EXPERIMENTAL DENTAL CARIES

I. The Effect of Orchiectomy and Ovariectomy on Dental Caries in Immature Rats

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EVIDENCE from the work of independent investigators indicates that gonadectomizing hamsters[1, 2] and rats[3] reduces the incidence of dental decay in immature males, while only a "biological" reduction appears in the females. Keyes[4] attempted to clarify this relationship by comparing the caries in a group of male and female gonadectomized hamsters with a similar group receiving testosterone. Under the conditions of his experiment, the data indicated that castrated males had slightly less caries than the noncastrated controls, while the castrated males receiving testosterone had a higher incidence than both former groups. In the females the controls and castrates had approximately the same amount of caries, while the castrates receiving testosterone had considerably less decay.

Previous work in this laboratory, using a strain of rats with a low degree of caries incidence,[5, 6] indicated that there appeared to be a difference in the incidence of caries in control male and female rats receiving the same diet. In the hope of clarifying this complex relationship between the sex of the animal and dental caries, this present study was designed to investigate whether the caries scores of castrated males would simulate the scores of uncastrated females, and if castrated female scores would resemble uncastrated males, as well as to determine whether the degree of caries would essentially return to its normal pattern in castrated animals by the administration of male or female sex hormones. A control group receiving the same concentration of sex hormones as the castrated group was tested on the basis that if removing the hormones would reduce caries, then increasing the hormone level might increase caries.

EXPERIMENTAL

A total of 134 animals,* of which 117 completed the experimental period, were divided as equally as possible as to sex, weight, and littermates into 8 groups. Each sex contained a control and a castrate group, and each control and castrate group was subdivided so that half received their respective sex hormones. The males received testosterone by implanting, subcutaneously, pellets weighing approximately 37 mg. between the scapulas. After 45 days pellets weighing approximately 75 mg. were implanted under similar conditions and those portions of these pellets remaining at the termination of the experiment

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*Carl Wilson strain, Beech Grove, Indianapolis, Indiana.

were removed and weighed in order that the dosage could be calculated. The females received 10 mg. of diethylstilbestrol in oil per week by subcutaneous injection.* All the animals were started on the experiment at 30 days of age and the investigation lasted for 117 days. All the animals received the caries-producing diet[5] and a fluoride-low water (0.1 μg per ml.) from birth. The method of preparing the heads for examination was reported previously.[5]

In the method for scoring caries, the extent or size of the various lesions was evaluated arbitrarily. For this purpose 3 different categories were recognized. A Number 1 classification was used for a very small cavity not penetrating the enamel, or for a superficial lesion on an exposed occlusal dentin surface. The use of the Number 2 designation was reserved for lesions which definitely extended into the dentin through the apex of the enamel-covered fissure and involved a relatively large area, but without extending beyond its own fissure or cusp. A Number 3 classification was used when the carious process had entirely destroyed its cusp or fissure and pulpal involvement was apparent. In the use of this system of classification the extent index for each animal was computed by determining the sum of the extent numbers and then taking the mean of those values. The mean value constituted the extent index for the series.

The expression "severity incidence" (SI) is a new method† for evaluating both the number of the lesions and the size of each lesion by one number. This figure is obtained by assigning a value of 1 to a tooth if less than one-fourth of it is destroyed by decay; a value of 2 if from one-fourth to one-half is destroyed; and a value of 3 if more than half is decayed. The arithmetic mean for each group is thus calculated.

At the end of the experimental period the animals were sacrificed and the heads removed in order to examine the teeth for caries, and the data are reported in this paper. In addition, the femurs were removed for breaking strength determination, calcium, phosphorus and fluorine determinations. The salivary glands were fixed by placing them immediately in Zenker's solution. The tongue, adrenal glands, and a section of long bones also were removed, as well as the lower jaw of each animal after caries had been scored, for histological examination. These data will be reported at a later date.

