activity vs. reciprocal of absolute temperature yielded energies of activation of 14.6 kcal/mole and 12.6 kcal/mole for the whole homogenate and acetone powder, respectively. While linearity of the Arrhenius plot was obtained over the range of 14–38 °C for the whole homogenate, the plot for the acetone extract deviated from a straight line above 22 °C, a feature noted previously[7] with some purified allantoinases from other sources. This is thought not to be an effect of denaturation of the enzyme at the higher temperatures[7] but may represent a conformational change in the enzyme.

Two classes of the phylum Annelida, the Oligochaeta and Hirudinea, are purinostatic and lack enzymes of uricolysis[14]. For the third class, the Polychaeta, allantoinase has been found in 4 of the 6 species of Sedentaria tested[8,9], but only 'traces' have been found in one of the 6 Errantia tested[8]. This report provides evidence for the occurrence of allantoinase in another species of Sedentaria. These discoveries of purinolytic activity in the polychaetes give evidence of a biochemical link with the related phylum Sipunculida, which has a complete system of purinolysis[14].

Polychaetes of the Sedentaria may be one of the few groups of organisms possibly possessing both complete purinolysis and ornithine-urea cycle enzymes as mechanisms for the production of urea. It would be of interest to determine whether or not L-ornithine-ketoacid aminotransferase (EC 2.6.1.13) is present in the Sedentaria since this enzyme provides a link between the ornithine-urea cycle and the purine-urea cycle. The question of why errant polychaetes lack enzymes of uricolysis and some sedentary polychaetes possess them might be answered by further examination of the adaptive significance of this pathway for polychaetes.

Allantoinase activity in extract of acetone powder of *Eudistylia vancouveri* for two typical experiments

System	Glyoxylic acid produced (μmoles)
Experiment 1[a]	
Complete	0.245, 0.249[c]
Complete (boiled extract)	0.035
Complete (zero time control)	0
Experiment 2[b]	
Complete	0.257, 0.266, 0.295[c]
Complete (boiled extract)	0.032
Complete (zero time control)	0

[a] 30 min incubation time, 7.70 mg protein/ml. [b] 45 min incubation time, 5.09 mg protein/ml. [c] Replicates.

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Evidence of an Essential Histidyl Residue in Arylsulphatase B[1]

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Summary. Diazotization and carbethoxylation studies of arylsulphatase B have indicated that a histidine residue is essential for arylsulphatase B activity.

Arylsulphatase B has recently been obtained in a homogeneous form from ox tissues[3,4] and human liver[5]. This enzyme differs from the corresponding arylsulphatase A both by its higher isoelectric point and lower molecular weight[6]. Although some information is available on the functional groups in the active site, and on the physiological role of arylsulphatase A[7–10], very little is known about the active site and the physiological importance of arylsulphatase B[11,12]. Recent studies of AGOGHUA and WYNN[5] have indicated that probably a histidine residue is involved in the reaction catalyzed by arylsulphatase B. The present study was undertaken to gain more information about the participation of a histidine residue in the hydrolysis of arylsulphates by arylsulphatase B.

Materials and methods. The homogeneous preparations of ox liver arylsulphatases $B1_\alpha$ and $B1_\beta$ were prepared

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and assayed by the method of FAROOQUI and ROY[4]. To 0.8 ml of 0.01 *M* nitrocatechol sulphate (dipotassium salt from Sigma Chemical Co; St. Louis, USA) in 0.5 *M* sodium acetate buffer, pH 5.4 was added the enzyme solution (20 µl). The mixture was incubated at 37 °C for 5 min and the reaction was terminated by the addition of 5 ml of 0.5 *M* sodium hydroxide. The amount of liberated nitrocatechol (ε 510 nm = 12,600) was determined spectrophotometrically. 1 unit of enzyme is defined as the amount which hydrolyses 1 µmole of nitrocatechol sulphate per min under usual assay conditions. The protein concentration was determined spectrophotometrically using an E 1% 280 nm of 17.0, and molar concentrations of arylsulphatase B were calculated from molecular weight[4] of 55,000. Diazonium salts of sulphanilic acid were prepared immediatly before use by the method of JERFY and ROY[7]. The concentration of the diazonium salts was normally between 10–25 m*M*. The monoazo *N*-acetylhistidine and monoazoacetyltyrosine were prepared by the method of TABACHNIK and SOBOTKA[13].

Some kinetic parameters of native and diazotized arylsulphatase $B1_\alpha$

	Native $B1_\alpha$	Sulphanilazo $B1_\alpha$
pH optimum	5.4	5.4
K_m value (m*M*)	1.85	1.87
V_{max} (mole/min/mg)	130	15

The enzyme was assayed as described in the text.

