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Androgen and the *myo*-Inositol Content of Male Accessory Organs of the Rat.* (23492)

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The function of inositol in animal and plant physiology has been the subject of many investigations since its discovery more than one hundred years ago by Scherer(1). Inositol is required for growth of certain yeasts(2) and mice(3), and a dietary deficiency of this factor in hamsters is associated with reproductive disturbances(4). The importance of inositol as a lipotropic substance has been established(5). Furthermore, it has been demonstrated that *myo*-inositol is an essential growth factor for normal and malignant human cells in tissue culture(6,7). Mann(8) has reported the occurrence of relatively large quantities of free inositol in the seminal vesicles of the boar. Inositol was present to the extent of 2 to 3 g per 100 ml of seminal vesicle secretion.

The investigation presented here deals with the *myo*-inositol content of the male accessory organs of the rat during growth and the influence of androgen on the *myo*-inositol content of these organs. The significance of inositol in male accessory organs and secretions is obscure. It may be involved in the carbohydrate metabolism of these organs. Stetten and Stetten(9) demonstrated that deutero-labeled inositol is converted to glucose by the rat. Daughaday *et al.*(10) using glucose uniformly labeled with C^{14} showed that in the immature rat and chick embryo endogenous synthesis of inositol occurs.

Methods. Male rats of the Holtzman strain were used in this investigation and were fed Purina Laboratory Chow and raw carrots. The seminal vesicles, coagulating glands, dorsal and ventral prostates, and Cowper's glands were used in this study. Secretion-free organs were prepared by splitting glands lengthwise and removing the secretion by blotting on filter paper. Bilateral castration was performed on animals in which the effects of gonadectomy and androgen replacement were studied. A period of at least 20 days was allowed following castration for the regression of the male accessory organs. Testosterone propionate in oil† was given subcutaneously in daily doses of 500 μg. The selective uptake of *myo*-inositol by the seminal vesicles and dorsal prostates of castrate rats was studied. *Myo*-inositol was administered by stomach tube (25 mg per 100 g body wt) to fasting castrate and hormone-treated castrates 12, 8 and 4 hours before they were sacrificed. *Myo*-inositol was isolated and

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† Peranderen, Ciba Pharmaceutical Products, Summit, N. J.

TABLE I. Effect of Acid Hydrolysis on Recovery of *Myo*-inositol Added to Rat Accessory Organs.

Organ	Sample	μg *myo*-inositol Added	Sample	Recovered	% recovery	Avg % recovery
Seminal vesicles	1	0	9161			
	2	1400	10492	1331	95	
Coagulating glands	1	0	1400			
	2	2800	4140	2740	98	
Dorsal prostates	1	0	3450			
	2	1000	4550	1100	110	101

identified from pooled accessory organs of mature rats using the method of Halliday and Anderson(11). The isolated compound gave a melting point of 222-223°C. Furthermore, *myo*-inositol hexacetate (mp 217°C) was prepared from the isolated *myo*-inositol. Microbiological assays for inositol were carried out using *Neurospora crassa* 37401 according to a procedure described by Beadle(12). Accessory organs to be assayed were removed from the animals, weighed and hydrolyzed in 4N sulphuric acid in an autoclave for 2 hours at 15 lb pressure. Hydrolyzates were adjusted to a pH of 5.5 with sodium hydroxide and diluted to volume prior to assay. Recovery assays were carried out in which different levels of *myo*-inositol were added to samples of seminal vesicles, coagulating glands, and dorsal prostates before hydrolysis. These data are presented in Table I. It is noted that the average recovery was 101%. These data would suggest that little, if any, *myo*-inositol is lost during the hydrolysis procedure. During this investigation it was found that the *myo*-inositol present in the male accessory organs of the rat is available to *Neurospora crassa* without prior acid hydrolysis. Tissue may be prepared for assay by homogenization and subsequent centrifugation or filtration or dialysis.

Results. Animals were selected at different stages of maturity to furnish data in the *myo*-inositol content of rat male accessory organs during development. These data are presented in Table II. The effects of castration and androgen replacement on the *myo*-inositol content of rat accessory organs are presented in Tables III and IV, respectively. Data are presented in Table V showing the influence of androgen replacement and stomach-feeding of *myo*-inositol on the seminal vesicles and dorsal prostates of the castrate rat.

