Prophenolase and the Role of Mehlis' Gland in Helminths

Enzyme phenolase is known to occur along with phenol and protein in vitelline cells of helminths having phenolically tanned eggs[1]. Yet the reasons as to why tanning does not occur before the shell globules coalesce to form the shell, and the nature of the association of the precursors of sclerotin while in vitelline cells, as well as the role of Mehlis' gland in this regard, have defied helminthologists[1-6], inspite of their realization that Mehlis' gland, by its location around the ootype, may have some role in the formation of the shell.

In an attempt at elucidating these problems, studies on the histochemistry of vitelline cells and Mehlis' gland of monogeneans *Pricea* and *Protomicrocotyle* were undertaken. As this study has yielded some interesting information bearing on the questions mentioned above, it is therefore being reported in this communication.

Materials and methods. Monogenetic trematodes *Pricea* and *Protomicrocotyle* obtained from the gills of marine fishes *Scomberomorus guttatus* and *Caranx sexfasciatus* respectively were employed in the study. Phenolase in these helminths was detected by following the method of SMYTH[7]. The experiments designed in the study were as follows: 1. Live specimens and specimens fixed and stored in 5% neutral formalin for 15 min were separately incubated in catechol. 2. Specimens stored in fixatives (5% neutral formalin and 70% alcohol) for 24 h were separately incubated in catechol. 3. Live specimens and specimens fixed and stored in 5% neutral formalin for 15 min were separately treated in 0.2% sodium oleate (prepared in phosphate buffer of pH 7) for 30 min and then separately incubated in catechol. 4. Live specimens and specimens fixed and stored in 5% neutral formalin for 15 min were injured by rendering them asunder and were separately incubated in catechol.

Results and discussion. Results obtained on specimens in experiment 1 reveal that vitelline cells in the vitellaria were negative whereas the shell precursors released from these cells and egg shell in the uterus after their passage through the ootype surrounded by Mehlis' gland were positive. The results suggest that the enzyme, while in vitelline cells in situ, remains unreactive to catechol but is enabled to react with it on coming under the influence of Mehlis' gland secretion.

The catechol negative reaction revealed by vitelline cells in situ in experiment 1 is at variance with the observations reported by previous workers[1-6]. To determine whether the catechol-positive reaction reported by previous workers was due to the influence of storage in fixative, experiment 2 was designed and the results obtained in the specimens revealed the vitelline cells in situ to be catechol-positive: thus agreeing with the observations reported by earlier workers and suggesting that storage in fixatives has influenced the enzyme in rendering it to be catechol-positive.

To determine whether the catechol-negative reaction revealed by vitelline cells in situ in experiment 1 was due to the enzyme existing as proenzyme, experiment 3 was designed employing sodium oleate treatment, as such a treatment is known to activate prophenolase of insects[8]. The results obtained on specimens in experiment 3 revealed vitelline cells in situ to be catechol-positive, suggesting thereby that not only the enzyme exists as prophenolase in them but is also activated by sodium oleate treatment.

To confirm the observation as regards the occurrence of phenolase as proenzyme in these helminths, experiment 4 was designed based on the evidence of injury causing an activation of cuticular prophenolase in insects[9]. Results obtained on specimens in this experiment revealed that vitelline cells at the regions of injury were catechol-positive whereas those in the intact regions were catechol-negative; this supports the observation made that phenolase in the vitelline cells in situ exists as prophenolase.

Conclusions. This study provides direct evidence not only of the existence of phenolase as proenzyme (to be reported for the first time in helminths) but also of the role of Mehlis' gland in activating the enzyme. By remaining a proenzyme it constitutes a factor responsible for preventing the precursors of sclerotin from tanning in situ.

The author has recently obtained evidence from a continuation of the study[10] for the presence of sulphated acid mucopolysaccharide in the vitelline cells in association with phenol which constitutes as an in-built inhibition system preventing the precursors from tanning in situ. Mehlis' gland secretion functions as a releasor of this inhibition, facilitating the process of tanning which will be published elsewhere[11].

Zusammenfassung. Die Phenoloxydase der Vittellinzellen von Trematoden wirkt als inaktive Prophenoloxydase und bedingt, dass die von Dotterstockzellen synthetisierten Vorläufer von Sklerotin in situ nicht tanniert werden. Das Sekret der Mehlis-Drüse beeinflusst die Fermentaktivierung.

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The Isolation and Identification of 2-Amino-3-Hydroxyacetophenone from the Urine of Rats

In a survey of aromatic metabolites in the urine of rats following ingestion of tryptophan, it was noticed that the urine contained a small amount of substance with a greenish blue fluorescence. This compound has now been identified as 2-amino-3-hydroxyacetophenone. It was reported that 2-amino-3-hydroxyacetophenone-*O*-sulfate were excreted in a small amount in some normal human urines, and in appreciably larger amounts in certain pathological urines[1]. But there are no reports on the occurrence of 2-amino-3-hydroxyacetophenone in any

other species. This paper describes the isolation and identification of 2-amino-3-hydroxyacetophenone from the urine of rats. The biosynthetic pathway by which the compound is formed from tryptophan was discussed by DALGLIESH[1].

