EFFECTS OF DIFFERENT CONCENTRATIONS OF ANDROGEN UPON SEXUAL BEHAVIOR IN CASTRATED MALE RATS[1]

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Several investigators have employed testosterone propionate to evoke copulatory behavior in castrated male animals (Shapiro, 1937; Moore and Price, 1938; Stone, 1939). The majority of studies have dealt with the rat, and the dosages have varied from 100 to 1250 micrograms of hormone per day (1 microgram = .001 mg). It is well established that large amounts of androgen will maintain or restore normal sexual behavior in the adult castrate but we still do not know just how much hormone is required to achieve this result.

The present experiment was conducted to measure the sexual behavior of castrated male rats receiving various amounts of testosterone propionate by daily injection. We hoped to define the "adequate maintenance dose" which would keep behavior exactly at preoperative levels, and in addition to observe the behavioral effects of holding the hormone level below or above the minimal concentration needed for maintenance.

PROCEDURE

Subjects for the investigation were male rats, 90 to 100 days of age at the beginning of the tests. The animals were drawn from a colony which for ten years has been inbred to the extent of avoiding the introduction of any new stock, although no systematic plan of sibling crosses or back crosses has been followed. The strain was derived from a cross between wild *Rattus norvegicus* and tame albino rats from the Wistar Institute. For five or six years preceding this experiment males used as sires were individuals chosen for their willingness to mate promptly and vigorously. If one assumes that such characteristics are determined in part by heredity it follows that our practice may have resulted in a gradual increase in the sexual excitability of the strain.

Selection of the experimental animals was preceded by a series of preliminary sex tests in which males were placed singly with a female in heat and observed for the execution of mating responses. Individuals which failed to show any copulatory activity after two or three tests were discarded. This procedure eliminated sexually-sluggish animals and perhaps some others which were emotionally disturbed by the general testing situation.

The regular sex tests began after 52 suitably active males had been selected. These tests were conducted once each week in a quiet room with adequate ventilation and lighting. The circular observation cage was 34 inches in diameter, 30 inches high, and had no cover.

[1] This investigation was supported by a grant from the Committee for Research in Problems of Sex, National Research Council. The experiment was conducted while the senior author held the Chairmanship of the Department of Animal Behavior at the American Museum of Natural History. The junior author conducted most of the tests, performed the operations, and made the hormone injections. The senior author's responsibility included planning the experiment, interpretation of the data and preparation of the manuscript.

Stimulus animals were spayed females which had been brought into heat by the administration of ovarian hormones (Beach, 1942). Each day the injected females first were tested with non-experimental males of known sexual vigor. Only those individuals that displayed normal heat behavior in response to the mating attempts of the "indicator male" were employed in the experimental tests.

A male was placed in the observation cage and allowed a three-minute adaptation period and then a sexually-receptive female was quietly deposited in the center of the cage. Each test lasted for 10 minutes from the time of the first complete or incomplete copulation by the experimental male. In a complete copulation intromission is achieved. Incomplete copulations involve mounting the female and executing pelvic thrusts but insertion is lacking. Ejaculation is not involved in either type of response. If mounting responses did not occur within 10 minutes after the introduction of the female, the test was terminated and scored as negative. The behavioral items noted and the various measures employed will be described in the presentation of experimental results.

At the conclusion of the sixth preoperative test all males were castrated, and hormone injections were begun 48 hours later.

TABLE 1

Schedule of androgen treatment after castration

GROUP	N	MICROGRAMS OF TESTOSTERONE PROPIONATE PER DAY	
		First post operative period (9 weeks)	Second post operative period (10 weeks)
I	11	0	1
II	10	25	75
III	10	50	0
IV	10	100	omitted
V	11	500	0

The operated animals were divided into five experimental groups which were equated as closely as possible in terms of the average frequency of copulations per preoperative test. Each male was injected subcutaneously once every 24 hours with .2 cc. of sesame oil. The concentration of testosterone propionate contained in this amount of oil ranged from 25 to 500 micrograms for various groups and a control group received plain oil with no hormone.[2]

Tests were continued for a period of nine weeks, at which time the hormone dosages were changed. A final ten-week period concluded the experimental tests. The amounts of androgen administered daily to males in each group are shown in table 1.

Animals in Group IV received 100 micrograms per day during the first nine weeks after operation and 5 micrograms daily for the remainder of the experiment. These rats showed no response to the smaller dose. Their behavior was quite similar to that of castrates in Groups III and V who were given no hormone in the second post operative period. However, Group I exhibited definite increases in sexual activity in response to daily injections of 1 microgram of testosterone propionate. This suggests that treatment at a high dose level may render animals insensitive to much lower concentrations but our data are insufficient to establish the point. We do not here report the scores of Group IV rats during the second post operative period because they were in no way different from those of Groups III and V and because inclusion of their records would have obscured an otherwise clear

[2] The hormone preparations used in this study were generously supplied by Dr. Edward Henderson of Schering Corporation, Bloomfield, N. J.

relationship between hormone dosage and frequency of sexual responses. It is felt that exclusion of these data is justified by the fact that Group IV was the only group in which androgen concentration was reduced but not brought to zero,—a procedure which evidently yields results quite different from (1) increasing the dosage or (2) withdrawing the hormone altogether.

All animals were sacrificed within 24 hours after the final test. Completeness of testis removal was checked by macroscopic inspection and samples of seminal vesicle tissue were removed for histological study.

RESULTS

The experimental results will be discussed in terms of the various behavioral items studied.

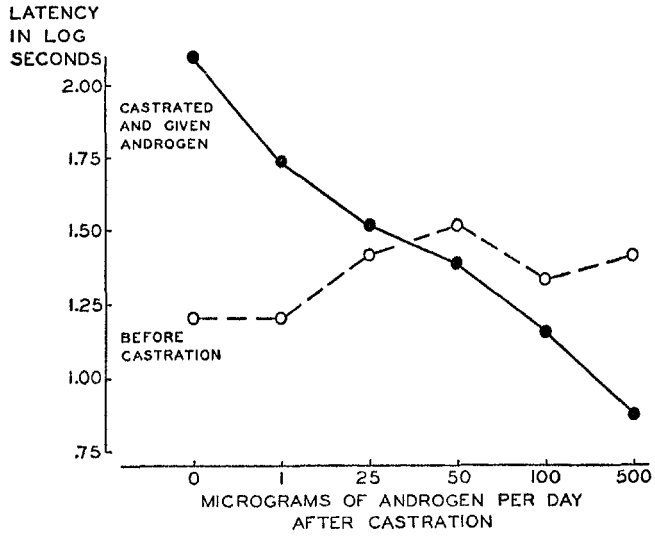

FIG. 1. AVERAGE DELAY PRECEDING THE FIRST SEXUAL MOUNT IN PRE- AND POST-OPERATIVE TESTS

Changes in Latency

Latency is defined as the number of seconds elapsing between the time that the receptive female was placed in the observation cage and the time that the male executed his first sexual mount (complete or incomplete copulation). This measure proved to be a very sensitive indicator of the amount of hormone administered to castrated males.