Discussion. The data presented in Table II show increases in weights and *myo*-inositol contents of male accessory organs during growth of the rat. The marked differences noted between Groups 4 and 5 occurred at the time of increased gonadal androgen production at puberty. The *myo*-inositol concentration increased during growth of the seminal vesicles, coagulating glands and dorsal prostates whereas a decrease in concentration was noted in the dorsal prostates and Cowper's

TABLE II. Changes in *Myo*-inositol Content of Male Accessory Organs during Growth.

		Seminal vesicles		Coagulating glands		Dorsal prostates		Ventral prostates		Cowper's glands	
Animals per group	Body wt (g)	Wt (mg)	Inositol (μg)	Wt (mg)	Inositol (μg)	Wt (mg)	Inositol (μg)	Wt (mg)	Inositol (μg)	Wt (mg)	Inositol (μg)
10	63*	8*	20*	3*	10*	13*	58*	29*	75*	1*	2*
10	88	12	26	5	14	18	81	42	97	2	3
10	122	22	59	7	29	26	136	46	135	3	5
10	147	27	69	10	49	32	162	59	162	5	7
5	190	90	269	23	87	57	332	119	261	19	21
5	219	271	763	55	203	101	576	174	366	33	31
5	262	466	1613	72	275	112	672	225	463	42	42
5	385	945	5239	151	664	244	1704	594	978	67	44

* Mean.

TABLE III. Effect of Castration on Weight and *Myo*-inositol Content of Secretion-Free Male Accessory Organs of the Rat.

Days after castration	Animals per group	Seminal vesicles Wt (mg)	Seminal vesicles Inositol (μg)	Coagulating glands Wt (mg)	Coagulating glands Inositol (μg)	Dorsal prostates Wt (mg)	Dorsal prostates Inositol (μg)	Ventral prostates Wt (mg)	Ventral prostates Inositol (μg)	Cowper's glands Wt (mg)	Cowper's glands Inositol (μg)
0	7*	218 ± 7†	520 ± 41	65 ± 3	226 ± 28	108 ± 10	790 ± 64	181 ± 14	327 ± 61	29 ± 3	40 ± 9
2	5	158 ± 11	426 ± 68	49 ± 2	225 ± 21	92 ± 6	564 ± 30	118 ± 17	326 ± 56	22 ± 1	27 ± 5
10	10	116 ± 10	221 ± 13	31 ± 3	90 ± 11	55 ± 5	233 ± 31	40 ± 4	98 ± 14	25 ± 1	12 ± 1
20	5	62 ± 1	145 ± 8	17 ± 1	41 ± 2	25 ± 2	90 ± 13	27 ± 2	61 ± 5	8 ± 1	

* Mean wt animals 319 ± 5 g. † Mean ± S.E.

TABLE IV. Effect of Testosterone Propionate on Weight and *Myo*-inositol Content of Accessory Organs of Castrate Rats.

Days of hormone inj., 500 μg daily	Animals per group	Seminal vesicles Wt (mg)	Seminal vesicles Inositol (μg)	Coagulating glands Wt (mg)	Coagulating glands Inositol (μg)	Dorsal prostates Wt (mg)	Dorsal prostates Inositol (μg)	Ventral prostates Wt (mg)	Ventral prostates Inositol (μg)	Cowper's glands Wt (mg)	Cowper's glands Inositol (μg)
0	5*	83 ± 6†	161 ± 7	24 ± 2	55 ± 7	45 ± 4	149 ± 7	35 ± 3	66 ± 10	12 ± 1	7 ± 1
5	5	390 ± 29	1646 ± 255	107 ± 5	651 ± 63	151 ± 8	1209 ± 103	136 ± 15	354 ± 33		
10	5	974 ± 33	7233 ± 732	160 ± 7	957 ± 47	232 ± 17	1577 ± 100	374 ± 31	970 ± 81	49 ± 5	39 ± 8
20	5	1540 ± 108	11613 ± 1607	236 ± 4	1539 ± 38	391 ± 37	3471 ± 147	631 ± 34	1513 ± 134	76 ± 7	52 ± 7

* Mean wt 340 ± 79 g. † Mean ± S.E.

glands. The average concentration of *myo*-inositol in the accessory organs expressed as μg/g of tissue in sexually mature males (Group 8) was as follows: seminal vesicles 5544; coagulating glands 4397; dorsal prostates 6984; ventral prostates 1646 and coagulating glands 657. The concentrations of *myo*-inositol in the seminal vesicles, coagulating glands and dorsal prostates are higher than that reported for other tissues of the rat. For example, Mitchell and Isbell(13) have reported the following concentrations of inositol for various male rat organs expressed as μg/g liver 640; kidney 1300; spleen 1000; heart 650; lung 960; brain 1800 and muscle 220.