RESULTS AND DISCUSSION

The data for this experiment are presented in Table I. This table indicates the various experimental groups, the number of animals, and the number of cavities in each group. The extent of the lesions, the severity incidence (SI) and molars affected in each group are also shown. The data indicate that the number of lesions in the castrated males is considerably less than in the control males. Statistically, this difference is significant. The values for S.I. and extent of the lesions in this group further bear this out. The castrated males receiving testosterone appear to have fewer cavities than the control group, but the values for S.I. and extent of the lesions indicate that there is little difference between

*The Schering Corp., Bloomfield, N. J., and Mattox and Moore, Indianapolis, Ind., generously supplied the hormones used in this study.

†The authors wish to thank Dr. Arthur W. Radike for developing this method of counting caries.

these two groups. The control males receiving testosterone have a greater degree of caries than any of the other three groups. The difference between this group and the control group is not significant statistically, but the extent, S.I. and M.A. all indicate that some "biological factor" appears significant in this group.

The data for the females are quite different. The ovariectomized females have an increase in caries as compared to the control females. This is indicated by the number of cavities, the extent of the lesions, and severity incidence and molars affected, although statistically the data do not indicate significance. While the control males receiving testosterone had an increase in caries, the

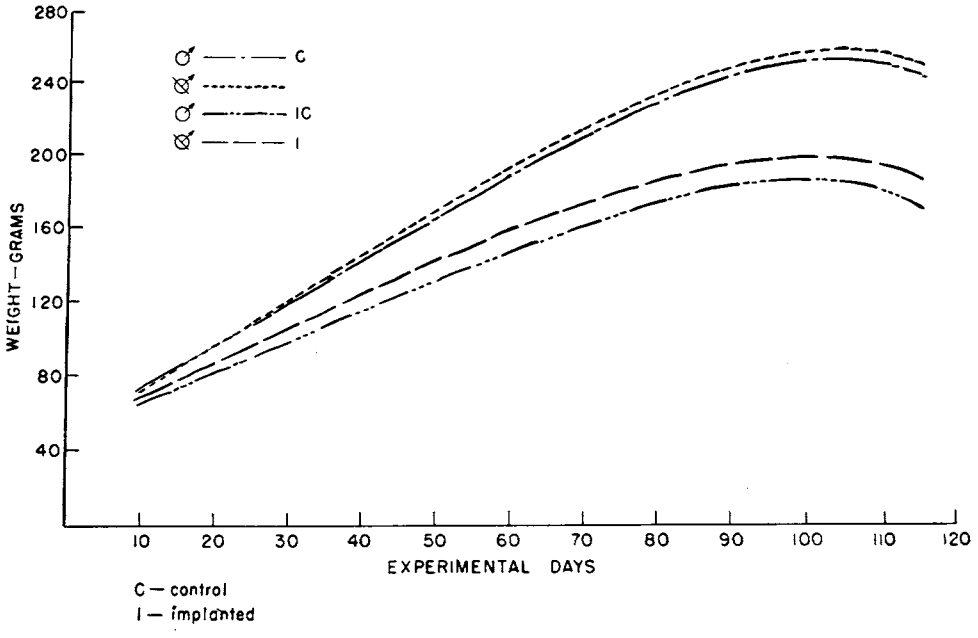
TABLE I

THE EFFECT OF ORCHIECTOMY AND OVARIECTOMY AND ADMINISTRATION OF TESTOSTERONE AND DIETHYLSTILBESTROL ON THE INCIDENCE OF DENTAL CARIES IN RATS RECEIVING A CARIES-PRODUCING DIET

