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SIZE CHANGES IN THE SEMINAL VESICLES OF THE MOUSE DURING DEVELOPMENT AND AFTER CASTRATION.

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I. Introduction.

The activity of testis hormone preparations might theoretically be demonstrated by reversing any of the various changes which follow castration, but the most hopeful results have naturally been obtained by restoring the structure or function of the accessory reproductive organs.

The sensitivity of the various tests which have been used is very different, but most of them appear to be satisfactory from a qualitative point of view. On the other hand, considerable difficulty has been experienced in developing methods of quantitative assay on mammals. The cytological appearance of the seminal vesicles, prostate and Cowper's glands of the rat and guinea-pig, and the motility of spermatozoa after castration, have all been used by the Chicago workers as qualitative or roughly quantitative indicators of activity, but for purposes of accurate assay they prefer the capon comb [Moore, Hughes and Gallagher, 1930; Moore, Price and Gallagher, 1930; Moore and Gallagher, 1930; Moore and Koch, 1932]. Voss and Loewe [1930, 1931], noting that cytological normality of the epithelium of the seminal vesicles was restored before macroscopic changes in the organ became appreciable, abandoned the attempt to use the gross size changes in favour of measurement of the height and mitotic activity of the vesicular epithelium. Martins and Rocha [1930], also working with mouse seminal vesicles as test objects, found them very variable in 16–21 g. mice, and used a length × breadth index to express their gross size. Laqueur and the Amsterdam school [Freud, de Fremery and Laqueur, 1932] and Schoeller and Gehrke [1931] appear to rely mainly on the capon

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comb technique. Korenchevsky [1932] writes: "An average increase of 40 p.c. in the weight of the prostate with seminal vesicles of the injected pair, as compared with the control pair, was suggested above as a quite definite proof of the activity of testicular extracts and perhaps as a basic figure for one rat unit."

Activity of a preparation may be demonstrated in two ways: (*a*) the atrophy or non-development of an organ or a function after castration may be prevented by administration of the hormone, or (*b*) atrophy already effected may be abolished by injection. The application of either principle to assay work is, however, complicated by the difficulty of assessing the physiological or morphological reactions in quantitative terms. This difficulty is due to the lack of a definite end-point in most of the reactions and to the great individual variation which is found both in the normal and atrophied organs and in capacity to respond to the hormone. Further, the gross size of the seminal vesicles or prostate, which could be most easily assessed quantitatively, appears to be the least responsive to the hormone. A practical disadvantage is that most of the tests involve killing the animals, which can therefore be used only once.

It is preferable that routine assay should be as simple and as rapid as possible, and it is clear that some method based on the gross size of the seminal vesicles would be highly desirable. Such a method is likely to require longer administration of the hormone than is necessary with more sensitive indicators, but in view of its increasing availability the larger amount of hormone required is not a serious drawback, and for laboratory routine the elimination of histological examination is a great convenience. As a further practical point it seemed desirable to devise a method in which the use of adequate numbers of animals would avoid the necessity of collecting litter mate controls at every test. It seemed essential, however, that any system of assay depending on the development, maintenance or restoration of the gross size of the seminal vesicles should be based both on a detailed investigation of the size of these organs in the normal male, and on the relation between the initial weight and the ultimate size after castration. The present paper records the results of this preliminary work.

II. Technique.

Mice were used from the albino colony previously maintained at University College, and now transferred to the National Institute for Medical Research. These have been colony-inbred for 11 years. Castration was performed in the ordinary way through the scrotum. At autopsy,

the animals were killed with chloroform, and the pelvic organs fixed whole in alcoholic Bouin's fluid. Preliminary fixation avoids the necessity of dissecting out the whole reproductive tract to prevent the loss of secretion which otherwise occurs when the glands or their ducts are cut in the fresh condition. After overnight fixation the glands were transferred to 70 p.c. alcohol, dissected out and weighed. The seminal vesicles of the mouse are

