at a constant temperature of 26° C in a thermostatically maintained room, where a daily period of light from 06.00 to 21.00 hours was alternated with a period of darkness from 21.00 to 06.00 hours by means of an automatic switch. The animals were allowed unrestricted access to food (Diet 41B, Dixon (Ware) & Sons Ltd) and water.

Experimental groups of animals

The experiment was designed so that male animals were divided into seven groups (see Table 1). The first of these consisted of six control animals, where both testes and epididymides were left intact. The first experimental group (Group A) contained twenty-five animals in which both testes were present but the epididymides were ligated. Group-B animals (twenty-eight) comprised

unilaterally gonadectomized males (the left testis was removed but both epididymides were ligated), whilst Groups C and D comprised bilaterally gonadectomized males (thirty-one and eighteen animals, respectively) with both epididymides ligated. Group-D animals received a replacement of testosterone (25 mg subcutaneous implant, Organon Laboratories Ltd, London). Thus, the term 'gonadectomy' is to be taken literally in the context of these experiments since the epididymides were always allowed to remain even when the testes were removed.

To test whether the viability of spermatozoa following bilateral gonadectomy was maintained by hypophysial hormones, an additional six gonadectomized and hypophysectomized males were allocated to two groups (E and F). The animals in one of these groups (Group F) received eight daily injections of 100 i.u. HCG and, in each case, animals of Groups E and F were killed after 8 days and the fertilizing capacity of the retained epididymal spermatozoa was determined.

Table 1. Number of male hamsters used in each experimental group at various times

Animal Group	Days														
	0	4	5	6	7	8	9	10	11	12	16	18	20	22	24
Controls	6	—	—	—	—	—	—	—	—	—	—	—	—	—	—
A	2	2	—	—	—	2	—	—	—	2	5	3	5	2	2
B	—	4	—	—	—	4	—	—	—	4	4	4	4	—	4
C	2	2	2	2	2	5	5	7	2	2	—	—	—	—	—
D	—	—	—	—	—	—	—	—	—	2	5	5	4	—	2
E	—	—	—	—	—	3	—	—	—	—	—	—	—	—	—
F	—	—	—	—	—	3	—	—	—	—	—	—	—	—	—

Characteristics of Groups (see text): (A) testes present, epididymides ligated; (B) unilateral gonadectomy, epididymides ligated; (C) bilateral gonadectomy, epididymides ligated; (D) bilateral gonadectomy, epididymides ligated, replacement therapy (testosterone); (E) bilateral gonadectomy, hypophysectomy, epididymides ligated; (F) bilateral gonadectomy, hypophysectomy, epididymides ligated and replacement therapy (HCG).

Sperm viability

To test the viability of mature spermatozoa, samples were generally taken from both epididymides of each male and placed into both uterine horns of three or four females. Since the variation between results in different females proved to be negligible, the contents of each cauda epididymidis were placed into a single female only when unilaterally gonadectomized males were used (Group B). In general, when the results were unambiguous, this procedure was carried out using only two males, but the experiments were repeated using several males when more variation was observed in initial results (see Table 1). This explains the variation in the numbers of males used in the different groups.

To determine oestrus in the females, a male and female hamster were placed together in a container between 19.00 and 21.00 hours. If the female displayed lordosis, this was taken as positive evidence that ovulation would occur between

01.00 and 03.00 hours (Ward, 1946; Strauss, 1956; Harvey, Yanagimachi & Chang, 1961).

Spermatozoa were always obtained by macerating the cauda epididymidis in 4 ml Hanks' solution and passing the material through sterile gauze. The motility of the spermatozoa was arbitrarily assessed from examination of the filtrate, and the percentage of live spermatozoa was estimated. For this, the sperm suspension (filtrate) was mixed with an equal volume of aqueous nigrosin-eosin and a smear was made on three separate slides. A total of 250 spermatozoa were counted on the three slides, 100 on each of two and 50 on the third slide. This number was chosen for convenience of calculation and permitted an estimate of variation in the sub-samples to be obtained.

