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# FURTHER STUDIES OF MALE SEX-STIMULATING, FEMALE SEX-REPRESSIVE FRACTIONS OF THE ADRENAL GLANDS OF COWS, STEERS AND BULLS[1]

F. M. POTTENGER, JR. AND D. G. SIMONSEN
MONROVIA, CALIFORNIA

IN A PREVIOUS PAPER, we (1) described a female sex-repressive, male stimulative factor prepared from adrenal glands. This work has been extended to a study of the differences of this fraction as obtained from cow, bull and steer adrenal glands.

The method of preparation described in the previous paper was used except for one minor change. The benzene-insoluble material (after agitation with acetic acid and sodium acetate) is rather difficult to filter. We found that the addition of a small amount of Celite (diatomaceous earth) aids greatly in the filtration. In some cases, however, even after addition of Celite, the solution may filter slowly. If this occurs the mixture should be frozen solid (−20° C. will suffice) for from 24 to 48 hours. The freezing breaks the colloid mass and upon thawing the aqueous solution separates from the fatty material and can be readily filtered.

The female sex-repressive, male stimulative factor (hereafter called the *S-S* fraction) was prepared separately from bull, cow and steer adrenals. The concentrations of the various preparations were as follows: bull gland preparation *190*, 1 cc. equivalent to 56 gm. of whole gland; *235*, 40 gm. of whole gland (bull adrenals are very difficult to obtain and hence the concentration had to be reduced in preparation *235*). Cow gland preparations *176* and *234*, 1 cc. equivalent to 56 gm. of whole gland. Steer gland preparations *120* and *159–60*, 1 cc. equivalent to 66 gm. of whole gland.

The male and female test rats were given injections (subcutaneous) of 0.5 cc. of these various fractions daily for 30 days. The control rats were litter mates (litter mates are designated by letters in the tables) of the test animals and were untreated. Both test and control animals were weighed at 10-day intervals. The rats were from 2.5 to 6 months of age. Daily vaginal smears were made of both the test and control female rats. The smears were graded on a scale of 0 to 4 as suggested by Frank (2). The scale is as follows: 0, leucocytes, mucus, a few nucleated epithelial cells; 1, predominance of leucocytes, increase in nucleated epithelial cells; 2, presence of numerous leucocytes, predominance of nucleated epithelial cells; 3, no leucocytes, nucleated epithelial cells; 4, epithelial non-nucleated scales exclusively. At the end of the 30-day period, the animals were sacrificed. The uteri and ovaries of the female rats and the testes of the male rats were removed and weighed.

## RESULTS

Tables 1, 2 and 3 show the weights of the animals and of the uteri, ovaries and testes both of the control rats and of those treated with the *S-S* fraction of steer, cow and bull adrenals respectively.

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*Steer Glands.* Although marked variations were observed among the individual rats, the uteri and ovaries of the test animals per group were smaller than those of the control animals. In the test animals the weight of the uteri and ovaries ranged from 0.136 to 0.225 gm. and in the controls from 0.170 to 0.224 gm. Calculated in

TABLE 1. S-S FRACTION FROM STEER ADRENALS[1]

| | Rat No. | Body Wt. | Wt. of Uterus and Ovaries | Wt. of Uterus and Ovaries / Body Wt. % | Average % |
|---|---|---|---|---|---|
| | | *Grams* | | | |
| | | *A. Female Rats* | | | |
| Prepn. 159–60 | S-54 | 109 | 0.144 | 0.132 | |
| Test animal | S-80 | 114 | 0.199 | 0.175 | 0.153 |
| Control animal | S-50 | 102 | 0.190 | 0.186 | |
| | S- 7 | 118 | 0.170 | 0.144 | 0.165 |
| Prep. 159–60 | | | | | |
| Test animal | M-1 | 88 | 0.136 | 0.155 | |
| | L-2 | 93 | 0.225 | 0.242 | 0.196 |
| Control animal | M-20 | 90 | 0.224 | 0.249 | |
| | L-13 | 110 | 0.198 | 0.180 | 0.214 |