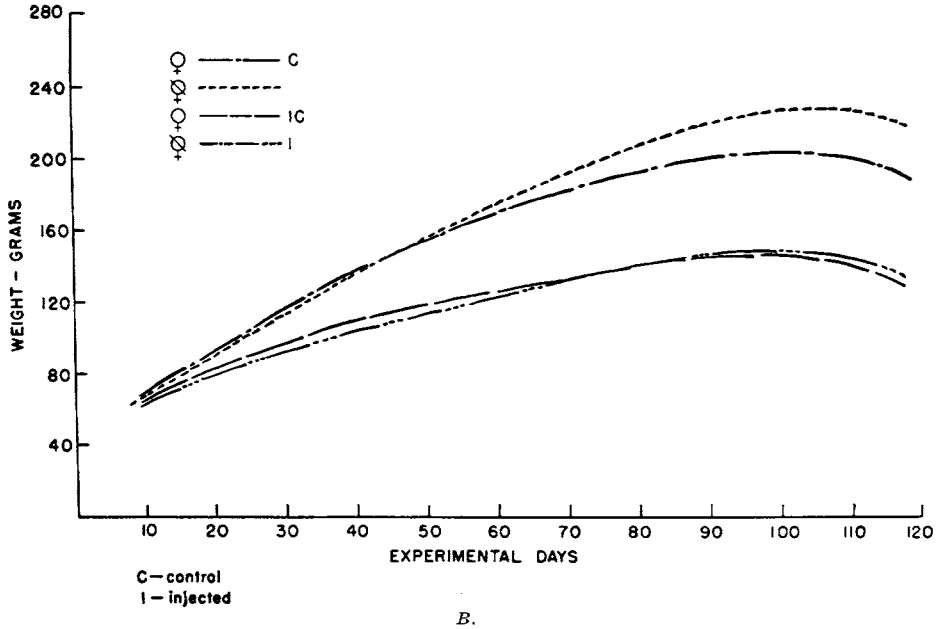
EXPERIMENTAL GROUP	NO. OF ANIMALS	NO. OF LESIONS	STANDARD DEVIATION	STANDARD ERROR OF THE MEAN	EXTENT	S.I.	M.A.
			Males				
Control	17	8.0	±0.93	0.23	1.82	10.40	4.70
Control+ Hormone	13	8.9	±0.69	0.19	2.12	11.82	5.15
Castrate	17	6.5	±1.28	0.31	1.27	8.21	4.31
Castrate+ Hormone	12	7.4	±0.80	0.23	1.71	10.30	4.50
			Females				
Control	16	7.1	±0.92	0.23	1.55	9.30	4.60
Control+ Hormone	12	6.5	±0.81	0.23	1.32	8.50	4.50
Castrate	17	8.4	±0.75	0.18	2.21	10.01	4.70
Castrate+ Hormone	13	9.0	±0.86	0.39	2.14	11.50	5.30

female controls receiving diethylstilbestrol have fewer lesions, smaller extent values and a lower caries incidence and molars affected. Thus, the decay pattern for castrated males receiving testosterone indicates fewer cavities as compared to the controls, while in the females the opposite situation occurred, there being a significant increase in caries in this group when compared with the control females. These data for the males corroborate the work of Keyes[4] on the hamster, but fail to substantiate the data for the female. This fact further indicates an inherent difference between the hamster and the rat in relation to their cariogenic activity. Our data in this laboratory using the Carl Wilson strain of rat have consistently shown a greater degree of caries in the male, while another strain (McCollum) has consistently shown the reverse.[5, 6] It is interesting that in this study, using the Carl Wilson strain, the control males again show at least a biological increase over the control females.

Our basic assumption that if castration should lower the incidence of decay, then the addition of the hormone might increase decay, appears to be true in the male but not in the female. These data compared with the discrepancy in the male and female castrate groups indicate that a more detailed study should be made pertaining to the effect of various hormones under different experimental conditions in the male and female rat before any definite conclusions are drawn.



A.



B.

Fig. 1.—The weight curves of the experimental animals. *A* indicates the weight changes in the four groups of male rates, while *B* indicates the increase in weight in the female animals.

A short discussion relative to the weights of the animals in each group should be presented. The initial mean weight values for the 8 groups of animals showed, at the maximum, a 4 Gm. difference between any 2 groups. By the end of the experimental period, during which bi-weekly weighings were made, the folllowing observations were noted: (1) no significant difference between control and castrated males; (2) a slight increase in weight of castrated females as compared to control females; (3) a marked diminution in weight of both males and females given testosterone and diethylstilbestrol, respectively, as compared to control males and females; (4) the same marked diminution in weight in castrated males and females given their respective hormones. In that large doses of these hormones have been shown to close prematurely the epiphysial center of ossification, this might account for a portion of the weight loss occurring in these animals. These comparisons are easily seen in Fig. 1.