In order to normalize the distributions, raw scores have been translated into logarithms. Figure 1 is a graphic representation of the average log of latencies for each group before and after castration. Some differences existed between the averages for the various groups in preoperative tests, and after castration the average latency was directly proportional to the size of the hormone dosage. During the first preoperative test many animals showed long latencies which seemed to reflect emotional disturbance due to the strangeness of the environ-

ment. Therefore the scores in this test were not used in computing group averages. Immediately after operation the latency scores tended to be quite variable, but they were stabilized after the castrates had been receiving hormone for a week or ten days. Accordingly the records of the first postoperative test have been omitted from these particular calculations. Preoperative averages are based upon the results of tests 2 to 6 and the values representing postoperative performances of all groups are based upon nine tests conducted from 2 to 10 weeks after castration. In addition we have inserted the average score for Group I during the second post operative period at which time these rats were receiving 1 microgram of testosterone propionate per twenty-four hours.

In order to determine the significance of changes in latency we have compared the average log latencies of each group before and after castration. Preoperative means are based upon scores made in the last three tests before castration, and post operative means are based upon the records of six successive tests conducted

TABLE 2

Mean log latency

GROUP	N	NORMAL (TESTS 4-6)	CASTRATE (TESTS 10-15)	DIFFERENCE	PROBABILITY	MICROGAMS OF ANDROGEN PER DAY
I	7	1.14	2.26	+1.12	<.01	0
I	8	1.22	1.66*	+0.44	.03	1
II	10	1.33	1.53	+0.20	.04	25
III	10	1.47	1.34	−0.13	.11	50
IV	10	1.28	1.15	−0.13	.06	100
V	11	1.30	0.78	−0.52	<.01	500

* Based on tests 19 to 25.

after the animals had been receiving androgen for one month. In addition there are included the records of Group I during the last six tests in the second postoperative period when 1 microgram per day was being injected. The post operative change in mean values for each group was evaluated in terms of Student's *t* test, and using Snedecor's tables we have calculated the probability that the differences could have occurred by chance.

Table 2 presents the results of these comparisons. Castration followed by no treatment resulted in an average increase in lateny which would be expected to occur by chance less than 1 time in 100 and is therefore clearly significant in the statistical sense. Apparently 50 micrograms of testosterone propionate per day was adequate to maintain latency scores at preoperative levels. Lower doses were associated with latencies which, while shorter than would be expected without any replacement therapy, were significantly longer than normal. One hundred micrograms of androgen per twenty-four hours produced a decrease in latency scores which was significant at the 6 per cent level of confidence, and under the influence of 500 micrograms of hormone per day castrated male rats showed latencies that were definitely shorter than normal.

Data collected during the second post operative period corroborate these conclusions. Males in Group II were given 25 micrograms during the first and 75 micrograms during the second post operative period. The increase in dosage occasioned a shortening of the average log latency which was significant at below the 1 per cent level. In fact, under the influence of 75 micrograms per day these animals showed latencies shorter than they had displayed before castration (significant at the 8 per cent level).

Males in Groups III and V received 50 and 500 micrograms of androgen respectively during the first post operative period and no hormone in the second post operative period. The withdrawal of hormone was followed by increases in log latencies which were highly significant in both cases.

Changes in the copulatory response

The rat's copulatory response includes five distinct elements. (a) The male mounts the receptive female from the rear, clasping his forelegs around her sides in the lumbar region. (b) The male's forelegs are pressed downward and backward and then brought forward again in a series of very rapid "palpation" movements which help stimulate the female to elevate the perineum and thus expose the genitalia. (c) Concomitantly with the forelimb palpations, the male executes a series of short pelvic thrusts which bring the penis into contact with the genitalia of the female. (d) After several such preliminary pelvic movements the male accomplishes the brief intromission by means of a single, deep thrust. (e) Insertion usually is maintained for only a fraction of a second and the male dismounts abruptly with a vigorous backward lunge which often carries him half a foot or more away from the female. This pattern is clearly recognizable under ordinary circumstances. Ejaculation does not occur with each copulation, and when it does take place the behavior is different as subsequent description will reveal.

Per cent of each group copulating in each test: The first noticeable post operational change in sexual behavior was the complete disappearance of the copulatory response from the behavior of some males in some tests. We shall first consider the results on a "present-or-absent" basis, paying no attention to the frequency of copulations during tests in which such behavior occurred.

The trends represented in figure 2 are based upon 3-point moving averages. Results of tests 1 and 2 are not shown, but the percentage of copulators increased for all groups in the first few preoperative tests and then tended to stabilize. This probably reflects gradual adaptation to the testing situation as well as some general "conditioning" effect. In the interest of legibility we have refrained from reproducing the curves for Groups III and IV which received 50 and 100 micrograms per day respectively. The performance of Group IV males was very similar to that of Group V and the curve for Group III falls midway between those of Groups II and IV.

During the first post operative period the records of the several groups fell in a definite order which corresponded to the magnitude of hormone dosages. Because of inter-group differences in preoperative performance, comparisons between behavior in the first and second periods of the experiment must be drawn

with considerable caution. However, comparing each group with itself in the two periods we may tentatively conclude that daily injections of 50, 100, or 500 micrograms sufficed to maintain the proportion of copulators at preoperative levels if not to increase it somewhat.