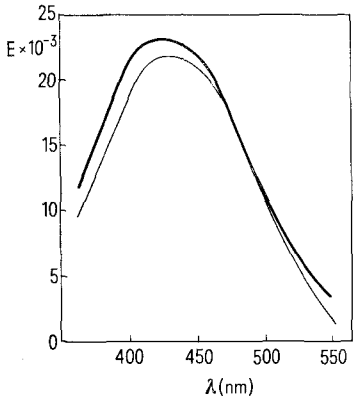

Fig. 1. The absorption spectrum of azosulphanilarylsulphatase $B1_\alpha$ (prepared at 4-fold excess of diazotized sulphanilic acid) in 0.1 *M* sodium hydroxide (heavy line). The thin line shows the absorption spectrum for the azoderivative of *N*-acetyl histidine.

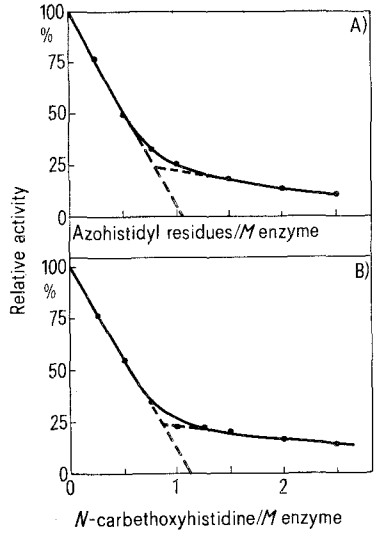

Fig. 2. Loss of arylsulphatase B activity (native enzyme 100%) as a function of the degree of diazotization (A) and carbethoxylation (B).

To 0.4 ml of arylsulphatases $B1_\alpha$ or $B1_\beta$ (100 µ*M*) in 0.1 m*M* sodium bicarbonate was added 0.04 ml of 0.56 *M* sodium bicarbonate and 0.02 ml of diazosulphanilic acid. After keeping the tubes for 30 min at room temperature, the reaction mixture was passed through a column (1.2 × 5 cm) of Sephadex G-25 in 0.1 *M tris* HCl, pH 7.4 and was eluted with the same buffer. The procedure resulted in a complete separation of light yellow azoarylsulphatase B_1 (yield 90%) from the other reactants. The azoarylsulphatase B_1 after concentration (Minicon A-25: Amicon Corporation, Lexington, Mass., USA) and filtration through 0.45 µ*M* filter was used for obtaining the absorption spectra. Arylsulphatases $B1_\alpha$ and $B1_\beta$ were also treated with diethyl pyrocarbonate by the method of LEE and VAN-ETTEN[8], and the formation of *N*-carbethoxy histidine residues was followed spectrophotometrically at 240 nm by comparison with the native enzyme by using ε 240 nm = 3,200.

Results and discussion. The treatment of arylsulphatases $B1_\alpha$ or $B1_\beta$ with 10-fold molar excess of diazotized sulphanilic acid resulted in 90% inhibition of enzyme activity. Similarly diethyl pyrocarbonate (10 m*M*) inhibited the activities of arylsulphatases $B1_\alpha$ or $B1_\beta$ by 90% in 3 min. Further the rate of inactivation of enzyme was proportional to diethyl pyrocarbonate concentration and exhibits pseudofirst order kinetics during the first 3–7 min of the carbethoxylation. Here it must be noted that arylsulphatase $B1_\alpha$ and $B1_\beta$ have the same kinetic parameters[14] and molecular weight[4]. The Table shows the kinetic properties of native and diazotized arylsulphatase B_1. It is conceivable that the decreased enzyme activity is not due to a change in the pH optimum or K_m value of this enzyme. The comparison of spectrum of sulphanilazoarylsulphatase B_1 with sulphanilazo *N*-acetylhistidine in 0.1 *M* sodium hydroxide is shown in Figure 1. It is obvious that the 2 spectra are very similar, and show that azohistidyl residues are the main chromophore in the visible region. The analysis of the spectra of sulphanilazoarylsulphatase $B1_\alpha$ or $B1_\beta$ showed the presence of 1 azohistidyl and 0.2 azotyrosyl residue per molecule of enzyme. A similar observation was also made by JERFY and ROY[7] for sulphanilazoarylsulphatase A. The absorption spectrum of enzyme treated with diethyl pyrocarbonate had a single peak at 238 nm characteristic of carbethoxyhistidine[15].