The sperm suspension (0.1 ml) was then diluted to 20 ml with 10% formol saline and the sperm concentration was assessed using a Fuchs-Rosenthal haemocytometer slide. Between 8×10^6 and 16×10^6 spermatozoa were deposited into both uterine horns of female hamsters between 20.00 and 22.00 hours as in the procedure previously described by Yanagimachi & Chang (1961). Each female was anaesthetized with 0.1 ml sodium pentobarbitone (Nembutal, 60 mg/ml, Abbott Laboratories Ltd)/100 g body weight, given intraperitoneally.

The fertilizing capacity of the spermatozoa was then estimated by the examination of tubal eggs flushed from the uterine horns with 0.2 ml Hanks' solution between 30 and 36 hr after ovulation. This was carried out with an ordinary record syringe using a fine needle (gauge-32) inserted into the infundibulum of the uterine tube. The tubes were flushed twice and, after transferring the eggs to a slide, whole mounts were made according to the method described by Chang (1952). The criterion of fertilization was the presence of a decapitate spermatozoon within the vitellus of a cleaved egg.

Recovery of eggs by this means was almost always 100% effective and, on this assumption, the fertilizing capacity was assessed as:

$$\frac{\text{Number of eggs fertilized}}{\text{Number of eggs recovered}} \times 100\%$$

Histological examination

Tissue from each cauda epididymidis was fixed for 24 hr in Bouin's fluid, embedded in paraffin wax, sectioned at $7 \mu\text{m}$, stained with Harris' haematoxylin and eosin and mounted in DPX. As part of this examination, the incidence of 'light' cells was determined on material from three males killed at different times after ligation of the duct. The number of these cells was counted for each animal in ten areas of tubule as revealed in a histological section.

Fructose estimations

The effects of gonadectomy and testosterone replacement were also tested by estimating the content of fructose in the seminal vesicle. For this, the seminal vesicles were drained and blotted on filter paper and stored at -20°C until required. The method used to determine fructose was basically the same as that described by Lindner & Mann (1960). Each gland was weighed, homogenized

and then centrifuged at 2900 rev/min for 3 min. Equal volumes (0.25 ml) of 5% zinc sulphate and 0.3 N-barium hydroxide were added to the supernatant and the mixture was recentrifuged. A 1-ml vol. of resorcinol (0.1% in ethanol) and 3 ml 30% HCl were next added to 1 ml of the resulting supernatant. The total mixture was then incubated for 10 min at 90° C, allowed to cool and the percentage of light transmission was determined at a wavelength of 520 nm using a CE 303 grating spectrophotometer (Cecil Instruments, Cambridge). Fructose concentration was expressed as mg/g dry weight of tissue by determining the percentage dry weight of seminal vesicles from animals in each group.

All data obtained in the experiment were analysed by using the simple Student *t* test.

TEXT-FIG. 1. Sites of ligation of the hamster excurrent duct before gonadectomy and suture of the inverted ligamentum testis to the tunica vaginalis and scrotal skin. A, spermatic blood vessels; B, caput epididymidis; C, ductuli efferentes; D, corpus epididymidis; E, ductus deferens; F, cauda epididymidis; G, tunica vaginalis, H, scrotal skin; I, ligamentum testis (inverted); l, ligation sites.

Male gonadectomy, hypophysectomy and ligation of the epididymis

For gonadectomy, the testes of each animal were exposed through a small incision in the scrotum and tunica vaginalis, and were removed after ligation of the spermatic blood vessels and the ductuli efferentes. To do this, a single ligature was appropriately placed close to the surface of the testis so that it came to enclose both the blood vessels and the efferent ductules (see Text-fig. 1), and

left the epididymal vasculature intact. Next, the cauda epididymidis was isolated from the rest of the duct by means of a ligature round the distal part of the corpus epididymidis and round the ductus deferens near to the cauda epididymidis (Text-fig. 1). Whenever possible, ligatures were placed beneath blood vessels so that the circulation to the isolated area was minimally impaired.

