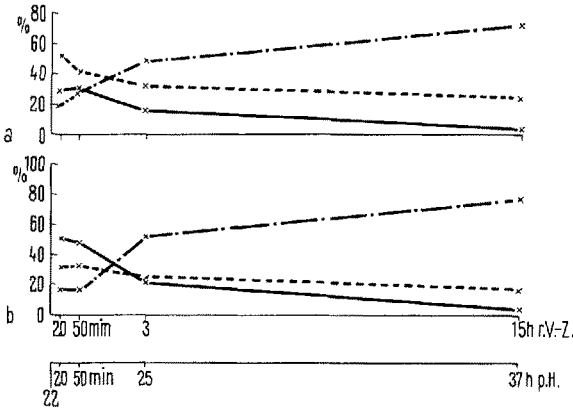
die nukleoläre Aktivität im Versuchskollektiv etwas höher, was mit der starken Grössenzunahme und besonders intensiven Markierung des Kernkörperchens zusammenhängt. Auch das Verhältnis der gesamten cytoplasmatischen zur gesamten Kern-EW-Synthese[12] unterliegt keinen nennenswerten Schwankungen (Aktivität Cytoplasma/Aktivität Kern: Kontrollen = 6,15; 24 h pH 7,05; 40 h pH 5,85). Bei einer starken Steigerung der gesamten RNS- und EW-Synthese der Zelle bleibt demnach die Relation der metabolischen Aktivität der einzelnen Zellbestandteile zueinander weitgehend erhalten.

Durch eine Doppelinjektion von ^{3}H-Cytidin + ^{3}H-Thymidin bzw. von ^{3}H-Phenylalanin + ^{3}H-Thymidin



Mittlerer Prozentsatz der ^{3}H-Cytidin-RNS-Aktivität/Zellstruktur (Schnittfläche; —— Nukleolus; --- Karyoplasma; -·- Cytoplasma) als Funktion der radioaktiven Versuchszeit (r.V.-Z.) und der Zeit nach partieller Hepatektomie (pH) für Kontrollen (a) und Versuchstiere (b).

sind solche Zellen, deren Kerne sich in der S-Phase befinden oder sie durchlaufen haben, auf Grund einer wesentlich stärkeren nuklearen Markierung eindeutig zu erkennen und von Zellen mit Kernen ausserhalb der DNS-Synthesephase abzugrenzen. Dabei läuft in Zellen mit DNS synthetisierenden Kernen die RNS-Migration ins Cytoplasma und die cytoplasmatische EW-Synthese genau so ab wie in den Zellen, deren Kerne nicht in die DNS-Synthese eingetreten sind (Tabelle II; vgl.[13] Lit.).

Summary. During the first wave of parenchymal liver regeneration in adult rats after partial hepatectomy, the cellular synthesis and migration of RNA and the metabolism of protein were studied by autoradiography following an injection of ^{3}H-cytidine or ^{3}H-l-phenylalanine and double injections of 1 of these precursors + ^{3}H-thymidine. The following results were obtained: the synthesis and migration of RNA and the metabolism of protein are enhanced under these conditions of proliferation. In spite of this, the relation of metabolic activity in nucleolus, karyoplasm and cytoplasm remains constant. By double injection techniques it is proved that no differences exist in migration of RNA into the cytoplasm and cytoplasmic protein synthesis between cells with or without DNA synthesizing nuclei.

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Metabolism of Pyridoxal Phosphate in the Liver of Hypo- and Hyperthyroid Rats

Several investigators have observed that in the liver of hyperthyroid rats there is a reduction of the activity of enzymes known to require pyridoxal phosphate as prosthetic group[1–3]. This holds for L-serine dehydratase, L-threonine dehydratase, cysteine desulphydrase, but not for aspartate aminotransferase. L-serine and L-threonine dehydratase activity is restored by in vitro addition of pyridoxal phosphate to the enzyme preparations[3]. These modifications are supposed to be due to a decrease in the liver pyridoxal phosphate content[4,5]. According to Mascitelli-Coriandoli and Boldrini[4], such a reduction is related to an impairment of the phosphorylation processes. In contrast to this hypothesis is the observation made by Horwath, according to whom even pyridoxine, added in vitro, restores L-serine and L-threonine dehydratase activities[3]. Therefore it seems more likely that the rate of catabolism of the prosthetic group is increased in hyperthyroidism rather than its rate of synthesis decreased.

In the attempt to explain how thyroid hormones control pyridoxal phosphate content in liver, we have measured the activity of the enzymes which synthesize the cofactor, pyridoxal kinase and pyridoxaminephosphate oxidase, and of the first enzyme which catalyzes its degradation, a phosphatase, in the liver of normal, hypo- and hyperthyroid rats. Determinations of the amount of pyridoxic acid excreted in urine have also been made.

Male rats, strain Wistar, weighing 50–60 g, have been used for the experiments. One group was fed for 6 weeks a normal diet (Diet Z, Zoofarm, Padova); a second group received for the same time the normal diet plus 0.15% of propylthiouracil; a third group was fed the same normal diet, plus 2% of thyroid powder (Costantino, Favria Canavese) in the last 10 days. All the animals were fasted for 8 h before the experiment.

