propanal; the N^1-monoacetylspermidine can further react with PAO to form putrescine and the second molecule of 3-acetamidopropanal[3]. This complicated two-step reaction seems undesirable for PAO assays. The PAO activity with N^1-monoacetylspermine was higher than that with N^1,N^{12}-diacetylspermine. These are reasons why we have adopted N^1-monoacetylspermine rather than N^1,N^{12}-diacetylspermine as substrate for PAO in the present assay system.

We added pargyline, a potent MAO inhibitor, and semicarbazide, a DAO inhibitor, to the mixture, to ensure specific assays for PAO. These inhibitors had almost no effect on PAO activity at the concentrations used. We have reported that N^8-monoacetylspermidine, monoacetylcadaverine and monoacetylputrescine can be substrates for rat liver mitochondrial MAO although their affinity for this enzyme is very low, but N^1-monoacetylspermine, N^1,N^{12}-diacetylspermine and N^1-monoacetylspermidine are absolutely not oxidized by rat liver MAO[9]. All monoacetylpolyamines and diamines are able to be oxidized by hog kidney DAO although their K_m values are more than 5 mM[9]. Therefore, both inhibitors might be omissible in the assays of PAO in most mammalian tissues with 0.2 mM N^1-monoacetylspermine as substrate.

This is the first report to describe the occurrence of PAO in human tissues. Seiler et al.[2] measured PAO activities in rat tissues and reported that the highest activity was found in the pancreas, followed by the liver, spleen and kidney; the activity in the testis was much lower than in the kidney. In human tissues, however, the highest activity was observed in the liver and the activity in the pancreas was lowest (table), although the latter might be partly due to the post-mortem degeneration of PAO by proteolysis. We can recommend human liver as a good PAO source for purification studies. The present assay method seems very useful for enzymological studies on PAO because of its simplicity and sensitivity.

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Stabilization of the egg-shell of a monogenean *Dionchus remorae*

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Summary. The phenolase of *Dionchus remorae* exists as proenzyme and is not substrate specific oxidising mono, di and polyphenols. The egg-shell is stabilized by quinone tanning.

The egg-shell of helminths is usually stabilized by quinone tanning[2]. Recent studies have shown that the egg-shell of monogeneans *Pseudomicrocotyle* sp., and *Pricea multae* are stabilized by dityrosine linkage and S-S bonding and that quinone tanning is absent in these species[3]. The present investigation reports the results obtained on the egg-shell stabilization of *Dionchus remorae.*

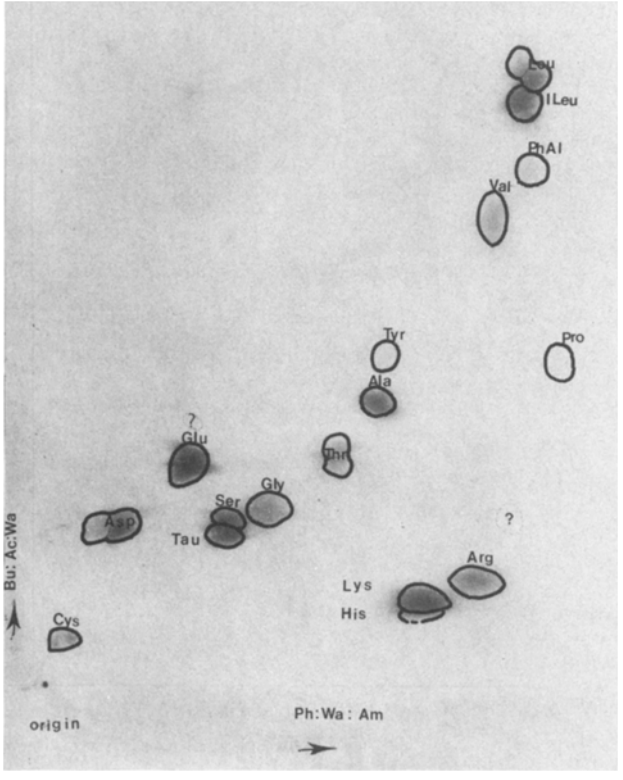
Materials and methods. The eggs of *D. remorae* were detached from the terminal ends of the gill filaments of *Scomberoides tol* and washed repeatedly in distilled water to free them of mucus. A 5 mg sample of eggs was hydrolyzed in 5 ml of 6 N HCl in a sealed test tube in an oven for 12 h at 105 °C. Excess HCl was evaporated in vacuo and by heating in a water bath at 65 °C. The sample was diluted with a few drops of water and further evaporated. The resultant amino acids were dissolved in 10% (v/v) isopropanol and spotted on Whatman No. 1 chromatography paper. Any acid remaining in the sample was neutralised by placing the paper over ammonia vapor. 2-way descending chromatograms were run using solvents butanol:acetic acid:water (12:3:5) for the first run (18 h) and phenol:water:ammonium hydroxide (80:40:1) in the second run. The amino acids were located using 0.2% ninhydrin in acetone. Phenols present in acid hydrolysate were visualized by their reaction with diazotized sulphanilic acid on a single dimensional chromatogram run with butanol:acetic acid:water[4]. The solubility of the eggs was tested by incubating samples in 1 N hydrochloric acid, 1 M sodium hydroxide or 0.75 M sodium hypochlorite for 48 h.

