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.

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The glutathione status of the rat liver*

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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-

Table 1. Total glutathione (TG) and free glutathione (GSH)

	GSH added (µg per 80 mg liver) 0	45	90	113	135
TG (µg per 80 mg liver)	195±6.4	238±2.9	284±4.4	297±9.3	326±5.9
Expected value	-	240	285	308	330
Recovery (%)	-	99	99	96	98
GSH (µg per 80 mg liver)	139±5.0	174±1.2	226±3.4	250±0.4	262±2.4
Expected value	-	184	229	252	274
Recovery (%)	-	95	99	99	96

Means±SD of 14 liver homogenates from 14 male Wistar rats (120 g b.wt); GSH was added before the homogenation.

thione was done according to the method proposed by Habeeb for splitting smaller amounts of disulfides with $NaBH_4$[15]; however, this method was modified for the conditions necessary for fluorometric GSH measurement: 80 mg liver powder in 1.75 ml Tris buffer (0.1 M, pH 8.0), in which immediately before the experiment 100 mg $NaBH_4$ was dissolved, and 2.0 ml guanidine (8 M) were homogenized for 20 sec in an Ultra-Turrax (Jahnke and Kunkel, Staufen, Germany); 0.05 ml n-octanol was used to prevent foaming. The homogenate was incubated (40 °C) for 30 min and then cooled in an icebath. 1.0 ml 50% ice cold $(HPO_3)_n$ was used to deproteinize the sample, which was then centrifuged for 15 min at 104,000 × g in a vertical rotor (TV 865, Du Pont-Sorvall). 0.5 ml of the supernatant was diluted with 4.5 ml phosphate EDTA-buffer (0.1 M potassium phosphate, 0.005 M EDTA, pH 8.0) Then 0.1 ml of the above dilution, 1.8 ml phosphate EDTA buffer and 0.1 ml o-phthalaldehyde (OPT) solution (100 µg OPT) were combined and mixed well. After 15 min at room temperature, the mixture was transferred to a fluorescence cuvette. The fluorescence intensity was measured in a Shimadzu RF 500 at 423 nm (activation at 350 nm).

GSH measurement: The GSH measurement corresponded to that of Hissin and Hilf[12]. GSH values obtained by the fluorometric method coincide with those determined by the glyoxalase method[1,2]. Other low molecular thiols reacting with OPT are quantitatively negligible[12].

GSSG measurement: As the proposed method from Hissin and Hilf for measuring fluorometric GSSG[12] is not reliable[16], disulfide glutathione was measured using the enzymatic optic method with glutathione reductase and NADPH[17].

Results and discussion. Glutathione parameters show diurnal[18] and probably also annual rhythms, and they are dependent on body weight[2]. Data on glutathione in the literature are hardly comparable as they were seldom obtained under identical conditions.

1. Data on TG (table 1) are rather sparse in the literature[3,6]. The only exception is the lens of the human eye[19]. Reduced glutathione (GSH) (table 1) is well investigated. Our data are in good agreement with the liver GSH-values obtained with other methods[12,20–23]. 2. XSSG is calculated by the formula XSSG = TG-(GSH + 2 GSSG); XSSG is expressed as SH-glutathione and all data are given in µmoles per g liver (table 2). Our results are in coincidence with those of Harrap[3] which were obtained with a different method, and they are in agreement with the results found by Modig with aszites tumour cells[6]. 3. The same GSSG-values were obtained by Tietze[20] with a different method. Some authors try to prevent possible GSH oxidation occuring during tissue preparation and they report lower GSSG-values[24,25]. 4. The relation between free GSH, XSSG and GSSG will be designated the glutathione status[2] (table 2). In a similar manner, Kosower[26] described a thiol disulfide status.

Table 2. Glutathione status

	TG (as GSH)	GSH	XSSG* (as GSH)	GSSG
µg per g liver	2445±80	1741±62	636	67±4
µmoles per g liver	7.96	5.67	2.07	0.110
Number of animals	14	14		14

The values are means±SD; male Wistar rats with 120 g b.wt; *see 'results and discussion' section.

Abbreviations: TG = total glutathione, GSH = glutathione, GSSG = disulfide glutathione, XSSG = unsymmetrical glutathione disulfide (as SH-glutathione expressed), $NaBH_4$ = sodium borohydride, OPT = ortho-phthalaldehyde, $(HPO_3)_n$ = meta-phosphoric acid.

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