Administration of 25 micrograms per day resulted in a decrease in the per cent of a group copulating in each test. Control injections of sesame oil had no recognizable effect, and the proportion of copulators in Group I progressively decreased until only approximately 10 per cent of the animals were responding in any given test. It should be stated that this did not reflect the persistence of copulatory behavior in a single member of the group. It was not always the same individual who copulated in successive post operative tests. Various males executed the

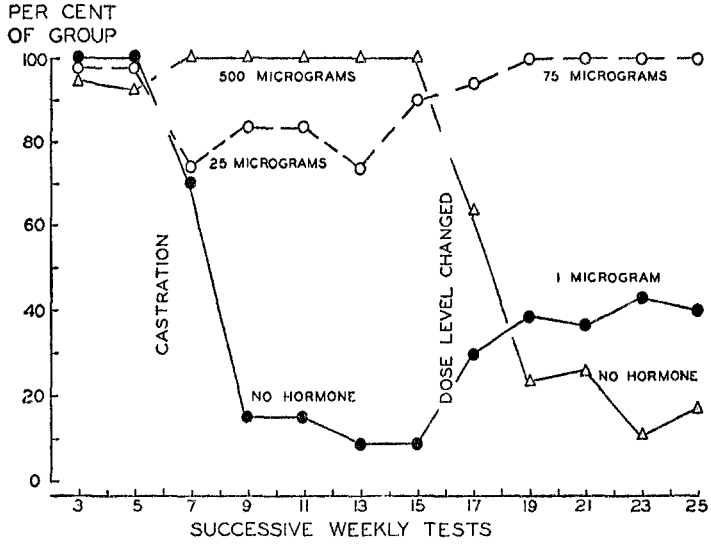

FIG. 2. PROPORTIONS OF THREE EXPERIMENTAL GROUPS SHOWING THE COPULATORY RESPONSE

response at different times, and the post castrational decline in behavior was not a regularly progressive change in individual animals although it was for the group as a whole. For example, one male in Group I exhibited no sexual activity for the first seven weeks after castration and then, in the eighth postoperative test, displayed 3 complete and 20 incomplete copulations. Another rat's scores were negative until the fifth test after castration at which time copulation and ejaculation occurred. Similar irregularities appeared in the individual records of males in Groups III and V during the second post operative period when androgen treatment was discontinued.

Hormone dosages were changed nine weeks after castration and subsequent alterations in behavior fully supported the foregoing conclusions. An increase from 25 to 75 micrograms in the daily dosage for Group II occasioned a prompt rise in the proportion of copulators per test, and within three weeks the record

of this group was equal to its own preoperative scores. Groups III and V were deprived of androgen in the second post operative period and the change in their performance was comparable to that of Group I during the first period after castration when plain oil was injected. The scores for Group I during the second post operative period show that as little as 1 microgram of testosterone propionate per day was sufficient to increase the percentage of copulators.

Frequency of copulations in positive tests: Thus far we have paid no attention to the number of times an animal copulated in a particular test. As long as the response occurred once the individual was scored as positive. We are now ready to consider the effects of castration and subsequent androgen administration upon copulatory frequency during those tests in which such behavior appeared.

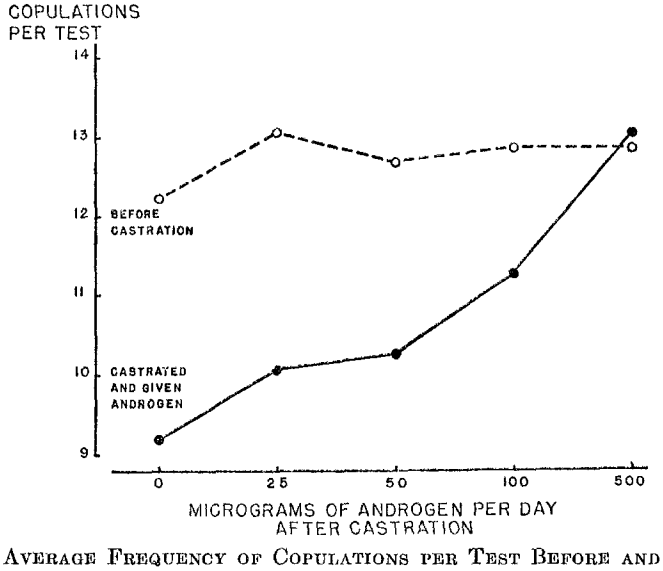

FIG. 3. AVERAGE FREQUENCY OF COPULATIONS PER TEST BEFORE AND AFTER CASTRATION

The average copulation frequency tended to increase in all groups during the first 2 or 3 preoperative tests and then to level off at approximately 12 to 13 copulations per test. During the last 4 tests before operation no major changes occurred in the group averages. Following castration the scores for all groups declined progressively. The drop from preoperative levels was quite marked in Groups II and III which received 25 and 50 micrograms of androgen respectively, and somewhat less pronounced in Groups IV and V receiving 100 and 500 micrograms. The average scores for Group I (no hormone) were extremely variable, due to the small number of males copulating, but obviously tended to decrease sharply.

In order to determine the statistical significance of these changes we have compared the average copulatory frequencies for each group during the last 3 preoperative tests with the mean scores during tests 10 to 15 which took place 4 to 9 weeks after castration. The comparisons are graphically represented in figure 3.

It is plain that in every group save one the average frequency of copulation was lower after castration, and the extent of the decrease was inversely proportional to the amount of androgen administered. Only those males which received 500 micrograms per day maintained their behavior at preoperative levels.

Application of the *t* test to differences between pre- and post operative means revealed that the difference was not statistically significant in the case of Group I, presumably because of the small N (5) and the high test-to-test variability shown by these untreated castrates. The decreases were significant, however, in the case of Groups II, III and IV (at the 1 per cent level for II and III and the 3 per cent level for IV).

During the second post operative period when Groups III and V were deprived of hormone the average frequency of copulations per positive test decreased markedly for both groups, but because relatively few males copulated at all the group averages were too variable to justify further statistical analysis. Group I males which had been receiving plain oil during the first post operative period showed no increase in copulation frequency when they were given 1 microgram of androgen per day although, as pointed out earlier, this treatment did produce an increase in the proportion of the group displaying the copulatory response at least once in a test.

During the first period after operation males in Group II were given 25 micrograms of hormone per day and copulated much less frequently during positive tests than they had before castration. In the second post operative period when the dosage was increased to 75 micrograms copulation frequency gradually increased until the group was performing as well as it had prior to operation. During the last 5 tests under the influence of 75 micrograms these animals copulated an average of 13.5 times per positive test which was slightly higher than their preoperative average. The average increase consequent to raised hormone dosage amounted to 3.4 copulations per rat per test and this difference proved to be significant at below the 1 per cent level of confidence.