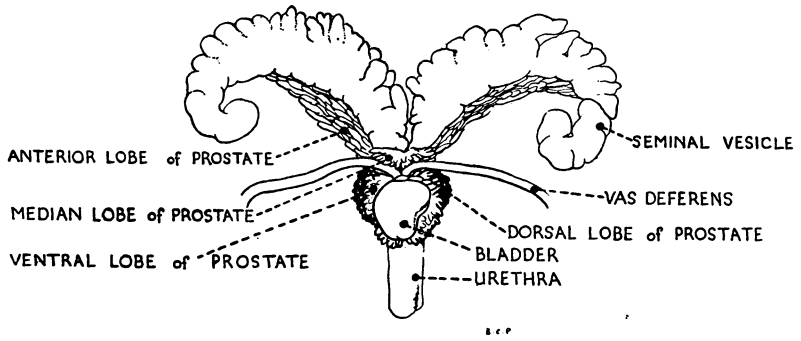

Fig. 1 A.

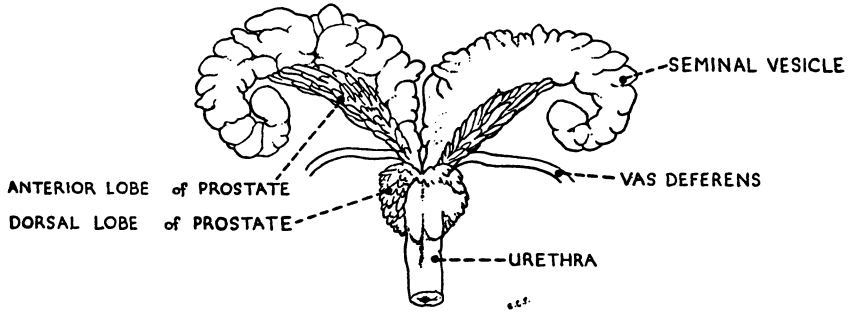

Fig. 1 B.

Fig. 1. A, ventral view of seminal vesicles, prostate and bladder of mouse; B, dorsal view of same region.

comparatively free in the abdominal cavity, being anchored only where they enter the urethra. The prostate (Fig. 1 A and B) consists of paired anterior lobes and a small inconspicuous median lobe attached to the base of the seminal vesicles, together with paired ventral lobes behind the neck of the bladder and paired dorsal lobes. The gland differs from that of the rat in the larger proportionate size of the anterior lobes, which in the mouse constitute about two-thirds of the total prostate. In the rat and guinea-pig the anterior lobes are said to differ histologically and function-

ally from the rest of the prostate, and to regress rather less rapidly after castration [see Moore, Price and Gallagher, 1930]. Complete separation of the prostate requires careful dissection even in the rat [Korenchevsky, 1932]; in the mouse it is difficult. When the glands are fixed *in situ*, however, there is little to be gained by taking the whole prostate. As a rapid and easily reproducible dissection we have therefore made a clean cut just in front of the bladder at the level of the insertion of the vasa deferentia, thus removing the whole of the seminal vesicles, and also the anterior lobes of the prostate. When the glands are fully grown, the prostate comprises up to 20–30 p.c. of the weight of the total prostate-vesicle complex. Since our technique removes only the anterior lobes of the prostate, only 15–20 p.c. of the removed tissue is prostate where the glands are fully grown. For brevity in this paper, the term "seminal vesicles" is used to denote all the removed tissue. Owing to the more fibrous nature of the prostate, its size atrophy after castration is proportionately less than that of the seminal vesicles, so that the atrophied glands include a rather larger percentage of prostate.

Statistical methods. The errors given are standard errors which, together with the coefficients of correlation, regression formulæ, etc., have all been calculated in the ordinary way. The abbreviation s.v.w. is used throughout for "weight of the seminal vesicles."