| | | | Wt. of Testes | Wt. of Testes / Body Weight % | |
|---|---|---|---|---|---|
| | | *Grams* | | | |
| | | *B. Male Rats* | | | |
| Prepn. 120 | | | | | |
| Test animal | F-9 | 87 | 1.30 | 1.49 | |
| | F-6 | 95 | 1.60 | 1.68 | |
| | H-50 | 81 | 1.10 | 1.36 | |
| | G-40 | 95 | 1.70 | 1.77 | 1.57 |
| Control animal | F-5 | 110 | 1.70 | 1.54 | |
| | F-4 | 94 | 0.70 | 0.74 | |
| | H-55 | 96 | 1.20 | 1.25 | |
| | G-44 | 89 | 1.60 | 1.79 | 1.33 |
| Prepn. 159–60 | | | | | |
| Test animal | R-84 | 118 | 1.63 | 1.38 | |
| | R-70 | 129 | 1.74 | 1.35 | 1.37 |
| Control animal | R-59 | 107 | 1.55 | 1.45 | |
| | R-90 | 131 | 1.42 | 1.08 | 1.27 |
| Prepn. 159–60 | | | | | |
| Test animal | M-11 | 104 | 1.58 | 1.52 | |
| | M-22 | 101 | 1.56 | 1.54 | 1.53 |
| Control animal | M-10 | 101 | 1.49 | 1.48 | |
| | M-21 | 95 | 1.38 | 1.45 | 1.47 |

[1] The figures in this table were compiled from a paper previously published by the authors. (1)

terms of per cent of body weight, the uteri and ovaries of the test animals average 10 per cent less than those of the controls.

Vaginal smears were not made of this group. However, in another experiment (3) vaginal smears were made for 60 days on a group of female rats used for tests with the S-S fraction prepared from steer glands. The controls showed a normal cycle, 5 to 7 days intervening between the four reactions. In the test animals there was usually

a delay in the first full reaction which on an average was 10 days. Then a marked stimulation occurred, the scale going from 0 to 4, then back to 2, and up again to 4, the rats never reaching a typical anestrous stage. This lasted for about 15 days. From

TABLE 2. S-S FRACTION FROM COW ADRENALS

| | Rat No. | Body Wt. | Wt. of Uterus and Ovaries | Wt. of Uterus and Ovaries / Body Weight % | Average % |
|---|---|---|---|---|---|
| | | *Grams* | | | |
| | | *A. Female Rats* | | | |
| Prepn. 176 | | | | | |
| Test animal | 1V-1 | 96 | 0.097 | 0.101 | |
| | 1W-5 | 86 | 0.078 | 0.091 | |
| | 1Y-13 | 80 | 0.046 | 0.057 | |
| | 1Z-15 | 87 | 0.068 | 0.078 | 0.082 |
| Control animal | 1V-2 | 82 | 0.138 | 0.169 | |
| | 1W-6 | 105 | 0.184 | 0.175 | |
| | 1Y-14 | 92 | 0.181 | 0.197 | |
| | 1Z-16 | 92 | 0.064 | 0.069 | 0.152 |
| Prepn. 234 | | | | | |
| Test animal | 2P-26 | 137 | 0.254 | 0.185 | |
| | 2Q-33 | 164 | 0.325 | 0.198 | |
| | 2Q-35 | 140 | 0.388 | 0.277 | |
| | 2R-42 | 66 | 0.104 | 0.158 | 0.205 |
| Control animal | 2P-30 | 153 | 0.313 | 0.204 | |
| | 2Q-34 | 152 | 0.323 | 0.212 | |
| | 2Q-36 | 131 | 0.423 | 0.323 | |
| | 2R-43 | 127 | 0.279 | 0.220 | 0.240 |

