TABLE I.
Effect of Isuprel upon Level of Blood Glucose at the End of 2 Hours in Eviscerate Rats Given Glucose and Insulin by Constant Injection.

Exp.	Conc. isuprel	No. rats	Avg blood glucose	Avg*	Diff. in avg	Diff.*	Diff. / diff.*
1	1:25,000	12	161	7.9			
	0	12	84	6.9	77	10.5	7.3
2	1:10,000	12	185	9.1			
	0	12	84	5.2	101	10.5	9.6

* Standard deviation.

of isuprel in concentrations of 1:10,000 and 1:25,000 caused a marked decrease in the glucose tolerance of the eviscerate rats.

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17189. Effect of Testosterone Propionate on Phosphatases in the Seminal Vesicle and Prostate of the Rat.*

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The discovery by Kutscher and Wolbergs[1] that the human prostate gland and ejaculum contain high concentrations of acid phosphatase provided the stimulus for a series of investigations of this enzyme in the tissues and secretions of the male reproductive tract.

Gutman and Gutman[2] showed that the high level of acid phosphatase (AcP-ase) as well as alkaline phosphatase (Alp-ase) in the prostatic tissue of the adult rhesus monkey is not present in the prepubertal animal, but that these enzymes can be raised to the adult level by the injection of androgen.

Pazos and Huggins[3] found that administration of androgen to starving, castrate immature dogs caused an 8 to 11-fold increase in AcP-ase activity of the prostate gland over that of a control animal. The Alp-ase in the glands of these dogs decreased 15-45%.

When Gutman and Gutman[4] studied the rat, however, they found that the levels of both AcP-ase and AlP-ase in the prostate gland were extremely low compared to levels in the primates studied. Administration of testosterone to normal adult male rats produced no change in the activity of either enzyme.

Atkinson[5] has shown histochemically that the AlP-ase in the stromal elements of the mouse seminal vesicle diminishes after castration and returns to normal after androgen treatment. Dempsey, Greep, and Deane[6] recently demonstrated the same phenomenon in the rat, also by histochemical means.

It was decided to conduct investigations to (1) determine whether an enzymatic response to testosterone could be obtained in the

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1 Kutscher, W., and Wolbergs, H., *Z. physiol. Chem.*, 1935, **236**, 237.

2 Gutman, A. B., and Gutman, E. B., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 277.

3 Pazos, R., and Huggins, C., *Endocrinology*, 1945, **36**, 416.

4 Gutman, A. B., and Gutman, E. B., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 529.

5 Atkinson, W. B., *Anat. Rec.*, 1948, **100**, 731.

6 Dempsey, E. W., Greep, R. O., and Deane, H. W., *Endocrinology*, 1949, **44**, 88.

TABLE I.
Changes in Seminal Vesicle Following Castration and Treatment with Testosterone Propionate.

Experimental stage	No. of rats*	Wt of one seminal vesicle (mg)	AcP-ase†	AlP-ase†	Dry wt (%)
Normal	4	160 (150-167)	17 (16-18)	34 (22-46)	20
Castrate 4 days	3	120 (112-130)	13 (11-15)	25 (20-29)	19
" 8 "	3	80 (77-86)	12 (11-14)	17 (13-20)	22
Testosterone 4 days	3	375 (227-522)	14 (12-17)	33 (31-34)	20
" 8 "	3	275 (273-279)	18 (14-22)	33 (28-37)	21

* Pertains to enzyme values only. Gland weights and dry weights taken from two rats in each group.

† Expressed as Huggins-Talalay units per 100 mg dry tissue. Figures in parentheses indicate ranges.

rat prostate similar to that obtained in the monkey and in the dog, (2) to provide quantitative support for the histochemical findings on the AlP-ase of the seminal vesicle, and (3) to provide information on the hitherto uninvestigated AcP-ase in the seminal vesicle. An experiment was designed, therefore, to study both AcP-ase and AlP-ase in homogenates of prostates and seminal vesicles of normal rats, castrate rats and in castrate rats receiving testosterone propionate.

Methods. The animals used were male rats of the Sprague-Dawley strain, 2½ to 3 months old. The rats were divided into 3 groups: (1) Normal animals, untreated; (2) Castrates, untreated; (3) Castrates injected subcutaneously with 600 γ testosterone propionate per day in 2 daily doses. Animals of groups (2) and (3) were killed 4 and 8 days following castration.