III. S.V.W. IN RELATION TO BODY WEIGHT.

Fig. 2 shows s.v.w. plotted against body weight in 104 mice ranging from 14 to 32 g. A glance at the distribution of these points shows that the size increase has three phases. By 19 g. body weight the glands are very variable and often still insignificant; a period of rapid development then sets in, and by 24 g. body weight something approaching full size is attained. There is only a slight rise in s.v.w. as the body weight increases above 24 g. In view of these definite indications, the data were treated by calculating regression formulæ for the s.v.w. at the body-weight groups, 14–19 g., 19–24 g., 24–32 g. The results are given in Table I, which shows

TABLE I.

Body weight of group (g.)	Mean weight of seminal vesicles (mg.)	Coefficient of variation $\left(\frac{\sigma}{\text{mean}} \times 100\right)$	Coefficient of correlation	Regression formulæ for weight of seminal vesicles mg. (y) in terms of body weight g. (x)
14–19	42·5	66·3	0·454	$y = 8{\cdot}98\,x - 114{\cdot}9$
19–24	122·7	55·5	0·749	$y = 31{\cdot}88\,x - 543{\cdot}9$
24–32	246·3	30·5	0·219	$y = 7{\cdot}99\,x + 32{\cdot}5$

for each body-weight group, the mean s.v.w., the variability expressed by the standard deviation as percentage of mean, the weight of the seminal vesicles mg. (y) in terms of body weight g. (x), and the coefficient of correlation between the two.

The practical conclusion to be drawn from these results is that in mice of 24 g. and over, variation in s.v.w., though still considerable, is less than in smaller mice, and the average increase as body weight becomes greater is comparatively slight; the average s.v.w. considered as p.c. body weight is roughly constant. It follows that mice of 24 g. body weight or over are

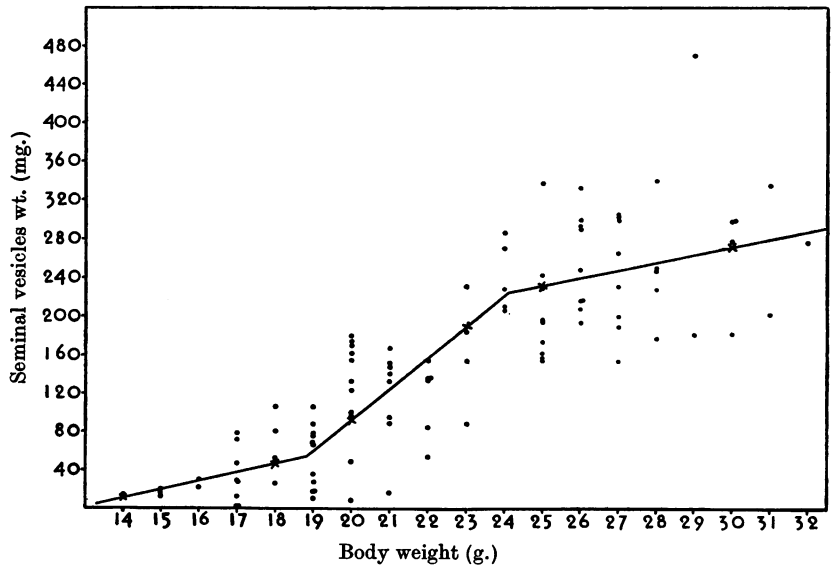

Fig. 2. Weight of the seminal vesicles of the mouse plotted against body weight.

the most homogeneous adults for experimental work involving seminal vesicle size. We have not ascertained to what extent, if any, the s.v.w. in mice above 24 g. is correlated with age or sexual activity, but we have observed a tendency for the vesicles of old mice to be small for their body weight. All of the mice dealt with here had been isolated from females.

IV. S.V.W. AFTER PRE-PUBERTAL CASTRATION.

Fig. 2 indicates that there is no appreciable development of the seminal vesicles below 14 g., corresponding to an age of 4–5 weeks. Twenty mice castrated below this age and weight were killed 8–12 months later. The s.v.w., in so far as it could be determined, varied between 1 and 3 mg.