The phenolase in *D. remorae* vitellaria, i.e. vitelline glands situated in the lateral parts of the fluke and believed to contribute shell and yolk materials for egg formation, was detected by the following method[5]. Fresh, unfixed specimens, specimens treated with 0.2% sodium oleate for 15 min (prepared in phosphate buffer pH 6.8) and fresh specimens injured at the vitellaria were incubated separately with 0.1% aqueous solution of the substrates L-tyrosine, tyramine, DL-Dopa, dopamine, protocatechuic acid, catechol, epinephrine (adrenaline), pyrogallol, resorcinol or hydroquinone. Control experiments were carried out by incubating sodium oleate treated and injured specimens with phenylthiourea or diethyldithiocarbamate for 15 min prior to incubation with substrates. The enzyme activity was determined based on the brown color development in the experiments and absence of colour development in the controls.

Results and discussion. The solubility tests revealed that the egg-shell protein was insoluble in dilute acid and alkaline solutions, suggesting that covalent bonds were involved in stabilization. On prolonged treatment (36 h) with the detanning agent, sodium hypochlorite, the egg-shell was rendered soluble, indicating the possible presence of quinone bonds[6]. Chromatograms of the acid hydrolysate of *D. remorae* eggs had 19 ninhydrin-positive spots. The relative color intensities of the identified amino acids were in the order of: glutamic acid > aspartic acid > lysine > arginine > glycine > cystine > isoleucine > leucine > alanine > threonine > valine > serine > taurine > phenylalanine > histidine > proline > tyrosine and 2 unidentified spots (fig.).

The single dimensional chromatograms with diazotized sulpha-

nilic acid had 2 spots with Rf values corresponding to dopa (0.2) and N-acetyl dopamine (0.67). This suggests that the tyrosine was hydrolyzed into dopa which in turn was converted into N-acetyl dopamine, as in insects[7]. The results on phenolase activity obtained from incubation experiments, using different phenolic substrates, revealed that the enzyme exists as prophenolase. This was inferred from the absence of color development in those specimens which were not treated with the activators. The enzyme was activated by sodium oleate and by injury. The activated enzyme reacted with the monophenol tyramine, the diphenolic substrates dopamine, catechol and epinephrine (adrenaline), the paraphenol hydroquinone, and the polyphenol pyrogallol. The enzyme showed no activity towards tyrosine, dopa, protocatechuic acid, and resorcinol. The enzyme activated by sodium oleate and injury was inhibited by phenylthiourea and diethyldithiocarbamate. The phenolase exists as the proenzyme in the vitellaria of *D. remorae* and is not substrate specific, oxidising mono, di and polyphenols. In contrast, phenolase of *Pricea multae* is substrate specific, reacting with only di and polyphenols without carboxyl- and amino-groups[8]. It would be interesting to extend the investigation to other monogeneans and determine whether the corresponding enzymes were substrate-specific or not. Although the amino acid cystine, considered to be involved in S-S linkages and stabilization of the egg-shell of *Fasciola hepatica, Pricea multae* and *Pseudomicrocotyle* sp.,[4,9] is also present in the acid hydrolysate of the eggs of *D. remorae,* further studies on the purified egg-shell would be necessary to confirm whether this amino acid is involved in the stabilization. In conclusion, evidence is presented for quinone tanning in the egg-shell of *D. remorae.*



2-dimensional chromatogram of acid hydrolysate of eggs showing ninhydrin-positive spots.

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Drug metabolism in spontaneously diabetic guinea pigs[1]

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Summary. Both sexes of spontaneously diabetic guinea pigs exhibit hyperinsulinemia (> 4-fold normal). This diabetic state is associated with the inhibition of hepatic drug metabolism in males but not females.

Although the guinea pig has been a useful experimental animal in the investigation of many pathologies, this has not been the case for diabetes. This is because the guinea pig is generally resistant to the production of sustained hyperglycemia after treatment with either alloxan[6] or streptozotocin[7-10]. Since hyperglycemia is the criterion most often used to indicate that experimentally induced diabetes has been established, attempts to use the guinea pig as a diabetic model in the traditional manner have been met with frustration. Consequently, the scant literature on biochemical studies in this model is limited to reports of acute studies[11]. Recently, however, spontaneous diabetes caused by an unknown infectious agent and characterized by non-ketotic glucosuria has been documented in a colony of guinea pigs[12,13]. This communication describes the effects of spontaneous diabetes in the guinea pig on hepatic drug metabolism, a process affected by diabetes in other laboratory animals[14].

Methods. Male and female spontaneously diabetic and normal