Finally, as a precaution, in case the cauda epididymidis might be withdrawn into the abdomen after removal of the gonad, the ligamentum testis was sutured to the tunica vaginalis and the scrotal skin as illustrated in Text-fig. 1. The incisions in the tunica vaginalis and scrotal skin were closed separately with nylon sutures. For all these procedures, the males were anaesthetized with sodium pentobarbitone in the same way as the females, and operations were performed in clean conditions. Sterilized braided silk sutures (size 5/0, Ethicon Ltd) were used for ligations and nylon sutures for the closure of wounds. (Two such sutures were also used to close the skin when testosterone implants were inserted.)

Table 2. The fertilizing capacity of epididymal spermatozoa from intact hamsters

<i>Untreated males</i>	<i>No. of females/male</i>	<i>Mean no. of eggs recovered</i>	<i>Mean no. of eggs fertilized</i>	<i>Mean % fertilization rate</i>
6	4	49.5	48.8	97.7

Hypophysectomy was performed on males through a transauricular approach by means of a Hoffman-Reiter hypophysectomy instrument (Model H-200, H. Neuman & Co., Skokie, Illinois, U.S.A.).

In each case, a macroscopic examination of the sella turcica was performed with the aid of a dissecting microscope to determine whether removal of the hypophysis had been successful. As an additional check on the completeness of the hypophysectomy, both adrenal glands were weighed to determine whether hypertrophy had occurred.

RESULTS

Table 2 shows that, when control males were used, 293 out of a total of 300 eggs (97.8%) were fertilized, and Table 3 shows that spermatozoa retained this high fertilizing capacity for 12 days when isolated in a ligated epididymis (Group A) (Text-fig. 2 and Table 3). This applied even when one testis was removed (Group B) and also when testosterone was administered after bilateral gonadectomy (Group D). In Group-B animals, the viability of the epididymal spermatozoa was maintained equally on either side, so the mean figures for this group in Table 3 are based on pooled data.

Thus, in all three groups, the mean fertilization rate decreased to approximately 55% on the 12th day after operation and, although a steady decline occurred thereafter, differences between Days 16 and 18 and between Days 18

and 20 were not significant. The difference between Day 16 and Day 20 was significant though ($P < 0.01$), and a drastic effect on fertilizing capacity occurred on and after the 22nd day.

In sharp contrast to these results, the mean fertilizing capacity of epididymal spermatozoa following bilateral gonadectomy began to decline after about 8 days ($87.3 \pm 4.2\%$) (Text-fig. 2 and Table 4), and was severely affected after 10 days ($32.5 \pm 19.9\%$), although animals varied greatly in their response about this time (Table 4). There was less variation, however, on Days 11 and 12

Table 3. The fertilizing capacity of retained epididymal spermatozoa in the presence of testicular androgens (Groups A and B) or exogenous androgens following bilateral gonadectomy (Group D) in hamsters