It seems paradoxical that 100 micrograms of testosterone propionate per day was insufficient to maintain normal copulatory frequency during the first 9 weeks after castration in the case of Group IV and that 75 micrograms constituted an adequate dosage at a later stage in the experiment for Group II. It is possible that the long period of treatment at a relatively low dose level somehow sensitized the males in Group II so that the increase to 75 micrograms was peculiarly effective. At the present state of knowledge it seems useless to speculate further.

Changes in the incomplete copulatory response

The male rat's incomplete copulatory response consists of mounting the female and displaying pelvic thrusts and palpating movements of the forelimbs. There is no intromission, and when he dismounts the male slides weakly off the female's back instead of throwing himself backward with the vigorous lunge which terminates the complete copulatory response. The difference between complete and incomplete copulations usually is quite obvious to the trained observer although in perhaps 5 per cent of the cases there may be some question as to the occurrence of intromission.

Per cent of each group showing incomplete copulations in each test. Castration without androgen replacement resulted in a decrease in the number of animals exhibiting the incomplete copulatory response. Groups I, III and V were injected with plain oil during either the first or the second post operative period and in all groups the proportion of tests in which this response occurred decreased by approximately 50 per cent from precastrate levels.

Castrates receiving 1, 25, or 50 micrograms of testosterone propionate per day showed incomplete copulatory reactions in about the same proportion of tests as they had before the operation. A daily dose of 100 micrograms caused some increase in the number of tests in which such behavior appeared; and 500 micrograms produced an even more marked rise. These results are summarized in table 3.

TABLE 3

Proportion of tests in which incomplete copulation occurred

GROUP	N	AVERAGE PERCENTAGE OF TESTS POSITIVE FOR GROUP*			MICROGRAMS OF ANDROGEN PER DAY
		Normal (tests 4-6)	Castrate (tests 10-15)	Difference	
I	11	48	46†	−2	1
II	10	53	62	+9	25
III	10	40	45	+5	50
IV	10	47	65	+18	100
V	11	54	82	+28	500
			(tests 20-25)		
I	11	48	21‡	−27	0
III	10	40	15	−25	0
V	11	54	30	−24	0

* A test is "positive" if the response in question occurs at least once.

† Refers to tests 20-25 for this one group.

‡ Refers to tests 10-15 for this one group.

It should be held in mind that we are considering here merely the proportion of tests in which incomplete copulation occurred one or more times. The question as to the number of responses executed per test is dealt with below.

Frequency of incomplete copulations in positive tests. There was a marked tendency for untreated castrates or those receiving small amounts of androgen to display an increase in the average frequency of incomplete copulations during those tests in which such behavior appeared. Mean values presented in table 4 show that when they were injected with bland oil animals in Groups I, III and V displayed more of these responses in positive tests than they had prior to castration. Administration of 1 or 25 micrograms per day was accompanied by a statistically significant rise in the average number of incomplete copulations per positive test (Groups I and II). Castrates receiving 50, 100, or 500 micrograms during the first post operative period exhibited no significant change in the average frequency of incomplete copulations. Furthermore, when the dosage for males

in Group II was raised from 25 to 75 micrograms per day the average frequency of this response decreased from 5.2 to 2.7 per test.

The data shown in tables 3 and 4 suggest that lack of testis hormone caused castrated male rats to show incomplete copulation in fewer tests but to execute the response a greater number of times during the tests in which it did occur. Administration of 1 or 25 micrograms of testosterone propionate per day induced no marked change in the proportion of tests in which incomplete copulations appeared, but the frequency of responses per positive test was increased by these dosages. This particular reaction was comparatively unaffected by castration if 50 micrograms of androgen were injected daily after the operation. Finally, large doses of 100 or 500 micrograms produced an increase in the number of tests in

TABLE 4

*Average number of incomplete copulations per positive test**

GROUP	N	NORMAL (TESTS 4–6)	CASTRATE (TESTS 10–15)	DIFFERENCE	PROBABILITY	MICROGRAMS OF ANDROGEN PER DAY
I	7	1.7	9.4†	+7.7	<.01	1
II	10	1.8	5.2	+3.4	.02	25
III	8	2.1	2.5	+0.4	.25	50
IV	9	3.2	2.7	−0.5	.32	100
V	8	2.9	3.7	+0.8	.35	500
			(TESTS 20–25)			
I	5	2.1	8.7‡	+6.6	.05	0
III	5	2.4	13.8	+11.4	.01	0
V	5	2.0	4.9	+2.9	.07	0

* A positive test is one in which the response in question occurred at least once.

† Refers to tests 20–25 for this one group.

‡ Refers to tests 10–15 for this one group.

which the behavior was present but did not change the frequency of incomplete copulations per test.

Changes in the ejaculatory response

When a male rat ejaculates the event is clearly reflected in the overt behavior. At first the pattern progresses in the manner described for the copulatory response, but when insertion is achieved the male does not release the female immediately. Instead he maintains the mating clasp with his forelegs and prolongs intromission, pressing tightly against the female's hind quarters. After several seconds the clasp is relaxed and the male raises his forelimbs rather slowly, rearing upwards and backwards and often coming to rest in a semi-sitting position. Occasionally instead of releasing the female in this manner the male may grip her more tightly and fall slowly to one side, pulling her with him. In either event the difference from a copulation without ejaculation is clear-cut and unmistakable.

In the present report the terms "ejaculation" or "ejaculatory pattern" refer exclusively to this sequence of overt responses and not to the occurrence of emission. Under certain circumstances male rats may display all of the outward signs of ejaculation even though they are incapable of emitting seminal fluid (e.g. after removal of the accessory sex glands), but in our records an animal was credited with an ejaculation each time the behavioral pattern appeared regardless of the presence or absence of a vaginal plug.

