compared with Na-TC or Na-TC/lecithin solutions. UCB oxidation: Some of the solubilized UCB became irreversibly oxidized during incubation. Transformation to biliverdin was checked by VIS-spectroscopy, which showed that less than 2% of UCB was oxidized to biliverdin during the whole experimental procedure. Standard deviations: Standard deviation of photometric UCB assay was calculated to be better than ±2%. Within a series of each 5 solubilization experiments, using the same bile salt concentration, standard deviation was about ±6.5% (with respect to UCB filtrate concentration).

Discussion and conclusion. The most striking result is the considerable solubilization effect of di- and trihydroxy bile salts on freshly precipitated but not commercial solid UCB. We used the precipitating procedure to obtain UCB in a physical state closely similar to the one in bile.

From the results we conclude that UCB solubilization by bile salts is based on micellar mechanisms, i.e. UCB is considered to enter the bulk of the bile salt micelle. Consequently, the solubilization effect of Na-DHC which does not form micelles, is expected to be small. In fact this was observed (figure 1). Furthermore, Na-TC/lecithin micelles which already have absorbed cholesterol, are somewhat prevented from solubilizing UCB (see above).

In bile, several lipids, especially cholesterol, compete for the mixed bile salt/phospholipid micelles. We postulate that a micelle which is saturated with cholesterol, will poorly solubilize UCB in bile. Thus, solubilization of UCB in bile very much depends on the degree of cholesterol saturation of mixed micelles. Biles where UCB is increased either by an elevated biliary excretion and/or by increased hydrolysis of conjugated bilirubin, will consequently become supersaturated with UCB if the solubilizing power of mixed micelles is mainly claimed by cholesterol and other insoluble lipids.

1 J.T.G. Overbeeck, C.L.J. Vink and H. Deenstra, Recl Trav. chim. *74*, 81 (1955).
2 R.C. Burnstine and R. Schmid, Proc. Soc. exp. Biol. Med. *109*, 356 (1962).
3 T.J. Devers, D. Gallo and J.D. Ostrow, Gastroenterology *69*, 816 (1975).
4 T.J. Devers, D. Gallo and J.D. Ostrow, in: The Liver. Quantitative Aspects of Structure and Function, p.429. Aulendorf 1976.
5 S. Yamamoto, Acta med. (Fukuoka) *28*, 28 (1958).
6 L. Jendrassik and P. Gròf, Biochem. Z. *297*, 81 (1938).
7 R. Paul and P. Balaram, Biochem. biophys. Res. Commun. *81*, 850 (1968).

Peroxidase activity in the sponge, *Iotrochota birotulata*[1]

M.D. Corbett and Bernadette R. Chipko

Rosenstiel School of Marine and Atmospheric Science, University of Miami, 4600 Rickenbacker Causeway, Miami (Florida 33149, USA), 4 September 1978

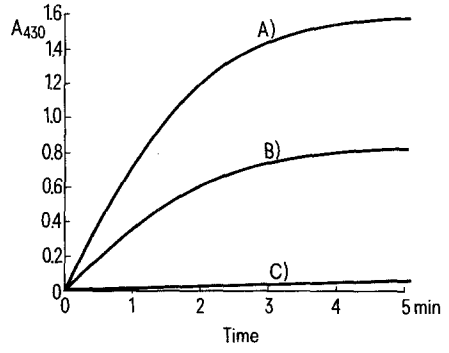
Summary. The sponge *Iotrochota birotulata* contains a peroxidase which was partially characterized. This is the first report of a peroxidase in Porifera, originally thought to be devoid of such enzymatic activity.

Sponges have proven to be a rich source of novel natural products, including brominated compounds[2] and compounds with interesting oxygenation patterns[3]. No attempts have yet been made to characterize those enzymes that are responsible for the production of such natural products, although it has been suggested that brominated marine natural products are the result of peroxidative enzymes similar to the well known terrestrial fungal enzyme, chloroperoxidase[4]. The only reported attempt to detect peroxidase activity in sponges concluded that peroxidases are absent in this phylum[5]. Unfortunately, this conclusion was based upon the investigation of only three genera of porifera. We now wish to report on the strong peroxidatic activity in a marine sponge, and to correct the phylogenetic conclusion that primitive animals, such as the sponges, lack this activity.

Our interest in invertebrate peroxidases arose from our finding that chloroperoxidase oxidizes arylamines to arylnitroso compounds[6]. The possible environmental consequences of such transformations were also recently proposed[7].

Our attention was drawn to *Iotrochota birotulata* (Higgins) which produces a dark purple colored, high mol. wt pigment when damaged, hence, its common name 'purple bleeding sponge'[8].

Materials and methods. Specimens were collected on 3 separate occasions in about 6 m of water on a coral reef 10 km southeast of Key Biscayne and returned immediately to the laboratory for processing. Identification of the species was made by us according to Wiedenmayer[9] and confirmed by J. Garcia-Gomez (University of Miami). Careful examination of the surfaces and of longitudinal sections of specimens indicated the absence of contaminating macroorganisms, such as commonly found in larger sponge species. In fact, we suspect that this sponge might contain toxic components, as we have never observed other benthic invertebrates or macroalgae growing in close asso-



Oxidation of pyrogallol by *I. birotulata* peroxidase. To a 1-cm quartz cuvette containing 20 µmoles of pyrogallol, 12 µmoles of H_2O_2 and 100 µmoles of potassium phosphate buffer, pH 6.0, in 2.0 ml of H_2O was added, to initiate the reaction: A) 0.20 ml of enzyme preparation; B) 0.10 ml of enzyme preparation and C) 0.10 ml of enzyme preparation that had been heated for 5 min at 80 °C.

ciation with this species. Examination at X500 magnification also failed to reveal the presence of obvious contaminant microorganisms.