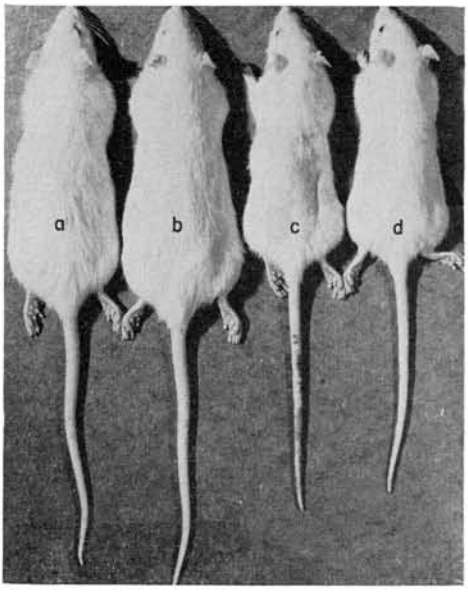


Fig. 2.—Comparison of females at termination of experimental period. *a* is control animal; *b*, castrate; *c*, castrate which has been injected with diethylstilbestrol; and *d*, control injected with diethylstilbestrol. Note loss of hair in animal *c*; skin in this group of animals is pigmented.

Figs. 2 and 3 demonstrate the gross weight changes indicated above, and in addition, show an additional experimental finding deserving some attention. In order to remove the ovaries, the hair on the dorsal surface of the back was removed. In those castrated females receiving the hormone the hair failed to return completely and, in addition, both the unoperated and castrated injected females had an extensive pigmentation of the skin. Similarly, in the castrated males the hair failed to grow back completely above the area operated upon for the implanting of the hormone, and the skin was also heavily pigmented. None

of the other animals presented this finding. Ginn and Volker[7] have indicated that rusting of the fur of the rat takes place in desalivated animals and correlate this finding with a faulty metabolism in some of the vitamins or sex hormones that were induced due to the removal of the salivary gland tissue.

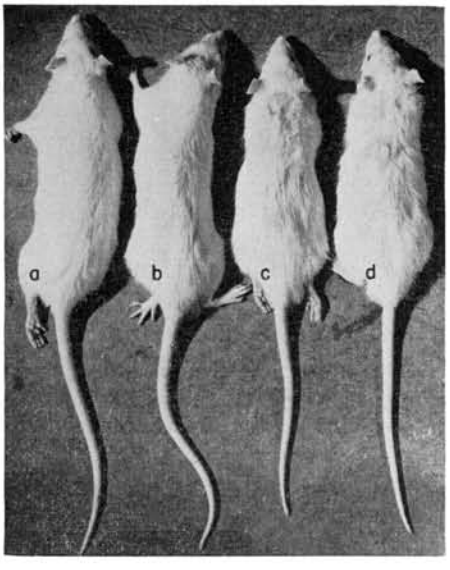


Fig. 3.—Comparison of males at termination of experimental period. *a* is control, *b* is castrate, *c* is castrate implanted with testosterone, and *d* is control implanted with testosterone. Note loss in hair in animal *c;* pigmentation of skin is marked in this group of animals.

CONCLUSIONS

1. The incidence of decay in immature orchiectomized rats is significantly less than control males, while ovariectomized rats show an increased amount of caries although this is not statistically significant.

2. The administration of testosterone to control males appears to increase decay, while the same hormone appears to reduce decay in gonadectomized males as compared to control rats. Conceivably, castration reduced the caries incidence in the male, but the hormone failed to return the decay incidence to normal.

3. The administration of diethylstilbestrol to control females appears to reduce the incidence of decay while similar treatment of ovariectomized animals significantly increases decay as compared to control animals.

4. Control females have less decay than control males, but the difference in the incidence of decay is not significant.

5. Failure to increase in weight in a normal manner was noted in both male and female castrate and control groups receiving testosterone and diethylstilbestrol, respectively.

6. Pigmentation of the skin and failure of hair growth on previously shaven areas were observed in castrated males and females receiving their respective hormones.

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