Days after treatment	Animal groups	No. of females/male	Mean no. of live sperm./uterine horn $\times 10^6$ ($\pm$ S.E.)	Mean no. of eggs recovered (with ranges)	Mean no. of eggs fertilized	Mean % fertilization rate ($\pm$ S.E.)
0	A	4	6.9 (± 0.0)	44.0 (44, 44)	43.0	97.7 (± 0.0)
	B	—	—	—	—	—
	D	—	—	—	—	—
4	A	4	4.1 (± 0.0)	46.0 (39, 53)	44.5	96.4 (± 1.1)
	B	1	5.6 (± 0.5)	25.5 (10 to 16)	25.0	97.9 (± 1.9)
	D	—	—	—	—	—
8	A	4	8.4 (± 0.5)	38.5 (37, 40)	38.5	100.0 (± 0.0)
	B	1	5.7 (± 0.3)	23.2 (9 to 14)	22.5	97.1 (± 2.7)
	D	—	—	—	—	—
12	A	4	6.3 (± 1.0)	52.0 (48, 56)	51.5	99.0 (± 0.7)
	B	1	6.9 (± 0.3)	22.5 (9 to 15)	22.0	97.6 (± 2.2)
	D	4	7.6 (± 0.0)	36.5 (35, 38)	34.5	94.2 (± 4.1)
16	A	4	3.5 (± 0.1)	42.8 (38 to 51)	22.6	53.9 (± 14.4)
	B	1	3.7 (± 0.3)	26.8 (8 to 15)	17.5	62.9 (± 12.7)
	D	3 or 4	3.6 (± 1.1)	37.8 (36 to 39)	21.6	58.7 (± 13.6)
18	A	3 or 4	1.1 (± 0.1)	33.3 (32 to 35)	13.3	39.2 (± 0.8)
	B	1	6.0 (± 0.8)	20.5 (8 to 13)	6.2	30.2 (± 7.1)
	D	3 or 4	1.1 (± 0.4)	30.6 (23 to 38)	11.8	38.7 (± 11.2)
20	A	3 or 4	2.5 (± 1.3)	39.0 (31, 47)	10.2	23.0 (± 12.1)
	B	1	2.2 (± 0.4)	24.5 (9 to 15)	2.7	11.1 (± 5.4)
	D	4	2.4 (± 0.5)	41.2 (33 to 51)	3.0	6.0 (± 4.0)
22	A	4	1.2 (± 0.2)	40.5 (40, 41)	2.0	4.8 (± 3.5)
	B	—	—	—	—	—
	D	—	—	—	—	—
24	A	4	1.0 (± 0.1)	42.0 (40, 44)	1.0	2.4 (± 1.7)
	B	1	0.5 (± 0.2)	19.7 (6 to 13)	0.0	0.0 (± 0.0)
	D	4	1.0 (± 0.1)	36.5 (36, 37)	0.0	0.0 (± 0.0)

when the adverse effects of removing the testes were unambiguous. When bilaterally gonadectomized animals were hypophysectomized, however, the fertilizing capacity of the spermatozoa was completely lost after 8 days (Group E) and HCG treatment after this operative procedure did not prevent the effect (Group F) (Table 5).

Sperm morphology

The cauda epididymidis of unmated animals always contained a high pro-

TEXT-FIG. 2. The fertilizing capacity of spermatozoa isolated in the hamster cauda epididymidis in the presence of circulating androgens (Groups A, B and D) or after the withdrawal of circulating testicular androgens following bilateral gonadectomy (Group C). ●, Group A; ○, Group B; ■, Group C; ▲, Group D.

Table 4. The fertilizing capacity of retained epididymal spermatozoa in the absence of circulating testicular androgens following bilateral gonadectomy (Group C) in hamsters

Days after bilateral gonadectomy (Group C)	Mean no. of live sperm./uterine horn $\times 10^6$ ($\pm$ S.E.)	No. of females/male	Total mean no. of eggs recovered (with ranges)	Total mean no. of eggs fertilized (with ranges)	Mean % fertilization rate ($\pm$ S.E.)
0	6.2 ($\pm$ 0.2)	4	51 (50, 52)	50 (48, 52)	98 ($\pm$ 1.4)
4	4.4 ($\pm$ 0.5)	4	46.5 (53, 40)	44.5 (49, 40)	96.2 ($\pm$ 2.7)
5	4.2 ($\pm$ 0.07)	4	46.5 (46, 47)	45.5 (45, 46)	97.8 ($\pm$ 0)
6	5.2 ($\pm$ 1.7)	4	53.5 (54, 53)	53 (54, 52)	99 ($\pm$ 0.7)
7	1.7 ($\pm$ 0.8)	4	37.5 (36, 39)	33.5 (35, 32)	89.6 ($\pm$ 5.4)
8	1.7 ($\pm$ 0.8)	4	47 (45 to 51)	41 (34 to 49)	87.3 ($\pm$ 4.2)
9	1.7 ($\pm$ 0.8)	4	46.6 (46 to 50)	25 (0 to 48)	54.7 ($\pm$ 17.4)
10	0.5 ($\pm$ 0.1)	3 or 4	42.3 (32 to 55)	16.5 (1 to 54)	36.8 ($\pm$ 14.9)
11	0.2 ($\pm$ 0.05)	4	28.5 (25, 32)	0 (0)	0 ($\pm$ 0)
12	0.1 ($\pm$ 0)	4	44 (44, 44)	1.0 (2, 0)	3.4 ($\pm$ 0.7)