| | | | Wt. of Testes | Wt. of Testes / Body Wt. % | |
|---|---|---|---|---|---|
| | | *Grams* | | | |
| | | *B. Male Rats* | | | |
| Prepn. 176 | | | | | |
| Test animal | 1W-3 | 119 | 1.45 | 1.22 | |
| | 1X-9 | 106 | 1.41 | 1.33 | |
| | 1Y-11 | 117 | 1.46 | 1.25 | |
| | 2A-20 | 130 | 1.63 | 1.25 | 1.26 |
| Control animal | 1W-4 | 125 | 1.58 | 1.26 | |
| | 1X-10 | 124 | 1.36 | 1.10 | |
| | 1Y-12 | 127 | 1.48 | 1.17 | |
| | 2A-21 | 114 | 1.28 | 1.12 | 1.16 |
| Prepn. 234 | | | | | |
| Test animal | 2P-22 | 183 | 2.38 | 1.30 | |
| | 2P-24 | 149 | 2.42 | 1.62 | |
| | 2Q-31 | 166 | 2.26 | 1.36 | |
| | 2R-40 | 161 | 2.27 | 1.41 | 1.42 |
| Control animal | 2P-23 | 177 | 2.41 | 1.36 | |
| | 2P-25 | 187 | 2.59 | 1.39 | |
| | 2Q-32 | 159 | 2.23 | 1.40 | |
| | 2R-41 | 190 | 2.39 | 1.26 | 1.35 |

then on the reaction diminished in intensity and the peaks of the cycles lengthened. In three-fourths of the rats the estrous cycle disappeared in from 30 to 35 days and at the end of 60 days the vaginal smear was graded 0. Although variations were

noted in the individual rats the above is a general picture for the group studies.

In the male rats, there was an increase in the weights of the testes (calculated as per cent of body weight) of the test animals as compared to those of the controls.

TABLE 3. S-S FRACTION FROM BULL ADRENALS

| | Rat No. | Body Wt. | Wt. of Uterus and Ovaries | Wt. of Uterus and Ovaries / Body Wt. % | Average % |
|---|---|---|---|---|---|
| | | *Grams* | | | |
| | | *A. Female Rats* | | | |
| Prepn. 190 | | | | | |
| Test animal | 1R-11 | 107 | 0.280 | 0.262 | |
| | 1S-15 | 98 | 0.250 | 0.255 | |
| | 1T-20 | 94 | 0.121 | 0.129 | |
| | 1U-22 | 80 | 0.119 | 0.149 | 0.199 |
| Control animal | 1R-12 | 108 | 0.404 | 0.374 | |
| | 1S-16 | 91 | 0.143 | 0.157 | |
| | 1T-21 | 96 | 0.107 | 0.112 | |
| | 1U-23 | 79 | 0.141 | 0.178 | 0.205 |
| Prepn. 235 | | | | | |
| Test animal | 2M-1 | 142 | 0.310 | 0.218 | |
| | 2N-11 | 132 | 0.339 | 0.257 | |
| | 2O-13 | 134 | 0.316 | 0.236 | |
| | 2O-15 | 152 | 0.377 | 0.248 | 0.240 |
| Control animal | 2M-2 | 150 | 0.403 | 0.269 | |
| | 2N-12 | 159 | 0.389 | 0.244 | |
| | 2O-14 | 141 | 0.350 | 0.248 | |
| | 2O-16 | 150 | 0.362 | 0.242 | 0.251 |

| | Rat No. | Body Wt. | Wt. of Testes | Wt. of Testes / Body Wt. % | Average % |
|---|---|---|---|---|---|
| | | *Grams* | | | |
| | | *B. Male Rats* | | | |
| Prepn. 190 | | | | | |
| Test animal | 1P-1 | — | — | — | |
| | 1Q-3 | 114 | 1.29 | 1.13 | |
| | 1Q-5 | 114 | 1.25 | 1.10 | |
| | 1S-13 | 95 | 0.72 | 0.76 | 1.00 |
| Control animal | 1P-2 | — | — | — | |
| | 1Q-6 | 98 | 0.78 | 0.79 | |
| | 1Q-40 | 117 | 0.79 | 0.68 | |
| | 1S-14 | 68 | 0.46 | 0.67 | 0.71 |
| Prepn. 235 | | | | | |
| Test animal | 2M-3 | 161 | 2.68 | 1.67 | |
| | 2N-5 | 195 | 2.49 | 1.28 | |
| | 2N-9 | 154 | 2.35 | 1.53 | |
| | 2O-20 | 135 | 2.01 | 1.49 | 1.49 |
| Control animal | 2M-4 | 186 | 2.71 | 1.46 | |
| | 2N-6 | 194 | 2.49 | 1.28 | |
| | 2N-10 | 187 | 2.63 | 1.41 | |
| | 2O-21 | 191 | 2.22 | 1.16 | 1.33 |