Analysis of ejaculatory frequency in normal animals. Before discussing the effects of castration and androgen treatment upon the ejaculatory response it will be profitable to consider briefly several aspects of this element in the sexual performance of the normal male. The data to be described bear directly upon

TABLE 5

Comparison of average scores of rats that ejaculated once with those that ejaculated twice in the sixth preoperative test

ITEM FOR COMPARISON	17 MALES THAT EJACULATED TWICE	29 MALES THAT EJACULATED ONCE	DIFFERENCE	PROBABILITY
Copulations preceding first ejaculation	8.5	12.2	+3.7	<.01
Seconds per copulation before first ejaculation	18.6	31.3	+12.7	<.01
Seconds to attain first ejaculation after mating began	152.4	349.2	+196.8	.05
Seconds latency	24.9	36.5	+11.6	†
Seconds recovery after first ejaculation	261.5	270.7*	+9.2	†

* Only 8 of the 29 males in this group recovered sufficiently to resume copulating before the test was terminated.

† Not significant.

questions of individual differences in sexual ability and possess considerably significance in relation to problems of sexual impotence.

The data upon which the following analysis is based were obtained during the last preoperative test for all rats. During this test a few animals failed to copulate, 5 copulated without ejaculating, 29 ejaculated once and 17 ejaculated twice. Disregarding those males which did not copulate or copulated without ejaculating we have attempted to discover such differences as might have existed between the 17 rats that ejaculated twice and the 29 males that did so only once.

The first and most obvious possibility was that a difference in general health was involved, but a comparison of body weights revealed no significant difference. Males ejaculating once weighed an average of 316 grams and the average weight of those ejaculating twice was 327 grams with a great deal of overlap between the two distributions.

Other possible explanations were analyzed and the results are summarized in table 5. Rats that ejaculated twice in a time-limited test were animals that reached the first ejaculation after fewer intromissions than did those individuals

who ejaculated only once in the same period. "Double-ejaculators" allowed less time to elapse between preejaculatory intromissions with the result that the average number of seconds per copulation was significantly lower, and, as a natural consequence of these two differences, "double ejaculators" achieved their first ejaculation earlier in the test than did "single ejaculators".

Males which were going to ejaculate twice tended to initiate sexual activity with less delay than rats that were going to ejaculate once, but this difference in latency scores was not statistically significant. Following the first ejaculation the "double ejaculators" resumed their copulatory activity after an average delay of about $4\frac{1}{2}$ minutes. Eight "single ejaculators" recovered in approximately the same length of time but failed to ejaculate again even though they completed as many copulations after the first ejaculation as did the other males. Twenty-one "single ejaculators" showed no copulatory behavior after the initial ejaculation. In their case ejaculation occurred so late in the test that not enough time remained for recovery and the renewal of sexual activity.

TABLE 6

Comparison of activity preceding first and the second ejaculations for 17 rats that ejaculate d twice during the sixth preoperative test

ITEM FOR COMPARISON	FIRST EJACULATION	SECOND EJACULATION	DIFFERENCE	PROBABILITY
Copulations preceding ejaculation	8.5	4.6	−3.9	< .01
Seconds preceding ejaculation after mating begins	152.4	97.7	−54.7	< .01
Seconds per copulation	18.6	21.5	+2.9	†

† Not significant.

One additional item of interest was seen in the fact that when a second ejaculation occurred it seemed to do so after fewer copulations and in less time than had been necessary for the first ejaculation. These conclusions are based upon data summarized in table 6.

Per cent of each group ejaculating in successive tests. Most of the sexually-active male rats of our strain will ejaculate at least once in the majority of a series of weekly tests, but not every male will ejaculate on every test. Figure 4, based on a 3-point moving average, shows the proportion of Groups I, II and V which achieved ejaculation at least once in successive tests. The curves for Groups III and IV fall between those of II and V but have been omitted so that the figure can be read easily.

There was an obvious relationship between the amount of androgen administered and the proportion of a group ejaculating. Comparison of figures 2 and 4 reveals that more hormone was necessary to evoke ejaculation than to call forth copulation.

Frequency of ejaculation in positive tests. Having seen the effects of castration and hormone treatment upon the presence or absence of the ejaculatory response, we are now in a position to consider the frequency of ejaculations during those

tests in which this reaction occurred at least once. Most rats which continued to ejaculate under the influence of very small amounts of androgen tended to do so only once within the 10-minute test. For example, before castration males in Group I ejaculated an average of 1.7 times per positive test. When these animals were castrated and injected with 1 microgram of testosterone propionate per 24 hours ejaculation occasionally occurred, but the average frequency was 1.0 per positive test. The difference between the means was significant at the 3 per cent level.

Animals in Group II also displayed a drop in the frequency of ejaculations when they were castrated and given daily injections of 25 micrograms, but the change was slight and the difference between the pre- and post operative means

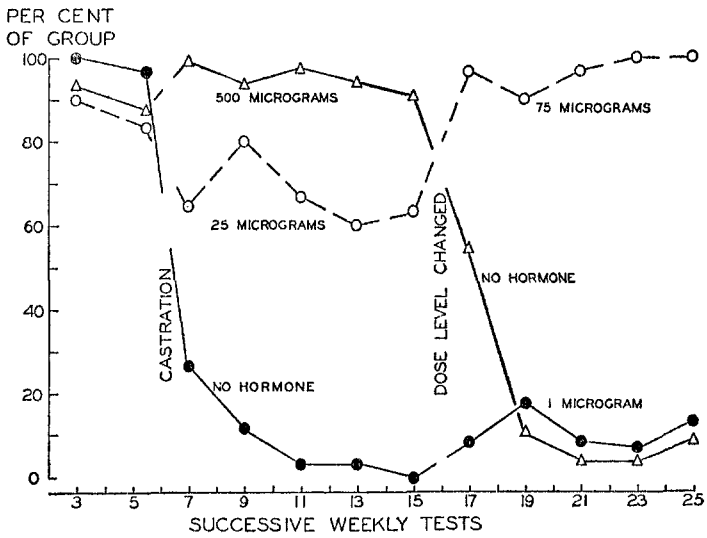

FIG. 4. PROPORTIONS OF THREE EXPERIMENTAL GROUPS EJACULATING AT LEAST ONCE DURING THE TEST

not statistically significant. Rats receiving 50 or 100 micrograms of androgen per day tended to ejaculate more frequently after castration although the increases were not marked. However, males in Group V ejaculated an average of 1.4 times per positive test before castration and 1.7 times after operation while receiving 500 micrograms of hormone per day. This rise in ejaculatory frequency was highly significant ($<.01$).