The rats were killed by decapitation and the livers were rapidly excised, chilled in ice, and homogenized in 4 parts

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Table I. Pyridoxal kinase, pyridoxaminephosphate oxidase and phosphatase activity in the liver of normal, hypothyroid and hyperthyroid rats

	μmoles pyridoxal phosphate formed or split/g liver/h		
	Normal	Hypothyroid	Hyperthyroid
Pyridoxal kinase	2.00 ± 0.54 (9)	1.60 ± 0.58 (9)	6.06 ± 0.53 (6)
Pyridoxaminephosphate oxidase	0.211 ± 0.017 (7)	0.199 ± 0.011 (7)	0.195 ± 0.014 (5)
Phosphatase	4.97 ± 1.14 (7)	5.14 ± 1.55 (7)	7.85 ± 1.59 (7)

In parentheses is shown the number of experiments.

Table II. Pyridoxic acid in urine of normal, hypothyroid, and hyperthyroid rats

	μg pyridoxic acid excreted/24 h
Normal	61.8 ± 8.1 (12)
Hypothyroid	43.9 ± 13.2 (10)
Hyperthyroid	83.7 ± 7.0 (11)

In parentheses is shown the number of experiments.

of 0.15 *M* KCl. The homogenate was used as such for measuring the phosphatase, and was centrifuged 15 min at 18,000 *g* for measuring the kinase and the oxidase in the supernatant.

Pyridoxal kinase activity was assayed as described by McCORMICK et al.[6] by measuring pyridoxal phosphate formed from pyridoxal with apotryptophanase prepared from dried cells of *E. coli*.

Pyridoxaminephosphate oxidase was assayed under the conditions described by McCORMICK et al.[6], but the pyridoxal phosphate formed from pyridoxamine phosphate was measured with apotryptophanase purified according to NAKAHARA et al.[7].

The incubation mixture for measuring the phosphatase activity contained: 0.75 μmoles pyridoxal phosphate, 20 μmoles of *Tris*(hydroxymethyl)aminomethane and 20 μmoles maleate at pH 6.25 and 6–8 mg protein of the liver homogenate. After 90 min at 37 °C, the reaction was stopped by addition of trichloroacetic acid, and the inorganic phosphate was measured according to YOUNGBURG and YOUNGBURG[8].

Pyridoxic acid was determined in urine with the method of HUFF and PERLZWEIG[9], standardized with a sample of pyridoxic acid generously given by Merck & Co., Rahway, N.J.

As shown in Table I, pyridoxal kinase activity is only slightly decreased in the liver of hypothyroid rats, but increased by 205% in the same tissue of thyroid-fed rats. On the contrary, no modification is observed in the activity of the enzyme responsible for the oxidation of pyridoxaminephosphate. The phosphatase is unchanged in propylthiouracil treated rats, whilst it is raised by 58% in the liver of hyperthyroid rats.

Less pyridoxic acid is excreted in the urine of hypothyroid rats than in that of normal animals. A higher amount is found in urine of hyperthyroid rats (Table II).

Our results show that none of the enzymes responsible for the synthesis of pyridoxal phosphate is reduced in hypothyroidism: the oxidase is unchanged, the activity of the kinase is on the contrary strongly increased. Therefore the drop in the pyridoxal phosphate content in the liver cannot be ascribed to a reduction in the capacity of its synthesis. However, the marked decrease of adenosine triphosphate content, known to occur in the liver of hyperthyroid rats[10], might have an influence on the pyridoxal phosphate synthesis.

On the contrary, the results obtained measuring the rate of dephosphorylation of pyridoxal phosphate support the view of a higher rate of degradation of the cofactor in hyperthyroid rats, since it is shown that the phosphatase is markedly increased. Even the following degradation of the cofactor seems to occur rapidly in the hyperthyroid rats, as the amount of pyridoxic acid excreted in urine is also increased.

As far as the mechanism of pyridoxal kinase elevation in hyperthyroidism is concerned, it appears that it proceeds by way of a control by pyridoxal phosphate: when its content in liver is low, the kinase, first enzyme of the pathway leading to the synthesis of the cofactor[11], increases. Consistent with this view is the observation that pyridoxal kinase activity is depressed in rabbit brain after administration of pyridoxine[12].

It may be concluded that the decreased content of pyridoxal phosphate in the liver of hyperthyroid rats is not due to a reduction in the rate of its synthesis, but rather to an increase of its breakdown. It follows that pyridoxal kinase is increased, so that it can be stated that this enzyme is controlled by pyridoxal phosphate[13].

Riassunto. È stata determinata nel fegato di ratti normali, ipotiroidei ed ipertiroidei l'attività degli enzimi che portano alla sintesi del piridossalfosfato, la piridossalcinasi e la piridossaminfosfatoossidasi, e del primo enzima che ne catalizza la demolizione, una fosfatasi. In condizioni di ipertiroidismo sono aumentate la piridossalcinasi e la fosfatasi, mentre la ossidasi è invariata; nell'ipotiroidismo invece non si osservano modificazioni significative. La quantità di acido piridossico escreta con l'urina è diminuita nell'ipotiroidismo e aumentata nell'ipertiroidismo.

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