In the test animals the average weight of the testes per group ranged from 1.10 to 1.74 gm. and in the controls from 0.70 to 1.70 gm. On an average group basis the testes of the treated animals were from 5 to 15 per cent larger than those of the untreated controls.

The weights of both test and control animals (male and female) increased during the time of injections. Variations were noted but based on group averages the changes were unimportant.

Histological observations of the female rats showed that the control animals had a greater follicular development than the test animals and also that the controls had a more highly developed type of uterus. In the uteri of the test animals degeneration of underlying epithelial cells was found. In the males, the test animals revealed a more mature spermatogenesis and the tubules showed greater activity as compared to those of the controls.

*Cow Glands.* There was a much greater degree of atrophy noted in the uteri and ovaries of the group used for tests with the *S-S* fraction of cow adrenals than with that of steer adrenals. In the test animals the weights of the uteri and ovaries ranged from 0.046 to 0.388 gm., and in the controls from 0.064 to 0.423 gm. Calculated in terms of percentage of body weight the uteri and ovaries of the test animals were from 15 to 45 per cent smaller than those of the controls.

Vaginal smears were made daily for 30 days in this group. The picture was somewhat similar to that obtained from rats treated with the *S-S* fraction of steer glands except that there was no stimulation of the cycle. The estrous cycle gradually diminished in intensity; after 10 to 15 days of treatment most of the rats showed only a 2 reaction. The cycle gradually disappeared and at the end of 30 days the reaction was graded 0, having been at that point for the last 10 to 15 days. The controls showed a 5 to 7-day cycle.

The female rats treated with *S-S* fraction *176* showed an average loss in weight of 1 gm. per rat as compared to an average gain of 5.5 gm. per control rat. These rats were approximately 6 months of age at the beginning of the experiment. In those treated with *234*, the test animals gained (average per rat) 30 gm. as compared to 46 gm. for the control rats. The rats in this group were 2.5 months of age at the beginning of the experiment.

The histological picture of the uteri and ovaries was similar to that obtained on rats treated with steer adrenals, except that the changes were much more marked.

In the male rats there was only a slight stimulation of growth of the testes of the animals treated with the *S-S* fraction of cow glands. In the test animals the weight of the testes ranged from 1.41 to 2.42 gm., and in the controls from 1.28 to 2.59 gm.

The test animals gained an average of 3 gm. per rat and the controls 13 gm. per rat during the course of treatment with *S-S* fraction *176*. In those treated with *234* the test animals gained an average of 56 gm. while the controls gained 67 gm. during the 30 day treatment. The first group averaged 6 months of age and the second, 2.5 months at the beginning of the experiment.

No histological observations were made on the testes of the rats treated with cow gland extract.

*Bull Glands.* There was only a slight difference in the weights of the uteri and ovaries of the test and control animals of this group. Those of the test animals ranged from 0.119 to 0.377 gm. and in the controls from 0.107 to 0.403 gm.

There was no inhibition of the estrous cycle of the female rats treated with the *S-S* fraction of bull glands. Although some variations in the individual rats were observed, both test and control animals showed a 5 to 7-day cycle.

The test females treated with *S-S* fraction *190* showed an average gain in weight of 3 gm., the controls, 9 gm. Those treated with *235* an average gain of 41 gm., the controls 59 gm. The groups of rats were 6 and 2.5 months of age respectively.

No histological examinations were made of the organs of the female rats of this group.

The testes of male rats treated with the S-S fraction of bull adrenals showed the greatest increase in weight compared to those of steer and cow adrenals. The weights of the testes of the treated animals ranged from 0.72 to 2.68 gm. and in the controls from 0.46 to 2.71 gm. Based on terms of percentage of body weight the testes of the treated animals were from 12 to 29 per cent larger than those of the controls.