portion of effete spermatozoa and they were classified into four groups as follows: (a) intact spermatozoa with a swollen acrosome (Pl. 1, Fig. 2); (b) spermatozoa with a partially detached acrosome (Pl. 1, Fig. 3); (c) spermatozoa bereft of an acrosome (Pl. 1, Fig. 4); and (d) decapitate spermatozoa.

These four categories represent successive stages in the degeneration of non-ejaculated spermatozoa.

Table 5. The fertilizing capacity of retained epididymal spermatozoa following gonadectomy and hypophysectomy (Group D) and HCG treatment (Group F) in hamsters

Days after treatment	Animal group	No. of females/male	Total mean no. of eggs recovered	Total mean no. of eggs fertilized	Mean % fertilization rate ($\pm$ S.E.)
8	D	3	31	0	0 ($\pm$ 0)
8	E	3	33	0	0 ($\pm$ 0)

TEXT-FIG. 3. Mean number of live spermatozoa injected/uterine horn in hamsters at various time intervals following treatment: (i) circulating androgen present (Group A: ●); (ii) after the withdrawal of circulating testicular androgens following bilateral gonadectomy (Group C: ■). The hatched bar represents the minimum number of spermatozoa required for maximum fertilization.

Number of live spermatozoa deposited in utero

It appears that when nigrosin-eosin is used to stain hamster spermatozoa, there is almost always some degree of staining irrespective of whether or not the spermatozoa are motile, and only a very few forms are seen to be completely

unstained. However, two distinct types of spermatozoa can be identified as those which are eosinophilic throughout their entire length, and those where only the posterior part of the sperm head is stained (Pl. 1, Fig. 1). We have classified the second of these as 'live' or 'non-eosinophilic' spermatozoa, and the others as 'dead' spermatozoa.

In all animals from Groups A, B or D, the mean number of live spermatozoa had significantly decreased when the cauda epididymidis was isolated for 20 days, but the mean number of live spermatozoa (24×10^6) still exceeded the

TEXT-FIG. 4. The incidence of 'light' cells in the isolated hamster cauda epididymidis in the presence of circulating androgens (Groups A, B and D) or after the withdrawal of circulating testicular androgens following bilateral gonadectomy (Group C). ●, Group A; ○, Group B; ■, Group C; ▲, Group D.

minimum required to support a high fertilization rate (see Text-fig. 3). Although bilateral gonadectomy induced a significant decrease in the mean number of live spermatozoa in the ligated cauda epididymidis between 6 and 10 days (Text-fig. 3), the fertilizing capacity of epididymal spermatozoa following bilateral gonadectomy only began to decline after 8 days ($87.3 \pm 4.2\%$; Text-

EXPLANATION OF PLATE 1

Aqueous nigrosin-eosin smears of golden hamster spermatozoa from the cauda epididymidis.

FIG. 1. Live unstained and partially stained spermatozoa. $\times 640$.

FIG. 2. Intact dead spermatozoon with a swollen acrosome (centre). $\times 480$.

FIG. 3. Dead spermatozoon with a ruptured acrosome. $\times 800$.

FIG. 4. Dead spermatozoon bereft of an acrosome. $\times 480$.