At autopsy the ventral lobe of the prostate was removed and weighed. The tissue was laid on moist, hard-surfaced filter paper and minced with a scalpel blade to rupture the majority of the gland lobules and permit escape of the contained secretion. The chopped tissue was washed in cold glass-distilled water, blotted on filter paper, weighed, and transferred to a chilled glass homogenizing tube. One seminal vesicle was removed and separated from the adherent coagulating gland; the secretion was expressed, and the gland was washed with cold saline solution, blotted, and transferred to an homogenizing tube.

Tissues were homogenized in glass distilled water, one per cent homogenates being used throughout the study. Solids and water content of the tissues were determined by drying pieces of tissue or homogenates at 75°C for 24 hours.

The Huggins-Talalay method[7] of phosphatase estimation, modified as previously reported,[8] was used on all tissues. This method measures phenolphthalein liberated from phenolphthalein phosphate. Both AcP-ase and AlP-ase were determined on tissues from at least three animals in all stages studied. The sequence in which the experiments were made was randomized in order to distribute any variation caused by minor differences in technic and condition of the animals due to seasonal factors.

Results. Seminal Vesicle. After castration the seminal vesicle rapidly loses weight, undergoing a 25% reduction in 4 days and reaching a level 50% below the pre-castration level by 8 days. The AlP-ase activity is reduced 30% in the first 4 days after castration, reaching a level 50% below the normal by the eighth day. The proportionality between weight loss of the gland and loss of enzyme activity is striking. Enzyme activity is expressed in terms of activity per unit weight of dry tissue, however, and the reduction in activity is real, not merely a reflection of the reduction in size of the gland.

The AcP-ase follows a trend slightly different from the gland weight and the AlP-ase, being reduced in activity by 25% after 4 days of castration, then undergoing very little further reduction through the eighth day.

[7] Huggins, C., and Talalay, P., *J. Biol. Chem.*, 1945, **159**, 399.

[8] Stafford, R. O., McShan, W. H., and Meyer, R. K., *Endocrinology*, 1947, **41**, 45.

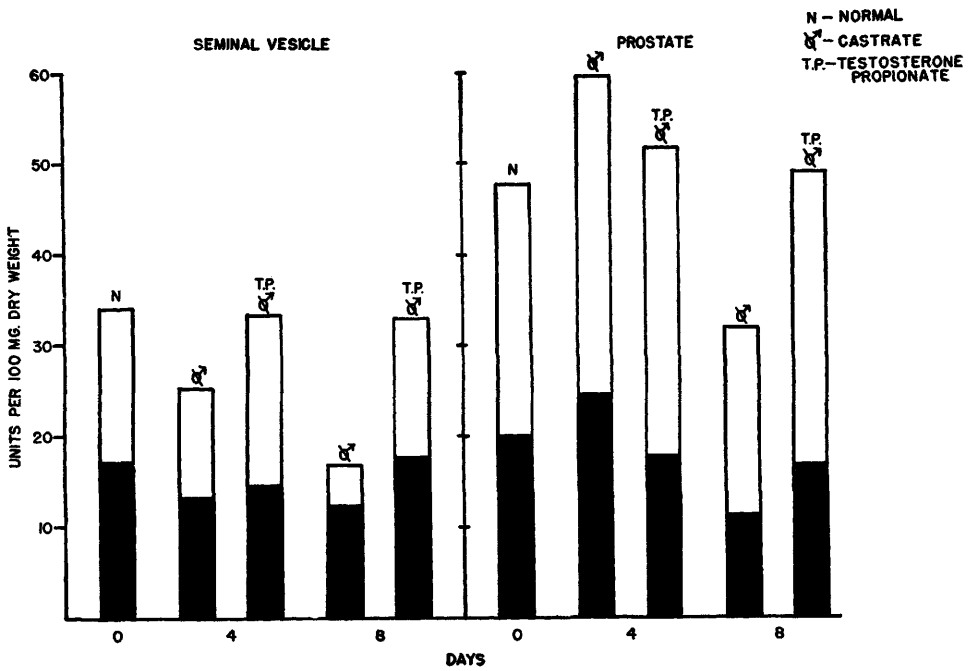

FIG. 1.

Changes in phosphatase activity produced by castration and treatment with testosterone propionate. AlP-ase activity is represented by total height of the bars. AcP-ase activity is represented by the black portions of the bars.