Homogenization of 300 g of freshly collected *I. birotulata* with 600 ml of 0.1 M, pH 7.4 potassium phosphate buffer, followed by centrifugation for 20 min at 11,000 rpm and recentrifugation of the supernatant for 30 min at 15,000 rpm (DuPont Sorvall SS-34) gave a dark purple-colored supernatant. The protein precipitate resulting from the 25–50% $(NH_4)_2SO_4$ fractionation of this supernatant was resuspended in the original volume of phosphate buffer to give the enzyme preparation of interest. Standard assays for peroxidase employing pyrogallol[10] and dianisidine[11] were used to monitor this partial purification. The enzyme preparation at this point was only a 2-fold purified product with respect to total protein, but was almost free of the contaminating purple pigment; thus, enabling us to employ spectrophotometric methods in studies of its peroxidase activities.

Results and discussion. The oxidation of pyrogallol by the enzyme preparation occurred only in the presence of H_2O_2, and was inhibited by both KCN and NaN_3 at 0.01% concentrations. Although the enzyme retained most of its activity upon standing for several days at room temperature, gentle heating destroyed the activity. The initial rate of oxidation and total amount of pyrogallol oxidized within 5 min was directly related to the amount of enzyme preparation employed (figure). The peroxidase activity was observed throughout the pH range of 4.0–8.0, with 7.5 being the optimal pH. Similar results were obtained with dianisidine as the substrate, although solubility problems prevented pH dependency studies above pH 6.0. The enzyme preparation was unable to effect chlorination of monochlorodimedon[10], nor did it convert 4-chloroaniline to 4-chloronitrosobenzene[6]. Thus, we conclude that the peroxidase in *I. birotulata* is similar in properties to horseradish peroxidase rather than chloroperoxidase.

Based on pyrogallol oxidation at pH 7.5, the level of peroxidase activity is estimated to be between 12 and 15 µM units per g of fresh sponge tissue. This represents an order of magnitude greater concentration of peroxidase in this sponge when compared to a marine red alga known to possess a high level of peroxidase[12]. This surprisingly high level of peroxidase activity that we observed in *I. birotulata* makes it highly improbable that this activity could be due to microalgae that might be present on the sponge surface.

Our demonstration of the presence of a peroxidase in this sponge necessitates a reconsideration of this biochemical parameter in the Porifera. A survey of sponges for peroxidative enzymes is now underway with the hopes of developing a potential chemotaxonomic tool for the classification of Porifera.

1 This study was supported by grant No. CA 21992 from the National Cancer Institute, DHEW.
2 L. Minale, G. Cimino, S. de Stefano and G. Sodano, Fortschr. Chem. org. NatStoffe *33*, 1 (1976).
3 R.J. Wells, Tetrahedron Lett. 2637 (1976).
4 W. Fenical, J. Phycol. *11*, 245 (1975).
5 K.H. Wurzinger and R. Hartenstein, Comp. Biochem. Physiol. *49B*, 171 (1974).
6 M.D. Corbett, B.R. Chipko and D.G. Baden, Biochem. J. *175*, 353 (1978).
7 M.D. Corbett, B.R. Chipko and J.H. Paul, J. envir. Path. Tox. *1*, 259 (1978).
8 G.L. Voss, "Seashore Life of Florida and the Caribbean," E.A. Seemann Co., Miami, 1976, p. 26.
9 F. Wiedenmayer, Shallow Water Sponges of the Western Bahamas, p. 138. Birkhäuser Verlag, Basel 1977.
10 P.F. Hollenberg, T. Rand-Meir and L.P. Hager, J. biol. Chem. *249*, 5816 (1974).
11 R. Bartha and L. Bordeleau, Soil Biol. Biochem. *1*, 139 (1969).
12 M.J. Murphy and C. Oh Eocha, Phytochemistry *12*, 55 (1973).

The glutathione status of the rat liver*

G. Harisch, J. Eikemeyer and J. Schole

Institute of Physiological Chemistry, College of Veterinary Medicine Hannover, Bischofsholer Damm 15, D-3000 Hannover (Federal Republic of Germany), 16 October 1978

Summary. For the determination of cellular total glutathione, a new method is presented based on a fluorometric procedure. The relation between reduced glutathione, mixed glutathione disulfides and disulfide glutathione will be designated the glutathione status.

The tripeptide glutathione is a structural component of most living cells[1]. It can be found in 3 forms, as reduced glutathione (GSH), as symmetrical glutathione disulfide (GSSG), and as unsymmetrical glutathione disulfide which we will call XSSG[2]. In the last form, the glutathione has combined with cysteine-SH groups of cellular proteins[3-9] or with low molecular SH compounds of the cell[10] to form a disulfide bond. By this means, a 'depot' is developed which, however, in certain situations can very rapidly be mobilized and then is available to the cell as GSH[11]. This characteristic makes it appear desirable to determine, in all investigations of cellular thiol-disulfide-status, the percent glutathione bound but mobilizable.

Normally this would be done by reducing the disulfide bonds and then measuring the increased GSH level. However, as there are often only very small quantities available in biological studies, a sensitive and selective GSH assay is necessary. A fluorometric method with these prerequisites was recently presented by Hissin and Hilf[12]. We have adapted this method to the conditions in liver tissues and expanded it to include the cellular total glutathione (TG), and thus the portion of bound glutathione can be measured as well.

Materials and methods. Male SPF rats, Wistar Han/Bö were kept under defined conditions (24 °C, light from 06.00 till 18.00 h) until they reached the desired 120 g. The animals were anesthetized with 40 mg/kg nembutal i.p. (Pentobarbital Sodium Abbott). The left lobe of the liver was removed using the freeze stop method[13,14].

TG measurement: The measurement of cellular total gluta-