The relationship between the loss of enzyme activity and extent of diazotization and carbethoxylation is shown in Figure 2. It is clear that the modification of a single histidine residue (out of 21)[3] by diazotization or carbethoxylation causes 85–90% inactivation of arylsulphatases $B1_\alpha$ or $B1_\beta$. Furthermore when diazotization or carbethoxylation was carried out in the presence of 40 m*M* UDP *N*-acetylgalactosamine-4-sulphate[11,12] a substrate for arylsulphatase B, the extent of inhibition was greatly decreased (10-fold molar excess of diazotized

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sulphanilic acid causes 40% of inhibition). This observation suggests that the modified histidyl residue is in, or is close to the active site of this enzyme. Here it must be recalled that BENKOVIC and DUNIKOSKI[16] have shown that the hydrolysis of 2,5-(5)-imidiazolylphenyl sulphate proceeds by an intramolecular catalysis, probably through the imidazole moiety acting as a general acid-base catalyst. Furthermore it has also been demonstrated[17] that a synthetic polymer (polyethyleneimine), containing only histidine residues as functional group, is capable of catalyzing a 10–12-fold rate acceleration of the hydrolysis of nitrocatechol sulphate. Thus it can be suggested that general acid-base catalysis by imidazole group represent a general feature of at least arylsulphatases A[7] and B.

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Increased in vitro Phosphorylation of Rat Liver Nucleolar Proteins Following Triiodothyronine Administration

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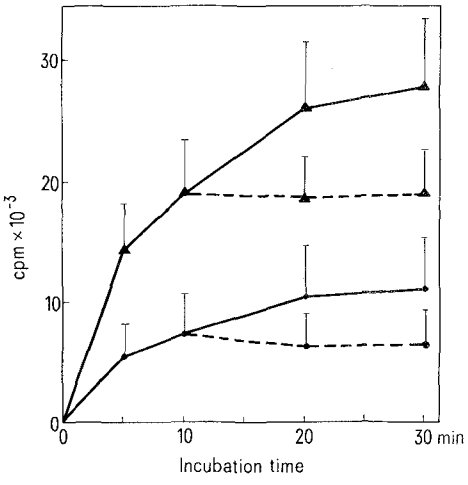
Summary. It has been shown that triiodothyronine (T_3) administration to thyroidectomized rats induces an increase in the in vitro net ^{32}P uptake into liver nucleolar proteins. Such an increase depends on a stimulation of the nucleolus-associated protein kinase activity and not on a lower dephosphorylation rate.

Thyroid hormones have been previously shown to stimulate the rate of synthesis of rapidly labelled nuclear RNA in hypothyroid rat liver[2]. Most of this newly synthesized RNA is preribosomal RNA which is specifically formed in the nucleolus and the increase in nucleolar RNA polymerase activity which takes place as early as 10 h after T_3 administration[3] appears to be in line with this observation. A consequence of the stimulated preribosomal RNA synthesis induced by thyroid hormones may be the accumulation of newly formed ribosomes in the cytoplasm of liver cells[4]. It is worth noting, however, that the mechanism by which thyroid hormones stimulate preribosomal RNA synthesis is still unclear.



Time-course of in vitro phosphorylation and dephosphorylation of liver nucleolar proteins from rats treated with T_3 for 24 h (▲) and controls (●). The nucleoli were incubated at 0.3 mg protein/ml as described in Materials and methods and 0.1 ml samples were withdrawn at the times indicated. After 10 min the reaction mixture was divided, a 10-fold excess of non-radioactive ATP was added to one part of the mixture and the incubation continued for a further 20 min. ▲----▲ and ●----●, incubations after addition of an excess of unlabelled ATP; ▲——▲ and ●——●, incubations without addition of unlabelled ATP. The points represent the mean ± SD of 8 experiments. In each experiment nucleoli pooled from 3 to 4 rats were used.

Evidence accumulated in recent years indicates that the phosphorylation of nuclear non-histone proteins may be a part of the mechanism regulating gene expression in higher organisms[5]. In fact, the extent of nuclear non-histone protein phosphorylation appears to be correlated to the transcriptional activity of several tissues[6,7]. In addition, the phosphorylation of proteins associated to nucleoli has been suggested to play a significant role in the assembly and processing of nucleolar preribosomal particles[8].

This study was undertaken to investigate whether the well-known increase in the amount of preribosomal RNA and ribosomes induced in rat liver by T_3 administration[4] is associated with an enhanced phosphorylation of liver nucleolar proteins in vitro.

Materials and methods. Animals. Thyroidectomized male albino rats (Wistar strain) weighing 140–180 g were kept on standard laboratory diet and tap water ad libitum. Surgical thyroidectomy was performed 4 weeks prior to the experiment. T_3 (Merck A.G., Darmstadt, Germany) was injected i.p. in a single dose of 30 μg/100 g body wt. at fixed times before death. All animals were fasted overnight prior to sacrifice.

Preparation of nucleoli. Nucleoli were isolated from liver nuclei by the sonication procedure of HIGASHINAKAGAWA et al.[9]. The purified nucleolar pellet was suspended in 0.25 *M* sucrose containing 1 m*M* dithiothreitol (DTT)

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