β-Glucuronidase, Lipase and Esterase in the Testis of the Cryptorchid Rat

Although considerable attention has been paid to lipase and esterase in the testis[1], comparatively little is known of β-glucuronidase in this organ. This communication is concerned with some relationships between testicular β-glucuronidase, lipase and esterase of the rat in artificial cryptorchidism.

Thirty-four albino rats of the Wistar stock bred in this laboratory were used and grouped as follows: normal control (13), unilateral cryptorchidism (9), bilateral cryptorchidism (12). Determinations of enzyme activity in tissue homogenates were made according to the method of Talalay *et al.*[2] for β-glucuronidase, of Nachlas *et al.*[3] for lipase and for esterase; the units used were the amount of enzyme which liberates 1 μgm. of phenolic substance in 1 hr. for β-glucuronidase and for lipase, and in 20 min. for esterase[4].

Table 1

	Normal control	Cryptorchidism		
		Unilateral		Bilateral
		Normally located testis	Cryptorchid testis	cryptorchid testes
No. of rats	13	9		12
Body-weight (gm.)	132±10*	136±6·8		160±8·9
Testicular weight (mgm.)	742±80	1,078±100	567±69	541±58
β-Glucuronidase activity†	146±14	222±44	722±242	1,333±136
Lipase activity†	12±1·6	29±4·2	52±3·8	34±4·2
Esterase activity†	7,341±126	7,284±447	9,179±502	9,178±639

* Mean ± standard error of the mean.
† Units per gm. of wet tissue.

In the testis of the cryptorchid rat four weeks after operation, the concentrations of all three enzymes showed an increase. The most striking change was seen in the β-glucuronidase level. The concentration of this enzyme in the testis increased nine-fold in the bilaterally cryptorchid rat and five-fold in the unilaterally operated animal. Lipase concentration, however, increased much more in the cryptorchid testis of the unilaterally cryptorchid animal than in the bilaterally operated animal. The increase in the concentration of esterase was slight and the same in both groups. It should also be noted that β-glucuronidase and lipase activities were significantly ($P<0·05$) elevated in the normally located testis of the unilaterally cryptorchid animal. Results are shown in Table 1.

A full account of this work will appear elsewhere.

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Inhibition of 5-Hydroxytryptophan Decarboxylase by Phenylalanine Metabolites

Evidence has recently been presented which demonstrated a lowered level of circulating 5-hydroxytryptamine in patients with phenylketonuria[1]. The condition is associated with production of an excess of certain aromatic acid metabolites of phenylalanine, which it has been claimed inhibit dihydroxyphenylalanine decarboxylase[2]. It was suggested that this inhibitory effect was an explanation of the low plasma levels of adrenaline found in phenylketonuria. As 5-hydroxytryptamine is derived from 5-hydroxytryptophan by the action of 5-hydroxytryptophan decarboxylase[3] it was decided to examine the effects of these aromatic acids on this enzyme system also.

Table 1. Inhibition of 5-Hydroxytryptophan Decarboxylase by Phenylalanine Metabolites

Additions	Conc. (μmoles/3 ml.)	μl.CO_2/40 min.	Inhibition (per cent)	Inhibition of dihydroxyphenylalanine decarboxylase* (per cent)
Control	—	75·7	—	—
Phenylpyruvic acid	100	15·7	79·3	100
	50	38·3	49·5	100
	10	74·2	2	77
Phenyl-lactic acid	100	36	52·5	100
	50	66	13	77
	10	71·2	6	50
Phenylacetic acid	100	12·5	83·5	50
	50	24·2	68	0
	10	—	—	0
L-Phenylalanine	100	65·7	13	0

Guinea pig kidneys were homogenized in ice-cold *M*/30 Sørensen's phosphate buffer *p*H 6·8. The homogenate was centrifuged in the cold for 5 min. and 2 ml. of the supernatant (equivalent to 2 gm. of original wet tissue) used in each Warburg flask. 25 μgm. of pyridoxal phosphate and the inhibitor, dissolved in phosphate buffer, was added. The volume was adjusted to 2·75 ml. with phosphate buffer. After gassing for 2 min. with nitrogen, the flasks were equilibrated at 37° for 10 min. and 0·25 ml. of DL-5-hydroxytryptophan (10 μmoles) added from the side-arm. Controls and blanks were also used. Carbon dioxide output was determined for 40 min.
* After Fellman (ref. 2). Dihydroxyphenylalanine (20 μmoles) as substrate.

Phenylacetic acid was found to be more active and phenylpyruvic and phenyl-lactic acids were less active as inhibitors of 5-hydroxytryptophan decarboxylase when compared with the results presented by Fellman[2] for dihydroxyphenylalanine decarboxylase, which is similar to but not identical with 5-hydroxytryptophan decarboxylase[3].

These results, although preliminary, suggest the possibility that such an inhibition, *in vivo*, might be responsible for the decreased production of 5-hydroxytryptamine observed in patients with phenylketonuria. As there is a large body of evidence to suggest that 5-hydroxytryptamine plays a part in normal brain function, inhibition of 5-hydroxytryptophan decarboxylase, present in some areas of the brain in high concentration[4], might thus contribute to the cause of the mental defect in this disease.

It is of interest to note that ingestion of phenylacetic acid in man is followed by transitory mental disturbance[5]. But before the inference can be drawn that this effect is due to inhibition of 5-hydroxytryptophan decarboxylase more work must be done on the mechanism of this inhibition, and the possible inhibitory action on other enzyme systems[6] must be studied.