In the castrate animals receiving testosterone propionate, the androgen largely prevents the castration changes in weight and enzyme activity. The AlP-ase is maintained at almost exactly the same level in the androgen-treated animals as in the normals, while the AcP-ase activity is fully restored to the normal level by the eighth day of treatment. These data are presented in Table I and Fig. 1. The results clearly show that deprivation of androgen results in a loss of activity of both phosphatases in the seminal vesicle and that replacement therapy with testosterone propionate restores the activity.

Prostate Gland. The enzyme pattern in the prostate is similar to that obtained in the seminal vesicle with one major difference. While the castration effects are manifested in the seminal vesicle by the fourth day after castration, in the prostate the effects are not clearly shown until the eighth day. There is greater variability in the prostate values, probably caused by the variation in amount of secretion present in the tissues, as the technic for removal of the secretion may not be completely effective.

By the fourth day after castration, 2 out of 3 animals did not show significant losses of weight in the ventral lobe of the prostate, while the AcP-ase and AlP-ase actually increased in activity by 20 and 25% respectively. By the eighth day, however, the weight of the gland was about one-third of the normal weight and the AcP-ase and AlP-ase activities were reduced by one-half and one-third respectively.

When testosterone propionate was administered to the castrate animals, the AlP-ase of the gland was restored to normal and the AcP-ase activity was partially restored. The dose of testosterone propionate used was sufficient to cause great prostatic hypertrophy by the eighth day. These data are presented in Table II and in Fig. 1.

Histochemical treatment (Gomori[9]) of the prostate to demonstrate localization of the AlP-ase revealed a possible reason why

[9] Gomori, G., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 23.

TABLE II.
Changes in Prostate Gland Following Castration and Treatment with Testosterone Propionate.

Experimental stage	No. of rats*	Wt of ventral prostate (mg)	AcP-ase†	AlP-ase†	Dry wt (%)
Normal	7	327 (303-366)	20 (14-28)	48 (30-64)	16
Castrate 4 days	6	266 (168-318)	24 (18-33)	59 (53-64)	15
" 8 days	6	103 (75-140)	11 (7-13)	32 (25-48)	22
Testosterone 4 days	6	434 (376-487)	18 (17-21)	51 (38-76)	16
" 8 "	6	728 (608-881)	17 (12-23)	50 (42-62)	17

* Pertains to enzyme values only. Gland weights and dry weights determined on 3 animals in each group.

† Expressed as Huggins-Talalay units per 100 mg dry tissue. Figures in parentheses indicate ranges.

the prostatic AlP-ase increases in concentration under the influence of androgen. The enzyme is localized in the epithelial elements of the gland, a positive phosphatase response being found at the basal and luminal borders of the epithelial cells. Since the androgen is trophic chiefly to the epithelium in this gland, causing an increased epithelial height, it is readily seen that androgenic stimulation should cause a relative increase in that amount of enzyme activity per unit weight of tissue.

Discussion. These data indicate that, contrary to previous belief,[4] the phosphatases in the prostate gland of the rat respond to androgen in a manner similar to that in the monkey and the dog. The quantitative data on the seminal vesicle presented here confirm the histochemical observations of Atkinson[4] and of Dempsey *et al.*[5] on the rat.

Davis, Meyer, and McShan[10] have shown that the succinic dehydrogenase (SDH-ase) and cytochrome oxidase in the prostate and seminal vesicle of the rat vary with the androgen supply in a manner somewhat similar to that shown here for the phosphatases. They found that in the castrate animal, the SDH-ase activity drops off as the weight of the glands decreases and returns when testosterone is administered. There is, however, one important difference, in the response of the two sets of enzymes studied. In both studies, dosage of testosterone used was sufficient to cause hypertrophy of the glands beyond the normal state. When this happened in the SDH-ase—cytochrome oxidase study, the enzyme activity rose beyond the level found in normal animals. In the case of the phosphatases, however, when the weight of the prostate was increased to more than twice that of the normal gland by 8 days of androgen treatment, the phosphatase activity did not rise beyond the normal level. The seminal vesicle under the same conditions hypertrophied to 70% above the normal weight, but the phosphatase activity did not rise above normal.

This phenomenon explains why Gutman and Gutman[4] were unable to show a response of the phosphatases to testosterone in their experiments; they were using adult, intact animals which presumably were at the maximal level of prostatic phosphatase activity before testosterone injections were begun.

