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# A MALE SEX-STIMULATING AND FEMALE SEX-REPRESSING FRACTION FROM THE ADRENAL GLAND[1]

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Recent work on the chemistry of the adrenal cortex has resulted in the isolation of several crystalline substances, some of which have been reported physiologically active in maintaining the life of bilaterally adrenalectomized animals. Reichstein (1), Kendall (2), Wintersteiner (3), and their collaborators have shown that some of these crystalline compounds have the ring structure characteristic of the sterols and sex hormones. Kendall (4) has reported that one of his crystalline substances stimulated comb growth in the capon.

Many workers have indicated a relationship between the adrenals and sex function. Adrenal cortical hyperfunction, particularly that resulting from tumor growth, stimulates the secondary sex characteristics not of the same sex but of the opposite sex. Women exhibit male hair distribution, deep voice, amenorrhea, atrophic breasts, and atrophic genitalia, while men exhibit a female type of obesity, breast hypertrophy, atrophy of testes and absence of libido. Saphir (5), Cahill (6), Lisser (7), Goldzieher (8) and their collaborators have reported some of this work. Still others (9, 10, 11) have reported intsances of precocious sexual maturity produced by cortico-adrenal extract.

In adrenal insufficiency there seems to be a reduction of the sexual functions with resultant gonadal atrophy (12, 13, 14). All of these observations indicate a close relationship between the adrenals and sexual function.

With respect to the preparation of adrenal extracts containing fractions which are responsible for the above phenomena, little work has been reported. Ovarian stimulation in rats (luteinization and endometrial hyperplasia) by an adrenal extract has been reported by Allen and Bourne (15), and Casida and Hellbaum (16). Hodler (17) has recently made a report concerning an alkaline aqueous extract of the adrenal cortex, which has a masculinizing effect on the genital organs of normal and ovariectomized female and on castrated male guinea pigs.

We report here the results of another fraction of the adrenal cortex used experimentally by the Research Department of the Pottenger Sanatorium. In a group of children receiving adrenal cortex extract, it was observed that a large number increased in weight during treatment. This led us to search for a growth-promoting factor, which ended in discovering a female sex-

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repressive and a male stimulative factor. Klein (18) prepared a saline extract of adrenal cortex which produced a stimulation in the development of the male sex organs and an inhibition of the female sex organs of rats. Our extract was prepared by an entirely different method, as described in the experimental procedure.

## EXPERIMENTAL

Adrenal cortex extract was prepared by the method of Swingle and Pfiffner (19). The benzene insoluble fraction (originally discarded) was treated by a modification of the Wallen-Lawrence and van Dyke (20) procedure for the extraction of gonad-stimulating substances from anterior pituitary powder and from pregnancy urine. Briefly, the procedure used is as follows. To the benzene insoluble fraction was added 500 cc. of N/5 acetic acid and 500 cc. of N/5 sodium acetate. The mixture was thoroughly agitated for 15 minutes by means of a mechanical mixer and then allowed to stand for 48 hours in the ice chest (O°C.). The material was filtered and the filtrate concentrated to a volume of 300 cc. by vacuum distillation. The concentrated solution was poured into 10 volumes of 95 per cent ethyl alcohol (containing 5 cc. of acetic acid per liter) and placed in an ice chest (—25 to —30°C.). A dark, gummy precipitate settled out. The solution was removed by filtration, the precipitate dissolved in water, filtered, and poured into 10 volumes of 95 per cent alcohol. The procedure was repeated from 4 to 7 times in order to remove inert material. In the last 2 reprecipitations, the acetic acid was not added to the alcohol. A light brown colored powder was isolated which was water soluble, alcohol insoluble, and apparently heat stable at 60°C. The color may be completely removed by treatment with charcoal, although care must be used, for the latter compound adsorbs some of the active portion. The powder is made up in physiological saline, filtered through permutit in order to remove the last traces of epinephrine and sterilized by Berkefeld filtration. The concentration is such that 1 cc. of the above solution is equivalent to 66.6 gm. of whole gland.

Evans and Simpson (21) have shown that pituitary growth hormone administered to rats caused not only a marked increase in weight but also stimulated sex activity. Since increase in the weight of rats is easily observed, the above-mentioned fraction was assayed for its growth-promoting property by this method. Female rats were given 0.5 cc. injections (subcutaneous) of this material daily for 20 days. The control rats were litter mates of the test animals and were untreated. Both control and test animals were weighed at 5-day intervals. At the end of the 20-day period, the animals were sacrificed and the uterus, fallopian tubes and ovaries were removed. Photographs were taken, and in later experiments the organs were weighed. Male rats were subjected to the same procedure and a study was made of the testicles of these animals.

As the results obtained from this male-stimulative, female-repressive fraction (hereafter called the *S-S* fraction) were rather unusual, the cortical extract was assayed by the same procedure. In our modification of the Swingle and

Pfiffner (19) procedure, the following fractions are obtained from the benzene residue: *A)*, the alcohol-soluble fraction; *B)*, the alcohol-insoluble fraction obtained by cooling the alcohol soluble to —25 to —30°C.; *C)*, the water-soluble fraction. The effect of each of these fractions and one commercial product (eschatin)[2] was studied on both male and female rats.