FIG. 1. Tubular epithelium from the cauda epididymidis of an intact golden hamster. Note high columnar principal cells interspersed with 'light' cells. $\times 220$.

FIG. 2. Tubular epithelium from the ligated cauda epididymidis of a hamster in which both testes were left *in situ* (Group A, 20 days). Note the flattened epithelium and the absence of 'light' cells. $\times 170$.

FIG. 3. Tubular epithelium from the ligated cauda epididymidis of a bilaterally gonadectomized hamster. $\times 170$.

TEXT-FIG. 5. The mean concentration of fructose in seminal vesicles of the seven groups of hamsters used in the study. (For an explanation of the characteristics of Groups A to F, see text.)

Table 6. Seminal vesicle fructose contents at various times under different experimental conditions (Groups A to F) in hamsters

Animal group	Days						
	0	4	8	10	12	16	20
Controls	8.11 (± 0.3)	—	—	—	—	—	—
A	—	—	—	—	—	—	7.8 (± 0.3)
B	—	—	—	—	—	—	8 (± 0.5)
C	—	6.02 (± 0.2)	5.3 (± 0.3)	4.8 (± 0.4)	2.7 (± 0.24)	—	—
D	—	—	—	—	—	—	8.1 (± 0.5)
E	—	—	2.1 (± 0.2)	—	—	—	—
F	—	—	3.2 (± 0.5)	—	—	—	—

Mean seminal fructose in mg/g dry wt ($\pm$ S.E.).
For characteristics of Groups, see footnote to Table 1.

fig. 2 and Table 4). This was not due to a paucity of live spermatozoa since the mean number of live spermatozoa deposited between 8 and 10 days after bilateral gonadectomy exceeded the minimum number (0.5×10^6) necessary to ensure maximum fertilization (see Text-fig. 3).

Histological effects

Examination of material from animals in Groups A, B and D revealed that, within 20 to 24 days, isolation of the cauda epididymidis resulted in flattening of the principal cells of the tubular epithelium and a gradual disappearance of so-called 'holocrine' cells, which are conspicuously interspersed between the principal cells of the epithelium (Pl. 2, Fig. 1). The incidence of these cells decreased significantly ($P < 0.001$) between 16 and 20 days in Groups A, B and D, and between 8 and 12 days following bilateral gonadectomy (Text-fig. 4). In each case, this decrease paralleled a loss of fertilizing capacity in the spermatozoa.

Fructose concentration of seminal vesicles

The results of these estimations (Text-fig. 5 and Table 6) show that in animals from Groups A, B or D, there was no significant change in the mean concentration of fructose in the seminal vesicles even after 20 days. In the animals from Group C, the fructose concentration decreased significantly after only 4 days, even though the spermatozoa retained a high fertilizing capacity for 8 days (Text-fig. 2). Later, both fructose concentration and fertilization rate decreased precipitously, and, within 12 days of removal of the gonads, they were very low indeed (Text-figs 5 and 2, respectively). The results from animals in Groups E and F, however, show that vesicular fructose was significantly lower ($P < 0.001$) after 8 days of hypophysectomy and gonadectomy than after gonadectomy alone.

DISCUSSION

It is well known that epididymal spermatozoa *in vitro* lose their motility twice as quickly if the testes have been removed. This has been shown in guinea-pigs (Benoit, 1926; Moore, 1927, 1928), rats (Moore, 1928; White, 1933) and mice (Benoit, 1926), but the present work has demonstrated that the maintenance of the fertilizing capacity of epididymal spermatozoa is also very dependent upon the presence of circulating androgen. After gonadectomy, spermatozoa were incapable of fertilization after about 10 or 12 days, compared to 20 to 24 days in intact animals. It appears, moreover, that only one testis is needed to preserve the spermatozoa because, irrespective of which testis is removed, the spermatozoa in both epididymides retain their fertilizing capacity for the normal period. If both testes are removed, the normal period of sperm survival may be ensured by the administration of exogenous testosterone.