None of the animals ejaculated more than twice in the same 10-minute test and changes in ejaculatory frequency during positive tests can therefore be illustrated by showing the proportions of the various groups which ejaculated twice in each test. This scheme has been followed in preparing figure 5 which is based upon a 3-point moving average. Curves for groups III and IV are not shown but they fitted in with the trends revealed by the other three groups. Group IV males received 100 micrograms and their scores fell approximately

midway between the 25 and 500 microgram groups. Group III with 50 micrograms showed two ejaculations about as frequently as rats receiving 25 micrograms.

There was some tendency for the percentage of a group ejaculating twice to increase in successive tests before operation. After castration if no androgen was given the ability to ejaculate twice in the same test was soon lost by all males. Administration of 25 or 50 micrograms of hormone per day maintained the proportion of "double ejaculators" at levels roughly comparable to those attained in the last preoperative tests. Higher dosages, particularly 500 micrograms per

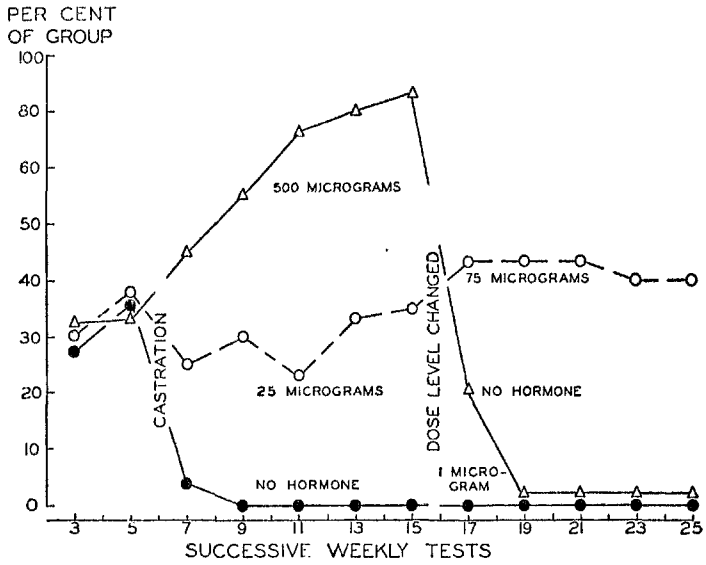

FIG. 5. PROPORTIONS OF THREE EXPERIMENTAL GROUPS EJACULATING TWICE DURING THE TEST

day, brought about a striking increase in the number of males capable of attaining two ejaculations within the time-limited test.

Number of copulations preceding ejaculation. Normal male rats display considerable individual variation with respect to the number of intromissions which are necessary to evoke ejaculation, and at the same time most individuals show a remarkable constancy in this function from one test to another. Some males regularly achieve the first ejaculation in each test after 5, 6 or 7 copulations whereas others just as reliably take 13, 14 or 15 intromissions to attain the ejaculatory peak.

Table 7 shows the effects of castration and subsequent androgen therapy on this particular aspect of the male's sexual behavior. Animals receiving 1 microgram per day or no hormone at all ejaculated so rarely that there are no reliable data concerning the number of copulations necessary for ejaculation in such cases. However, the values incorporated in table 7 strongly suggest that castrated rats receiving 25, 50 or 100 micrograms of testosterone propionate per day tended

to ejaculate after fewer copulations than they had before operation. In contrast, males injected with 500 micrograms per day showed no significant change in the frequency of copulations preceding the first ejaculation.

These mean scores suggest that relatively large amounts of androgen were necessary to maintain the preejaculatory copulation score at normal levels, and the performance of males in Group II during the three experimental periods supported this conclusion. Prior to operation these rats ejaculated after an average of 11.0 copulations. After castration while they were receiving 25 micrograms of testosterone propionate per day the mean number of copulations preceding ejaculation fell to 9.3. Then, when the daily dosage was increased to 75 micrograms the score rose again to an average value of 11.1. The increase occasioned by the higher dose level was highly significant ($p = <.01$).[3]

Time necessary to achieve ejaculation. The fact that castrated males receiving less than 500 micrograms of androgen per day tended to ejaculate after fewer intromissions during the first postoperative period than they had before gonad-

TABLE 7

Average number of copulations preceding the first ejaculation

GROUP	N	NORMAL (TESTS 4-6)	CASTRATE (TESTS 10-15)	DIFFERENCE	PROBABILITY	MICROGRAMS OF ANDROGEN PER DAY
II	10	11.0	9.3	−1.7	.05	25
III	10	11.1	8.5	−2.6	<.01	50
IV	10	11.7	8.7	−3.0	<.01	100
V	11	9.9	9.2	−0.7	.25	500

ectomy was an unexpected finding, and we proceeded to determine whether these rats ejaculated earlier in the tests after castration.

Basing our calculations upon the number of seconds intervening between the first sexual mount (complete or incomplete copulation) and the occurrence of the first ejaculation we found that rats receiving plain oil or 1 microgram of androgen invariably took longer to ejaculate than they had in preoperative tests. Group II males ejaculated in an average of 265 seconds before operation and 281 seconds after castration while they were receiving 25 micrograms of hormone per day. When the dosage was raised to 75 micrograms the average time needed to achieve ejaculation decreased to 257 seconds. Rats receiving 50 or 100 micrograms per day ejaculated more quickly in post operative tests than they had before castration; and under the influence of 500 micrograms of androgen per 24 hours castrated males displayed the most marked decrease in the number of seconds elapsing before ejaculation (255 seconds as normal and 211 as castrate, significant at the 5 per cent level).

Castrates receiving small doses of androgen tended to ejaculate after fewer

[3] Here, as at several other points in this report, it appears that Group II males responded more strongly to 75 micrograms than did Group IV animals to 100 micrograms. We are unable to explain this phenomenon but call attention to the fact that rats in Group II received 25 micrograms per day for 9 weeks before the higher concentration was employed.

copulations than normal, but it took them longer to reach the point of ejaculating. This suggests that the delay between copulations must have increased when the androgen level was low. Gonadectomized males injected with very large amounts of hormone ejaculated sooner than they had before operation, but the number of precjaculatory intromissions was unchanged. Here a shortened intercopulatory interval is indicated.