With such small organs the actual arithmetic mean of our figures (1·8 mg.) serves only as an indication, and for practical purposes the S.V.W. after pre-pubertal castration can be taken as 2 mg. The use of the undeveloped seminal vesicles as test objects will be discussed in a later paper.

V. S.V.W. AFTER POST-PUBERTAL CASTRATION.

The atrophy of the seminal vesicles after post-pubertal castration was studied in 182 mice, varying in body weight from 20 to 33 g. at the time of castration. They were killed in groups of approximately 20 at times ranging up to 15 weeks' post-castration. Most of the mice were over 24 g. at the time of castration, but two groups of mice of 20–23 g. were included for comparison. The data are given in Table II, in which the

TABLE II. Post-castration atrophy of the seminal vesicles.

	Group 24–33 g. body weight			Group 20–23 g. body weight		
Days after castration	No. of mice	Average body wt. g.	Average seminal vesicle wt. mg.	No. of mice	Average body wt. g.	Average seminal vesicle wt. mg.
0	45	27	246	29	21	126
7	21	28	129	—	—	—
14	20	25	70	—	—	—
21	21	25	44	—	—	—
24	—	—	—	15	21	20
35	23	26	34	—	—	—
52	22	26	22	—	—	—
70	19	27	26	—	—	—
78	—	—	—	22	21	10
105	19	27	18	—	—	—

two groups "0 days" after castration represent normal material taken from Fig. 1. In the 24–33 g. body-weight group it will be seen that the average weight for normal seminal vesicles sinks very rapidly after castration, about one-half of the weight being lost by the end of the first week. The decrease in weight seems to be caused chiefly by the loss of secretion from the seminal vesicles which accompanies the cytological degeneration described by Moore, Hughes and Gallagher [1930]. These workers find that almost the maximum amount of degeneration of the rat seminal vesicles is attained 10 days after castration. In the mouse atrophy is far advanced at 3 weeks after castration, but a further slight decrease takes place up to 7 weeks. Beyond this time there seems to be no further significant size change in the seminal vesicles. The top line in Fig. 3 shows the curve given by these results.

The 20–23 g. body-weight group shows a similar state of affairs, so far

as we have determined the curve. The seminal vesicles start at almost exactly half the size of those of the heavier mice. At 24 days after castration they weigh rather less than one-half those of the heavier mice, and at 78 days much the same relation is found (Fig. 3, bottom line). The weight of the glands at any given time after castration would thus appear to be roughly proportional to their original weight.