A total of 54 female rats was used in this experiment: 36 of them were treated with the *S-S* fraction, 6 with the alcohol-soluble fraction *A)*, 4 with the alcohol-insoluble fraction *B)*, 4 with the water-soluble fraction *C)*, and 4 with eschatin.

TABLE 1. FEMALE RATS.

| | Rat No. | Body Wt. gm. | Wt. of Uterus gm. | Wt. of Uterus / Body weight % | Average % |
|---|---|---|---|---|---|
| *S-S* Fraction, 159–60. | | | | | |
| Test | S54 | 109 | 0.144 | 0.132 | |
| | S80 | 114 | 0.199 | 0.175 | 1.53 |
| Control | S50 | 102 | 0.190 | 0.186 | |
| | S7 | 118 | 0.170 | 0.144 | 1.65 |
| *S-S* Fraction, 159–60. | | | | | |
| Test | M1 | 88 | 0.136 | 0.155 | |
| | L2 | 93 | 0.225 | 0.242 | 0.196 |
| Control | M20 | 90 | 0.224 | 0.249 | |
| | L13 | 110 | 0.198 | 0.180 | 0.214 |
| Water-soluble Fraction *C*). | | | | | |
| Test | O4 | 62 | 0.049 | 0.079 | |
| | Q50 | 52.5 | 0.028 | 0.053 | 0.066 |
| Control | O5 | 58.1 | 0.035 | 0.061 | |
| | Q31 | 59.8 | 0.043 | 0.072 | 0.066 |
| Eschatin | | | | | |
| Test | O3 | 31.3 | 0.026 | 0.082 | |
| | T60 | 52.7 | 0.028 | 0.054 | 0.068 |
| Control | O10 | 47.0 | 0.032 | 0.067 | |
| | T65 | 52.9 | 0.034 | 0.063 | 0.065 |
| Alcohol-soluble Fraction *A*). | | | | | |
| Test | 1A-20 | 103 | 0.165 | 0.161 | |
| | 1B-50 | 117 | 0.182 | 0.155 | 0.158 |
| Control | 1A-30 | 104 | 0.202 | 0.195 | |
| | 1B-13 | 113 | 0.220 | 0.195 | 0.195 |

Thirty-four male rats were used and divided as follows. 16 were treated with the *S-S* fraction, 6 with the alcohol soluble fraction *A)*, 4 with the alcohol-insoluble fraction *B)*, 4 with the water-soluble fraction *C)*, and 4 with eschatin. Half of the number of rats in each group were test animals, the other half control animals.

## RESULTS AND DISCUSSION

### *Female Rats (Gross Observations)*

The uteri of the test animals on the *S-S* fraction were long and slender, and showed marked atrophy as compared to those of the control animals. In a large number of test animals the horns were 5 or 6 mm. longer than those

[2] Part of the eschatin was donated by Parke Davis Company.

of the controls, while the diameter of the uterus (at the base) was from one-third to one-half of that of the control animals. In a few of the test animals, hyperemia of the ovaries was noted.

With the alcohol-insoluble fraction *B)* the reverse phenomena were noted,

TABLE 2. MALE RATS.

| | Rat No. | Body Wt. gm. | Wt. of Testes gm. | Wt. of Testes / Body Wt. % | Average % |
|---|---|---|---|---|---|
| *S-S* Fraction, 120. | | | | | |
| Test | F9 | 87 | 1.30 | 1.49 | |
| | F6 | 95 | 1.60 | 1.68 | |
| | H50 | 81 | 1.10 | 1.36 | |
| | G40 | 95 | 1.70 | 1.77 | 1.57 |
| Control | F5 | 110 | 1.70 | 1.54 | |
| | F4 | 94 | 0.70 | 0.74 | |
| | H55 | 96 | 1.20 | 1.25 | |
| | G44 | 89 | 1.60 | 1.79 | 1.33 |
| *S-S* Fraction 159–60. | | | | | |
| Test | R84 | 118 | 1.63 | 1.38 | |
| | R70 | 129 | 1.74 | 1.35 | 1.37 |
| Control | R59 | 107 | 1.55 | 1.45 | |
| | R90 | 131 | 1.42 | 1.08 | 1.27 |
| *S-S* Fraction 159–60. | | | | | |
| Test | M11 | 104 | 1.58 | 1.52 | |
| | M22 | 101 | 1.56 | 1.54 | 1.53 |
| Control | M10 | 101 | 1.49 | 1.48 | |
| | M21 | 95 | 1.38 | 1.45 | 1.47 |
| Alcohol-insoluble *B*). | | | | | |
| Test | L5 | 124 | 1.52 | 1.23 | |
| | L9 | 111 | 1.55 | 1.40 | 1.32 |
| Control | L7 | 128 | 1.61 | 1.26 | |
| | L60 | 120 | 1.16 | 0.97 | 1.12 |
| Water-soluble *C*). | | | | | |
| Test | P22 | 59.8 | 0.62 | 1.03 | |
| | P23 | 50.0 | 0.51 | 1.01 | 1.02 |
| Control | P30 | 57.2 | 0.58 | 1.02 | |
| | P13 | 55.5 | 0.36 | 0.65 | 0.84 |
| Eschatin | | | | | |
| Test | N1 | 87.0 | 1.04 | 1.19 | |
| | T55 | 48.8 | 0.42 | 0.85 | 1.02 |
| Control | N2 | 96.6 | 1.37 | 1.41 | |
| | T15 | 57.5 | 0.54 | 0.94 | 1.18 |
| Alcohol-soluble *A*). | | | | | |
| Test | 1A-7 | 158 | 2.52 | 1.60 | |
| | 1B-11 | 167 | 2.73 | 1.63 | |
| | 1C-26 | 144 | 2.18 | 1.52 | 1.58 |
| Control | 1A-4 | 140 | 2.30 | 1.65 | |
| | 1B-8 | 142 | 2.28 | 1.61 | |
| | 1C-29 | 86.2 | 1.08 | 1.26 | 1.51 |