Of the group used for tests with S-S 190 the treated animals showed an average gain of 11 gm., the controls 7 gm.; 235, the treated animals 48 gm., the controls, 66 gm.

Histological observation of the testes of the treated male rats showed a much more mature spermatogenesis and the tubules showed greater activity than those of the controls.

## GENERAL DISCUSSION

Table 4 gives a summary of the data in tables 1, 2 and 3. Briefly, the S-S fraction from cow adrenals produces a marked atrophy of the uteri and

TABLE 4. SUMMARY OF DATA

| | Wt. of Uterus and Ovaries / Body Weight Average per cent per group. Test | Control |
|---|---|---|
| | *A. Female Rats* | |
| Bull | 0.199 | 0.205 |
| | 0.240 | 0.251 |
| Cow | 0.082 | 0.152 |
| | 0.205 | 0.240 |
| Steer | 0.153 | 0.165 |
| | 0.196 | 0.214 |
| | **Weight of Testes / Body Weight Average per cent per group. Test** | **Control** |
| | *B. Male Rats* | |
| Bull | 1.00 | 0.71 |
| | 1.49 | 1.33 |
| Cow | 1.26 | 1.16 |
| | 1.42 | 1.35 |
| Steer | 1.57 | 1.33 |
| | 1.37 | 1.27 |
| | 1.53 | 1.47 |

ovaries of female rats, ranging from 15 to 45 per cent based on per cent of body weight and has little effect on the testes of male rats. That prepared from bull glands increases the weight of the testes of the treated animals from 12 to 29 per cent and has little or no effect on the uteri of female rats. That of steer glands is intermediate in effect, i.e., it causes some atrophy of the uteri and ovaries of female rats (about 10 per cent) and a slight stimulation (5 to 15 per cent) of the growth of the testes of male rats.

In the majority of the female rats, the *S-S* fraction of cow adrenals inhibits the estrous cycle in 30 days, steer glands in 60 days and bull adrenals have no apparent effect.

There was no evidence of masculinization of the female rats. Mammary development was normal and there was no hypertrophy of the clitoris. Testosterone propionate produces a masculinization of female rats as reported by Hamilton (4). Korenchevsky and collaborators (5, 6) have reported an hypertrophy of the uterus and ovaries of female rats treated with testosterone propionate. The male sex hormones testosterone, androsterone and adrenosterone are closely related chemically. Adrenosterone has been isolated from the adrenal by Reichstein (7). This compound, or one similar to it, may be responsible for the effects produced in the male rats, which have been reported here. It apparently is not the compound which causes atrophy of the female sex organs.

Histological observations of the female rats showed that the control animals had a greater follicular development than the test animals and also that the controls had a more highly developed type of uterus. In the uteri of the test animals degeneration of underlying epithelial cells was found. In the males, the test animals revealed a more mature spermatogenesis and the tubules showed greater activity as compared to those of the controls.

## SUMMARY

A female sex-repressive, male stimulative fraction has been prepared from cow, bull and steer adrenals.

The uteri and ovaries of female rats treated with this fraction prepared from cow glands were from 1/6 to nearly 1/2 the size of those of untreated controls. This same fraction had only a slight stimulative effect on the testes of male rats. This fraction from bull adrenals produced a 12 to 29 per cent increase in the weight of the testes of treated male rats as compared to those of the untreated controls. It produced only a slight atrophy of the uteri of female rats. This same fraction prepared from steer glands has an intermediate effect. On female rats the atrophy of the uteri amounts to about 10 per cent and the stimulation of testicular growth ranges from 5 to 15 per cent.

The estrous cycle is gradually inhibited in female rats treated with this fraction. Treatment for 60 days with steer glands or 30 days with cow glands produces a negative vaginal smear while that of rats treated with bull adrenals remains normal.

Histological observation reveals a functional atrophy of the uteri and ovaries of female rats and an increase of spermatogenesis in the testes of male rats.

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