Correspondingly, the morphology of the cells lining the cauda epididymidis seems to be extremely sensitive to circulating androgen, and all the indications are that mature spermatozoa depend, for the preservation of their fertilizing capacity, upon the epididymal cells and that these in turn are dependent on

circulating androgen. Voglmayr, Waites & Setchell (1966), White & Hudson (1968) and Appelgren (1969) have all discussed the subject of androgen and epididymal fluid but it is still not certain whether significant amounts of steroid are present in luminal fluid at the level of the cauda epididymidis. Any androgen that might be present must, in the light of present results, be regarded as less vital than systemic androgen for sperm preservation because unless the cells of the epididymis, which are dependent on circulating androgen, are functioning normally, the spermatozoa cannot retain their fertilizing capacity for the normal period.

Nevertheless, Mounib (1964) and, more recently, Murdoch, White & Seamark (1970) have suggested that testosterone might suppress the oxidative metabolism of spermatozoa in the male reproductive tract and that this might be important in preserving their viability. The suggestion receives some support from the work of Ericsson, Cornette & Buthala (1967) which showed that epididymal spermatozoa will bind labelled testosterone *in vivo*. Ejaculated spermatozoa from a wide variety of species are known to be capable of metabolizing testosterone and other sex steroids (Scott, Wales, Wallace & White, 1963; Seamark & White, 1964; Hathaway, 1965). If, however, epididymal spermatozoa need androgen in the luminal fluid in order to survive, then the bulk of it must be produced or transferred from the bloodstream by the epididymal cells.

The reason why epididymal spermatozoa soon become degraded after removal of the testes is unclear and requires investigation. In this context, it is of interest that, in the present work, loss of fertilizing capacity by the spermatozoa was always accompanied by a decrease in the incidence of 'light' cells or so-called 'holocrine' cells (Malone & Bower, 1962; Martan & Risley, 1963a, b) in the epididymal epithelium. It would be premature to suggest that the two events are related, but Martan & Risley (1963a, b) have suggested that renewal of these cells is both androgen- and pressure-dependent. In the present work, all spermatozoa were obtained from an isolated cauda epididymidis and in these circumstances, the duct swells and the epithelium becomes flattened, suggesting fluid accumulation and increased pressure in the lumen. Disappearance of these cells, therefore, may be nothing more than an artifact induced by the experimental procedure, but the influence of contained spermatozoa upon the cells lining the epididymis would seem to warrant further scrutiny.

Implicit in the rôle of the testes in sperm survival is some activity on the part of the pituitary, and present results have shown that this is not entirely a question of the testicular-hypophysial relationship. It seems that the pituitary exerts its own specific influence on sperm survival since fertilizing capacity in the spermatozoa is lost sooner if animals are hypophysectomized in addition to having their testes removed.

Recently, Goldman & Porter (1970) have shown that, in hamsters, the circulating titre of LH increases tenfold after 10 days of castration. Doubtless, this increase is due to the withdrawal of testosterone and in guinea-pigs, Resko (1970) showed that plasma testosterone had completely disappeared within 6 hr of castration. In our experiments, there was a significant reduction in the fructose content of the seminal vesicles 4 days after castration, and since seminal

fructose is a sensitive indicator of circulating testicular androgen (Mann, 1964), it appears that in hamsters also, testosterone declines very rapidly when the testes are removed. An increase in the level of LH might thus be predicted with the possibility that it might prolong the survival of spermatozoa to some extent. It might operate by stimulating androgenesis in the epididymis (see Hamilton, 1971) or androgen output by the adrenal (see Bullock & New, 1971) but the failure of HCG to prolong sperm survival should be noted. The observation that seminal fructose declined more rapidly after hypophysectomy and gonadectomy than after gonadectomy alone does suggest that a pituitary-dependent extragonadal source of androgen might be involved in sperm survival.

It is now important to determine precisely how circulating androgen produces the right conditions for the optimal survival of spermatozoa in the epididymis.

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