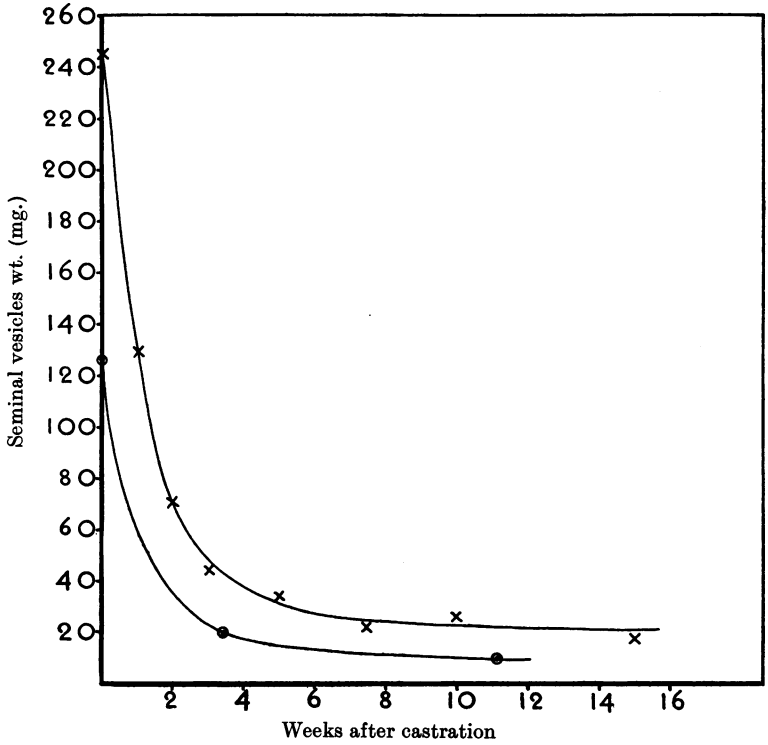

Fig. 3. Atrophy of the seminal vesicles after post-pubertal castration.

This relationship could be analysed in another way. We have shown (Table I) that there is a correlation in normal mice between body weight and s.v.w. A correlation between body weight at castration and the s.v.w. at any given time afterwards would almost certainly indicate a correlation of atrophied s.v.w. with original s.v.w. One would expect to be able to demonstrate this most easily on the smaller mice with the high correlation between body weight and s.v.w., but since we have concentrated on the heavier mice our material is inadequate to deal with this aspect of the question.

VI. Discussion.

The results described above show that, in spite of considerable variability, the atrophy of the seminal vesicles of the mouse after post-pubertal castration appears to be comparatively regular when studied on groups of about 20 mice, and it may therefore be used as a basis of quantitative assay of the testis hormone. There can be little doubt that the heavier mice offer the best possibilities for this work. As shown in Fig. 3, the percentage decrease in size of the small seminal vesicles of the 20–23 g. mice is as great as that occurring in the larger ones of the 24–33 g. animals, but two facts make the latter preferable, (*a*) the much smaller initial variation in the weight of the glands and its low correlation with body weight (see Table I), and (*b*) the greater accuracy of preparing and weighing the larger glands.

For the 45 normal mice of the 24–33 g. group the mean s.v.w. is 246 ± 11 mg. At 7 days after castration the mean s.v.w. in 21 mice of the same weight group is 128 ± 16 mg. The difference, 118 ± 19 mg., is highly significant. The mean s.v.w. for the groups of mice at later stages after castration have errors of about the same order, and the greater differences of the means from the pre-castration size are correspondingly more significant. At 15 weeks after castration the mean s.v.w. is 18 ± 2 mg., the difference from normal being 228 ± 11 mg. Allowing for a similar order of variability and for using groups of mice of about the same size, statistically significant results could be obtained by the elimination of (1) 33 p.c. of the atrophy at 7 days after castration, or (2) 20 p.c. of the atrophy at 15 weeks after castration; such results could be used as a basis of testis hormone assay. The first would involve starting injections immediately after castration, with the aim of preventing the usual degree of atrophy; the second would constitute the partial or entire restoration of the already atrophied glands. The relative merits of these two procedures from the point of view of assay will be dealt with in a later paper.

VII. Summary.

1. The normal size increase and post-castration atrophy of the seminal vesicles and attached anterior lobes of the prostate have been studied in 286 albino mice.

2. At 14 g. body weight the seminal vesicles are still minute, and in mice castrated below this weight the s.v.w. is only about 2 mg.

3. The seminal vesicles of the mouse undergo their main enlargement

at 19–24 g. body weight; over this range their size shows a high correlation (0·749) with body weight. In mice above 24 g. the glands are less variable and show a low correlation with body weight (Table I and Fig. 2).

4. The average weight of the seminal vesicles (including the anterior lobes of the prostate) from 45 mice of 24–33 g. body weight was 246 mg., that of the glands of 29 mice of 20–23 g. body weight was 126 mg.

5. After post-pubertal castration, the glands lose about half their weight within 7 days, and then gradually sink to a nearly constant level of less than 10 p.c. of their original weight (Fig. 3).

6. There is a reasonable hope that the gross size of the mouse seminal vesicles may be used as a basis for assaying the testis hormone, provided that adequate numbers are used to allow for the considerable variation.

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