Marked decreases were observed in the weight and *myo*-inositol content of secretion-free male accessory organs following castration as shown by the data in Table III. The concentration of *myo*-inositol remained approximately the same in the seminal vesicles and ventral prostates but decreased in the coagulating glands, dorsal prostates and Cowper's glands. The *myo*-inositol concentration of the seminal vesicles and ventral prostates was higher 2 days following castration than in the normal animal indicating that the inositol content of these organs does not decrease as rapidly as does the organ weight.

Testosterone propionate (500 μg per day) was administered to groups of 20-day castrate males for periods of 5, 10 and 20 days. The data are presented in Table IV. The weights of male accessory glands and their *myo*-inositol content returned to normal following 10 days of hormone injection. However, after 20 days of treatment the weights of the seminal vesicles had increased 18 times, coagulating glands 10, dorsal prostates 8, ventral prostates 18 and Cowper's glands 6. During the same period the *myo*-inositol increased 72 times in the seminal vesicles, 28 in the coagulating glands, 23 in the dorsal prostates, 23 in the ventral prostates and 7 in the Cowper's glands. A comparison of the data obtained from castrates receiving androgen for 20 days (Table IV) with that from mature, intact males (Group 8, Table II) indicates that the weights and *myo*-inositol contents of the seminal vesicles, coagulating glands, dorsal prostates and ventral prostates of the hormone treated

TABLE V. Effect of Androgen and Feeding of *Myo*-inositol on *Myo*-inositol Concentration in Seminal Vesicle and Dorsal Prostate Tissues of Castrate Rats.

Animal wt (g)	Animals per group	Treatment†		Seminal vesicles		Dorsal prostates	
		Testosterone propionate	Inositol	Wt (mg)	Inositol (μg/g)	Wt (mg)	Inositol (μg/g)
389 ± 4*	9	—	—	63 ± 1*	1570 ± 133*	31 ± 2*	2058 ± 162*
388 ± 10	9	—	+	61 ± 3	1705 ± 148	30 ± 2	2439 ± 188
376 ± 6	9	+	—	148 ± 9	1394 ± 110	70 ± 3	2591 ± 199
406 ± 6	9	+	+	155 ± 7	1606 ± 95	72 ± 5	3489 ± 227

* Mean ± S.E.

† Testosterone propionate, 500 μg, 60, 36 and 12 hr before killing; *myo*-inositol, 25 mg/100 g wt, 12, 8 and 4 hr before killing.

animals were greater than those of the intact males.

The *myo*-inositol present in male accessory glands may be synthesized by these organs as well as selectively accumulated from the blood. In the latter case, it may be produced by bacterial action in the alimentary tract or synthesized in other organs. Halliday and Anderson (11) isolated *myo*-inositol-C^{14} from carcasses of rats which had received injections of glucose-1-C^{14}. Data on the selective uptake of *myo*-inositol by the seminal vesicles and dorsal prostates of castrate animals are presented in Table V. The *myo*-inositol contents of the seminal vesicles and dorsal prostates of the *myo*-inositol fed group were not significantly different from the control group not receiving this substance. However, when similar *myo*-inositol fed animals were given androgen, the dorsal prostates showed a significant increase ($P = 0.01$) in concentration. There was no significant increase in *myo*-inositol concentration in the seminal vesicles of hormone treated animals receiving *myo*-inositol. It is of interest that Gunn *et al.*(14) have observed that the dorsolateral prostate of the rat concentrates Zn^{65} from 15 to 25 times more than any other organ.

Summary. The *myo*-inositol content of male accessory organs was determined in growing, adult, castrate and androgen treated castrate rats. The *myo*-inositol concentration (μg/g/gland) was 5544 in the seminal vesicles, 4397 in coagulating glands, 6984 in dorsal prostates, 1646 in ventral prostates and 657 in Cowper's glands of mature animals. The *myo*-inositol content of the male accessory organs is dependent upon androgen. This relationship may be either a direct effect of male hormone on *myo*-inositol synthesis or an indirect response resulting from its action upon the total activity of the male accessory organs. It was demonstrated that the dorsal prostates can selectively accumulate *myo*-inositol in androgen treated castrate rats.

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