Isolation and Identification of 2-amino-3-hydroxyacetophenone. Male Wistar albino rats, weighing 150 to 200 g, received daily for 10 days 600 mg of L-tryptophan per kg of body weight by i.p. injection. Ten 24-hour urine collections from 6 rats (1000 ml) were pooled, centrifuged and filtered. The filtrate was acidified to pH 3 with acetic acid and shaken with ethyl ether-ethanol (3:1). The organic phase was evaporated to dryness under nitrogen. The residue was dissolved in 100 ml of 1*N* HCl and hydrolyzed at 80 °C for 1 h under nitrogen gas. The solution was adjusted to pH 3–4 with sodium hydroxide and treated with ethyl ether. The ether extract was washed with 0.1% Na_2CO_3 solution, dried over sodium sulfate, anhydrous, and concentrated in vacuo. The sticky dark brown residue was dissolved in a small volume of dried ether and applied to a column of silicic acid, 1.5 cm in diameter and 9 cm long, which was then eluted by 30 ml of dried ether free from peroxide. The eluate was evaporated to dryness under nitrogen. The residue was dissolved in a small volume of methanol and partially purified by ascending paper chromatography. Whatman No. 3 MM papers were used with the solvent system of MASON and BERG[2] containing 1 ml of glacial acetic acid per 100 ml of solvent. *n*-Butanol-acetic acid-water (4:1:1) was also used. The fluorescent area corresponding to 2-amino-3-hydroxyacetophenone of the chromatograms was cut out and treated with methanol : ether (1:2) to extract the fluorescent material. The extract from paper strips was concentrated in vacuo. The residue was further purified by thin-layer chromatography. Thin layers of silica gel G (Merck, Darmstadt, Germany) were prepared on glass plates. The solvent systems of ethylacetate-isopropanol-28% ammonia water (9:6:4) and chloroform-ethylacetate-formic acid (60:40:1) were used. The details of thin-layer chromatography have already been described[3,4]. The isolated material was pure on both thin-layer and paper-chromatograms.

The comparison of the new compound from rat's urine with authentic sample. The isolated compound had Rf values identical with synthetic 2-amino-3-hydroxyacetophenone in the various solvent systems used for paper and thin-layer chromatography (Table). When spots of natural and synthetic 2-amino-3-hydroxyacetophenone were subjected to the color reactions of Ekman's reagent[5], diazotized sulfanilic acid (DSA)[6] and Ehrlich's reagent[5], identical color development was obtained with both natural and synthetic samples, with absorption maxima at 233, 270 and 378 nm in methanol. When the isolated compound was converted to a volatile derivative with diazomethane and trifluoroacetic anhydride and analyzed by gasliquid chromatography according to the method described before[7], the retention time of the volatile derivative of the isolated compound was indistinguishable with that of the volatile derivative (2-trifluoroacethylamino-3-methoxyacetophenone) of authentic sample.

Results of paper and thin layer chromatography of the product with authentic sample

	Product	Synthesized
Paper chromatography[a]		
Mason-Berg with 1% acetic acid, Rf	0.91	0.91
n-Butanol-acetic acid-water (4:1:1), Rf	0.88	0.88
Thin layer chromatography		
Ethylacetate-isopropanol-28% ammonia water (9:6:4), Rf	0.88	0.88
Chloroform-ethylacetate-formic acid (60:40:1), Rf	0.72	0.72
Fluorescence at 3650 Å	Greenish blue	Greenish blue
Diazotized sulfanilic acid	Pink orange	Pink orange
Ekman's reagent	Purple	Purple
Ehrlich's reagent	Pink orange	Pink orange
Absorption maxima at ph 7.0 (nm)	233	233
	270	270
	378	378

[a] Whatman No. 3 MM filter paper.

Zusammenfassung. 2-Amino-3-hydroxyacetophenon wurde aus Rattenharn nach Verabreichung von L-Tryptophan isoliert und identifiziert.

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Effects of Estradiol on the in vitro Incorporation of Acetate-1-^{14}C and Choline-1,2-^{14}C into the Phospholipids of Human Peripheral Arteries

Synthesis of total phospholipids by the human arterial wall has been demonstrated in vivo from phosphate-^{32}P[1] and in vitro from acetate-^{14}C[2,3]; recently the synthesis of the individual phospholipid classes in human arteries has been investigated[4].

Estrogenic hormones have been shown to increase phospholipid synthesis in liver[5], uterus[6] and human and canine arterial intimas[7]. Estrogen treatment in humans specifically increases serum lecithin levels[8], and in rats specifically increases the lecithin species containing stearic and arachidonic acids[9].

The present study is a preliminary investigation into the effects of estradiol on the incorporation of precursors into the individual phospholipids and phospholipid fatty acids of human peripheral arteries.

Material and methods. Above the knee amputated legs were obtained immediately after surgery, and the distal femoral artery and popliteal and tibial arteries dissected