The observations that the seminal vesicle is more sensitive to androgen deprivation than the prostate, morphologically[11] and with respect to oxidative enzyme activity[10] is borne out here again with respect to the phosphatases. While the seminal vesicle shows marked decrease in weight and phosphatase activity by the fourth day after castration, the prostate does not show a clear-cut response until sometime after this stage.

Summary. The acid and alkaline phosphatases of seminal vesicles and prostate glands of rats were estimated quantitatively in normal animals, castrates, and castrates receiving testosterone propionate.

In both glands the enzymes decrease in activity after castration, are restored to essen-

[10] Davis, J. S., Meyer, R. K., and McShan, W. H., *Endocrinology*, 1949, **44**, 1.

[11] Korenchevsky, B., Dennison, M., and Brovsin, I., *Biochem. J.*, 1936, **30**, 558.

tially normal levels by androgen therapy. The seminal vesicle is more sensitive than the prostate gland in this respect.

Androgen therapy caused hypertrophy of the glands beyond normal size, but under the conditions of these experiments, the acid and alkaline phosphatase activity was not increased beyond normal levels.

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17190. Investigations on the Use of Eserine for the Differentiation of Mammalian Esterases.*

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In most mammalian tissues there exist 3 types of esterase[1] capable of facilitating the hydrolysis of noncholine aliphatic esters, *i.e.*, ali-esterase,[2] pseudo-cholinesterase[1] (s-type)[3] and true cholinesterase[1] (e-type).[3] Most esters of aliphatic acids can be hydrolysed by enzymes from two of these 3 groups, certain non-choline esters (*e.g.*, triacetin) seem to be hydrolysed by enzymes of all 3 types[4-6] and, further, esterases of all 3 types are present in most crude tissue preparations. To distinguish between that portion of the hydrolysis of aliphatic esters which is due to the cholinesterases and that portion which is due to the ali-esterases in tissue preparations, Easson and Stedman[7] employed low concentrations of eserine or prostigmine to inhibit selectively any activity due to the cholinesterases (ChE's). Later investigators, *e.g.*, Richter and Croft,[2] Mendel and co-workers,[1] and others, also found low concentrations of eserine to be a very satisfactory tool for differentiating cholinesterase and ali-esterase activity. Thus it has been found that a concentration of 10^{-6}M eserine inhibits completely, or almost completely, the activities of both the true and the pseudo-ChE's of most mammalian tissues while the activity of the ali-esterases remains relatively unaffected by as much as 100 times this concentration.

Recently, however, McNaughton and Zeller[8] have concluded from their observations that "the use of eserine to decide whether a hydrolysis is catalysed by a cholinesterase or another esterase is of limited value because the s-type (pseudo-cholinesterase) is inhibited by this substance only in the presence of acetylcholine but not in that of ethyl chloroacetate." The evidence obtained in the present investigation has indicated, however, that eserine in low concentrations (10^{-6}M) can, in fact, be used to inhibit completely the activity of pseudo-ChE toward ethyl chloroacetate (ECIA) and that differentiation of cholinesterase activity from ali-esterase activity is therefore still possible by this means. Moreover, even though this concentration of eserine may not be adequate to effect complete inhibition of the ChE's from certain non-mammalian organisms,[9] in no case tested has the sensitivity of any cholinesterase to eserine been found to be altered significantly when one

* Supported by a grant from the National Research Council of Canada.

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2 Richter, D., and Croft, P. G., *Biochem. J.*, 1942, **36**, 746.

3 Zeller, E. A., and Bissegger, A., *Helv. chim. acta*, 1943, **26**, 1619.

4 Bodansky, O., *Ann. N. Y. Acad. Sci.*, 1946, **47**, 521.

5 Hawkins, R., Ph.D. Thesis, Toronto, 1947.

6 Adams, D. H., and Whittaker, V. P., *Biochem. J.*, 1948, **43**, xiv.

7 Easson, E. H., and Stedman, E., *Biochem. J.*, 1937, **31**, 1723.

8 McNaughton, R. A., and Zeller, E. A., PROC. SOC. EXP. BIOL. AND MED., 1949, **70**, 165.

9 Hawkins, R. D., and Mendel, B., *J. Cell. Comp. Physiol.*, 1946, **27**, 69.