These indications are borne out by values presented in table 8 which shows the mean recovery period in seconds for each group before and after castration. Post operative changes in the amount of time elapsing between copulations were directly proportional to the amount of androgen given daily to the castrated animals. Only very large doses were sufficient to maintain copulation at normal speeds. The significance of all the differences shown in table 8 cannot be demonstrated statistically, but the regularity of progression in the Difference column argues against the belief that this is a chance effect. For Groups II, III, IV and

TABLE 8

Average number of seconds intervening between copulations which precede the first ejaculation

GROUP	N	NORMAL (TESTS 4–6)	CASTRATE (TESTS 10–15)	DIFFERENCE	MICROGRAMS OF ANDROGEN PER DAY
I	3	18.3	32.6	+14.3	1
II	10	24.1	30.2	+6.1	25
III	10	25.3	30.9	+5.6	50
IV	10	26.0	29.0	+3.0	100
V	11	25.7	22.9	−2.8	500
III	2	26.0	39.8	+13.8	0
V	2	21.7	44.8	+23.1	0

V in which the N was large enough to permit application of the *t* test the probabilities were .07, .02, .05 and .11 respectively. Finally, in Group II the mean intercopulatory interval increased by 6.1 seconds while the castrates were receiving 25 micrograms of androgen per day, and then decreased by almost the same amount when the dosage was raised to 75 micrograms.

Number of incomplete copulations preceding ejaculation. We have already seen that the frequency of incomplete copulations increased in castrated males given relatively small amounts of androgen. In the case of these animals if we combine the incomplete and the complete copulations preceding the first ejaculation we arrive at the following tentative conclusions. Copulations with intromission occurred less frequently after castration and in their place there appeared more of the incomplete copulatory responses. The increase in incomplete reactions equalled or exceeded the decrease in complete ones with the result that under the influence of low hormone doses gonadectomized males actually mounted the female at least as frequently to achieve ejaculation as they had before operation or as did other castrates which received larger amounts of androgen.

Changes in the recovery period after ejaculation

In male rats of our experimental strain ejaculation is followed by several minutes of relative inactivity during which the animal appears refractory to sexual arousal. The total refractory period can be thought of as including an "absolute" and a "relative" phase. During the absolute phase the male seems incapable of copulatory performance. No amount of stimulation will evoke mounting behavior and very little attention is paid to the female. During the relative phase of the refractory period sexual responsiveness is gradually regained. This period may be shortened if the female spontaneously resumes the exhibition of heat responses. It seems as though the copulatory threshold becomes progressively lower and can be crossed before it reaches normal levels if the stimulation is sufficiently intense.

We have defined the post ejaculatory refractory period as the number of seconds intervening between an ejaculation and the occurrence of the next complete or incomplete copulation. Inspection of the experimental records revealed that in the first test after castration the length of this period was greatly increased unless at least 75 micrograms of testosterone propionate were supplied each day. If no androgen was injected the refractory period was very long in tests given one week after gonadectomy or cessation of hormone treatment. In such cases, however, ejaculation was soon eradicated and consequently there are not enough data on duration of the refractory period to permit statistical treatment. A similar situation obtains in the case of Group I rats which received 1 microgram per day during the second period after operation.

Males in Groups II to V ejaculated with sufficient frequency after operation to permit quantitative analysis of changes in the post ejaculatory refractory period. Prior to gonadectomy the average recovery periods for these 4 groups varied from 251 to 280 seconds. During the first postoperative period the group averages ranged from 229 to 309 seconds. The longest mean refractory period was that of Group II whose members received 25 micrograms of hormone per 24 hours, and the shortest was that of rats in Group V which were given 500 micrograms daily. Animals treated with 50 micrograms showed a slight increase in average recovery time and those receiving 100 micrograms a minor decrease. The average times for Group II in the three periods of experimentation were as follows: 281 seconds before operation, 309 seconds after castration while receiving 25 micrograms per day and 256 seconds after castration while receiving 75 micrograms per day.

In order to measure the statistical significance of changes in the post ejaculatory refractory period the raw scores were transformed into logarithms and differences between the pre- and postoperative averages for each group were evaluated by means of the t test. The results appear in table 9. It will be seen that the increased mean refractory periods shown by animals receiving 25 or 50 micrograms of androgen per day were statistically significant. The shortened recovery times for Groups IV and V were not significant although of course the possibility of a true difference is not disproven.

When the dosage for Group II was increased from 25 to 75 micrograms the mean log refractory period decreased from 2.49 to 2.41 and the change was significant at the 2 per cent level.

Morphological changes and general health

Condition of seminal vesicles: Within 24 hours after the final test all animals were sacrificed and samples of seminal vesicle tissue were removed from several members of each group for sectioning and histological study. It was regarded as undesirable to subject the rats to abdominal operation at the conclusion of the first postoperative period and therefore no information is available concerning

TABLE 9

Average duration of recovery period after first ejaculation (in log seconds)

GROUP	N	NORMAL (TESTS 4–6)	CASTRATED (TESTS 10–15)	DIFFERENCE	PROBABILITY	MICROGRAMS OF ANDROGEN PER DAY
II	5	2.40	2.49	+.09	<.01	25
III	7	2.39	2.45	+.06	.02	50
IV	6	2.42	2.37	−.05	.14	100
V	11	2.38	2.34	−.04	.13	500

TABLE 10

Average body weights

GROUP	N	NORMAL WEEKS 4–6	CASTRATE WEEKS		PER CENT INCREASE OVER PREOPERATIVE WEIGHT		MICROGRAMS OF ANDROGEN PER DAY	
			10–15	20–25	First post-operative period	Second post-operative period	First post-operative period	Second post-operative period
I	11	300	344	350	15	2	0	1
II	10	297	339	367	14	8	25	75
III	10	306	366	376	19	3	50	0
IV	10	318	369	382	16	—	100	—
V	11	304	349	375	15	7	500	0

the response of accessory glands to hormone dosages used during the first 9 weeks after castration.

Males in Groups III and V were given 50 and 500 micrograms per day respectively in the first period and plain sesame oil during the second period after castration. At the end of the second period the seminal vesicles were typical of the long-term castrate. Gross size was markedly reduced and there was no evidence of secretory activity. Much of the epithelium was totally desquamated and such cells as remained covering the connective tissue were very low with quite small, spherical nuclei.

Group I males received no hormone for 9 weeks after operation and then were give 1 microgram per day for the next 10 weeks. This amount of testosterone propionate exerted no observable effect upon the seminal vesicles, and the cyto-

logical picture in these animals was exactly the same as that described for Groups III and V after 10 weeks without androgen. This finding is particularly interesting in view of the fact that 1 microgram per day did produce a distinct increase in some aspects of the sexual behavior.

