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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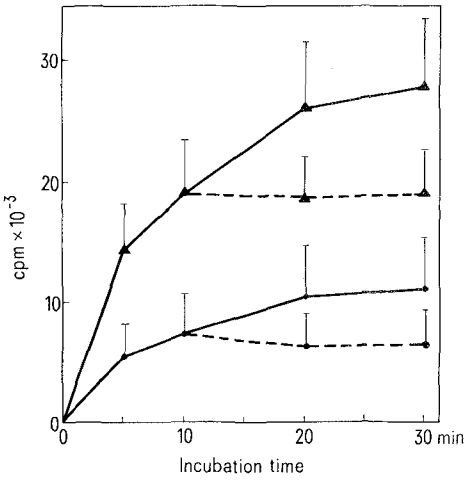
Summary. It has been shown that triiodothyronine (T_3) administration to thyroidectomized rats induces an increase in the in vitro net ^{32}P uptake into liver nucleolar proteins. Such an increase depends on a stimulation of the nucleolus-associated protein kinase activity and not on a lower dephosphorylation 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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and 50 µg/l of phenylmethane sulfonyl fluoride. The purity of the preparation was checked by phase contrast microscopy and by biochemical analysis (protein/DNA/RNA ratio).

In vitro phosphorylation and dephosphorylation of nucleoli. Nucleoli (0.3 mg protein/ml) were incubated at 30 °C in a reaction medium containing 250 m*M* sucrose, 100 m*M* *Tris*-HCl, pH 7.5, 20 m*M* $MgCl_2$, 100 m*M* NaCl, 6 m*M* NaF, 10 m*M* DTT and 0.005 m*M* (γ-^{32}P) ATP (1–1.5 Ci/mmole)[10]. At appropriate time intervals, aliquots were used for protein-bound ^{32}P determination according to the method B of REIMANN et al.[11]. The dephosphorylation rate due to phosphatase activity was determined by diluting the (γ-^{32}P)ATP 10-fold with unlabelled ATP in the reaction mixture after 10 min of incubation and measuring the radioactivity retained in the nucleolar proteins during the following 20 min. Radioactivity was measured in a Beckman LS 100 liquid scintillation spectrometer. All assays were performed at least in duplicate.

Analytical methods. Proteins were determined by the technique of LOWRY et al.[12] with bovine serum albumin as a standard. DNA and RNA were estimated according to the methods of BURTON[13] and CERIOTTI[14], respectively, by using appropriate standards.

Results and discussion. The effect of T_3 administration on in vitro net ^{32}P uptake into liver nucleolar proteins is shown in the Table. The results indicate a marked increase in ^{32}P incorporation into liver nucleolar proteins (to about 74%) which takes place 12 h after T_3 administration to thyroidectomized rats. A larger increase has been found after 24 and 48 h of treatment. The time course of in vitro phosphorylation and dephosphorylation of liver nucleolar proteins from control and T_3-treated rats is illustrated in the Figure 1. The results obtained in these experiments are in agreement with the observation of GRUMMT[10] who demonstrated that the *in vitro* turnover of phosphate groups under these conditions is very low. In addition, it can be seen that T_3 administration does not induce appreciable changes in the phosphatase activity of nucleoli incubated in vitro. This excludes the possibility that the increased amount of ^{32}P incorporated into liver nucleolar proteins from T_3-treated rats may depend on a lower dephosphorylation rate. Therefore, it can be assumed that the increased in vitro ^{32}P uptake into nucleolar proteins is due to a stimulation of the nucleolus-associated protein kinase activity induced by T_3 administration.

In vitro net ^{32}P uptake into liver nucleolar proteins

Time of treatment (h)	No. of experiments	Net ^{32}P uptake	Increase (%)	Student's *t*-test (*p*)
—	16	405 ± 195		
12	6	705 ± 248	+ 74	0.001
24	6	810 ± 328	+ 100	0.01
48[a]	4	778 ± 307	+ 92	0.01

The net ^{32}P uptake is expressed as pmoles ^{32}P incorporated into 1 mg nucleolar proteins /10 min. The values represent the mean ± SD. In each experiment, nucleoli pooled from 3 to 4 rats were used. [a]The rats received a second dose of T_3 24 h before sacrifice.

In another set of experiments (manuscript in preparation), the substrate specificity of the nucleolus-associated protein kinase activity was investigated. It has been shown that this kinase activity is able to phosphorylate casein and phosvitin, does not utilize to a significant extent histones or protamine as substrate, and is not stimulated by cyclic AMP.

So far the physiological role of the phosphorylation of nucleolar proteins has not yet been defined, although studies with isolated nucleoli support the suggestion that several highly phosphorylated proteins are involved in the assembly and processing of preribosomal particles[8]. According to this hypothesis, the increased phosphorylation of liver nucleolar proteins induced by T_3 administration to thyroidectomized rats may result in a stimulated maturation of preribosomal particles and, eventually, in the well known accumulation of newly formed ribosomes in the cytoplasm of liver cells.

Studies are in progress to investigate whether the phosphorylation pattern of liver nucleolar proteins is altered by T_3 administration.

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Influence on the Trypsin Activity by the Side Chain of Arginine Homologues

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Summary. The N-α-tosyl-*p*-nitroanilides of homoarginine and of the two shorter arginine homologues were synthesized. These compounds behave as specific, chromogenic substrates for trypsin.

In previous studies[1,2] the question arose to what extent longer or shorter homologues could replace L-arginine in its function as a part of biologically active molecules. In addition to other systems, the behaviour of trypsin toward suitable Arg-analogues presented itself as a simple model for this purpose.

In this study, use was made of *N*-α-tosyl-*p*-nitroanilides of homoarginine and the Arg-homologues shortened by 1 and 2 methylene groups, because, on the one hand, the tosyl group causes no racemization during its attachment as in the case of the benzoyl residue[3]. On the other hand, the *p*-nitroanilides are the more specific substrates than the corresponding esters[4].

Materials and methods. The shorter homologues with the tosyl group were synthesized in accordance with the method of RUDINGER[5], tosyl-homoarginine as described