i.e., the uteri of the test animals were short and thick as compared to those of the controls. No significant change was noted in the uteri of the test animals as compared to those of the controls when the test animals were treated with eschatin or with the water-soluble fraction *C)* which is comparable to eschatin.

*Weight observations.* Table 1 shows the weights of the rats and of the sex-organs of a small group of female rats. The lack of a suitable balance pre-

vented the recording of these weights in the early experiments. The weight of the uterus was calculated in terms of per cent of body weight. As the chart shows, in the average percentage of each group, the uterus of the test animal on the *S-S* fraction was smaller than that of the control. It is a small difference yet a significant one. In the rats treated with the water-soluble fraction *C)* and with eschatin, these percentages were practically equal.

The weights of both test and control animals increased during the 20-day treatment period. Variations in individual rats were noted but, based on group averages, these changes were insignificant.

*Histological Observations.* Microscopic examination of sections of uteri revealed the following facts. The control animals showed more follicular development than the test animals. In several rats a large amount of luteinization was found, but the control animal showed a much more highly developed type of uterus. The chief observation in the uteri of the test animals was a degeneration of underlying epithelial cells.

### *Male Rats (Gross Observations)*

No marked difference was observed in the testicles of the test and control animals other than that of size, which will be discussed under weight changes.

*Weight Observations.* Table 2 gives the weight chart of all male rats used in this experiment. In 6 of 7 groups of male rats, there was an increase in the weight of the testicles (average weight of each group) of the test animals as compared to that of the controls. In the test animals, the average weight of the testicles per group ranged from 0.57 to 2.73 gm. and in the controls from 0.47 to 2.30 gm. Although this weight change is small, the significant fact is that a positive correlation was found consistently in the test animals. The variation in the weights of the test and control animals (average weight per group) was insignificant. In the sixth group, eschatin was used, and a negative correlation was found, i.e., the testicles of the control rats were heavier than those of the test animals.

*Histological Observations.* Microscopic examination of sections of the testicles revealed the following facts. In the test animals, the tubules showed a greater activity than in those of the controls, and the test animals showed a more mature spermatogenesis.

## DISCUSSION

The histological changes observed, both in the male and female rats, seem to be functional rather than structural. Continued treatment might bring about a pathological condition. The age of the rats seems to play an important rôle. In the female rats, the atrophy is much more marked in the mature than in the young animal (5 to 8 weeks of age). In the young animal, the *S-S* fraction may cause only a temporary inhibition of uterine and ovarian growth. In the male rat the stimulating action is about equal in both young and old animals.

One interesting observation was the homosexual tendencies displayed by these rats. It was more pronounced in the male than in the female animals.

The test animals showed erection of the penis as soon as they were handled, while the controls did not. Broster and Vines (22) have isolated a crystalline compound from the urine of a patient suffering from hypersecretion of the adrenal. This compound seems to be specific to virilism, for the patient who exhibited homosexual tendencies before operation returned to normalcy afterwards.

The female sex-repressive fraction is confined in the discard portion of the adrenal extract prepared by the method of Swingle and Pfiffner (19). With the exception of the alcohol-insoluble fraction which seems to possess stimulative properties, the fractions possessing cortical activity are inactive in producing this repressive effect.

The male-stimulative effect is obtained from the various fractions of the adrenal gland, both the cortical and noncortical. It is probably related to the chemical compound cortin.

## SUMMARY

By purifying the benzene discard of the Swingle and Pfiffner method of extraction of adrenal glands, by a technique described, a white amorphous powder is obtained. When tested on female rats, it causes a functional atrophy of the uteri and the uterine horns. When tested on male rats, it causes an increase in weight of the testes and an increase in spermatogenesis. These bisexual effects are not obtained from the benzene soluble fractions. In one fraction the opposite effect was obtained on the female tract.

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