Daily injections of 75 micrograms of hormone maintained normal seminal vesicles in males of Group II. In their gross and microscopic aspects the accessories of these animals were indistinguishable from those of intact rats.

General health: The health of the experimental males was checked by observation and by recording bodily weight once each week. There was no indication that either the operation or the hormone treatment had any adverse effects. All animals gained weight during the first 9 weeks after castration and group averages increased from 14 to 19 per cent with no apparent relationship between magnitude of increase and size of hormone dosage. During the second postoperative period 41 rats gained a little more weight and 11 lost an average of 1 to 15 grams. These minor decreases were shown by some rats in every group and probably reflect the fact that the animals were getting to an age at which the weight curve tends to level off. Average weights are shown in table 10.

DISCUSSION

In interpreting the foregoing results it is necessary to hold in mind certain limitations of the conditions under which they were obtained. First, the experimental population was a highly selected group drawn from an inbred strain. Second, our own results show that the behavioral effects of a given concentration of androgen tend to vary depending upon previous hormonal treatment. Castrated rats which have never been given androgen show an improvement in some aspects of their sexual performance when they are injected with 1 microgram per day; yet five times this amount has no appreciable effect upon the behavior of castrates previously treated with 100 micrograms. Finally, although it is probable that other forms of androgen would facilitate sexual performance in the castrated male, it is entirely possible that the magnitude of such an effect is a function of the form of the hormone and the method of its administration.

Within these limits the results of our experiment are reasonably consistent and clear cut. It has long been known that sexual behavior can be maintained in castrated rats by the administration of androgen but it can now be added that if the androgen is testosterone propionate, and if it is administered in the form of daily injections, the amount needed to hold performance at or near preoperative levels is approximately 50 to 75 micrograms. This is considerably less than the amounts employed in most of the earlier studies. We have also obtained new evidence concerning the effects of hormonal concentrations falling well above or below this "maintenance level."

In a very general way it is correct to state that the strength of the "sex drive" in a castrated male rat is roughly proportional to the amount of exogenous testicular hormone which is injected. But any such generalization must be followed by the qualification that "sex drive" is a meaningful concept only when it is operationally defined, and that when this is done the strength of the drive tends

to vary according to the behavioral criterion selected to measure it. For example, if we choose the frequency of intromissions per 10 minutes as *the* criterion our results indicate that sex drive decreases after castration unless more than 100 micrograms of androgen are given each day. In contrast it would be entirely permissible to state that sex drive will be measured in terms of the number of intromissions necessary to cause a sexual climax, or the speed of sexual arousal as measured by initial latency scores. In either instance the minimum androgen concentration sufficient to maintain normal sex drive would be considerably below 100 micrograms.

We are impressed with the need for an unambiguous, operational definition of sex drive,—a definition based upon actual mating performance rather than some less direct type of behavior such as the tendency to cross an electrically charged grid to reach the receptive female. Present data are insufficient to establish the point but they contain some suggestion that initial latency, time to achieve the first ejaculation, and duration of the post ejaculatory recovery period may be positively correlated. If such a correlation were high enough it would be entirely feasible to devise a combined score which would incorporate these measures and would serve as a valid and reliable index to sex drive in the individual male. When this can be done it seems likely that there will prove to be a fairly high positive relationship between sex drive and concentration of exogenous androgen administered to the castrated male.

A word of caution should be addressed to the reader who is unfamiliar with endocrinological data. The results herein reported *do not* signify that individual differences in the sexual responsiveness and potency of *unoperated* male rats are due to differences in levels of endogenous hormone. This particular problem cannot be approached until there is available some sensitive and reliable method of determining the amount of hormone present in the blood. Nothing of this sort can be done as yet with an animal as small as the rat. There are many potential sources of variability in sexual excitability and differences between intact males are quite possibly entirely independent of any hormonal basis.

The results of this experiment tell us nothing with respect to the locus or specific nature of hormonal action. They are, however, harmonious with the general hypothesis that one important function of the hormone is to lower the threshold in those nervous mechanisms specifically concerned with the mediation of sexual responses. For instance, the reduced initial latencies characteristic of castrated males receiving high doses of androgen suggest that such animals reach the threshold of arousal necessary to mounting activity with less preliminary stimulation than do other males whose blood contains less male hormone. Similarly, the abbreviated period of sexual refractoriness after an ejaculation is suggestive of a lowered threshold and a consequent reduction in the duration or intensity of the liminal stimulus.

SUMMARY

Fifty-two male rats were observed in six weekly mating tests with receptive females and then castrated. Sex tests were continued after operation while the

males received daily injections of testosterone propionate. The amount of androgen administered to different groups varied from 1 to 500 micrograms per day. A control group was treated with plain sesame oil.

The amount of hormone necessary to maintain sexual performance at or near preoperative levels varied somewhat depending upon the behavioral criterion selected as a measure. In the main, however, 50 to 75 micrograms per day represented a maintenance dose.

Castrated rats receiving no hormone and those injected with less than 50 micrograms were less likely to show any sexual responses toward the female than were normal animals or other castrates given higher concentrations of male hormone. When they did display copulatory reactions the low dose castrates were slow in initiating sexual contact, and mounting activity was apt to be preceded by relatively protracted periods of inattention or investigation of the estrous female. Such mating behavior as did occur consisted to a large extent of copulatory attempts which failed because of lack of intromission. Successful intromissions often were widely spaced in time. Ejaculation rarely was achieved but when it did appear it usually occurred after fewer intromissions and was followed by a longer period of sexual inactivity than is the rule in normal rats or in castrates receiving larger amounts of hormone.

Castrated male rats injected with 100 or 500 micrograms of testosterone propionate per day exhibited sexual behavior that was equal or superior to that shown prior to operation. They were more likely to copulate in every test than they had been before gonadectomy. At the same time the occurrence of incomplete copulations increased. Castrates receiving these larger doses of hormone tended to initiate sexual relations after shorter delays than they had before operation. The frequency of intromissions in a 10-minute test was not increased but multiple ejaculations occurred more frequently, and the first sexual climax was reached at an earlier point in the test. Finally, the castrates supplied with large amounts of androgen tended to recover more rapidly from the sexually depressing effects of an ejaculation.

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