In uricotelic species, such as the rat, adaptive changes in metabolic flux and enzyme levels occur in the purine metabolic pathway when cells are rapidly growing. This is observed in both regenerating liver and in malignant tissues. The enzymes P-Rib-PP amidotransferase and IMP dehydrogenase increase in activity in both high uricotelic species, such as the chick, when compared to uricotelic animals. By treating immature roosters with the hormone β-estradiol, it is possible to induce rapid liver growth, allowing comparison of the regulation of purine biosynthesis and interconversion in high metabolic rate cells with different roles for purine metabolism. The tissue activities of P-Rib-PP amidotransferase, xanthine dehydrogenase, adenylosuccinate synthetase and lyase, AMP deaminase, IMP dehydrogenase, and GMP synthetase did not rise in livers from estradiol-treated chicks, as compared to controls. However, the rate of de novo purine synthesis triples and the intracellular level of P-Rib-PP doubles within 24 h of treatment. The biosynthesis of GMP is elevated at 12 and 24 h, but the levels of soluble nucleotide pools do not change. These data indicate that regulation of the de novo purine pathway in uricotelic species in a high metabolic situation is at the level of substrate availability (P-Rib-PP) and not due to changes in enzyme level or to feedback inhibition.

Uricotelic animals use synthesis of the purine ring system as a pathway for the excretion of excess nitrogen. In such animals, the domestic chicken for example, the level of de novo purine synthesis is 15-fold higher than that found in the rat (1). When birds are subjected to stress situations such as starvation or diets high in proteins (both conditions that lead to higher nitrogen excretion), the rate of purine synthesis is increased (2). Burns and Butterly (3) have demonstrated that a number of amino acids stimulate both total and de novo urate biosynthesis. They also found that both nitrogenous precursors and a readily available energy source were necessary for optimal urate biosynthesis in isolated chicken hepatocytes. Brand and Lowenstein (4) have shown that the activity of two enzymes (adenylsuccinate lyase and xanthine dehydrogenase) involved in the synthesis of urate, the end product of purine biosynthesis from small molecule precursors, increases in the livers of chickens starved for 4 days. In addition, the level of the first committed enzyme in purine biosynthesis, amidophosphoribosyltransferase, is elevated in livers from chicks fed diets high in protein (2). These studies show that, though normal levels of both purines and purine synthetic enzymes are high in the chick, the flux through the de novo pathway and the level of the enzymes can be increased by high levels of the amino acid substrates.

Livers from chickens treated with β-estradiol can provide a rapidly growing normal cell as a model system. Treatment with this hormone causes a rapid increase in liver weight and protein, RNA and DNA content (a 60% increase in 48 h) in preparation for the production of egg proteins (5). Jackson et al. (6, 7) and Katonuma and Weber (8) have shown that, in regenerating rat liver, another system that is used as a model for normal rapid growth, the level of certain purine biosynthetic enzymes is increased. This is also true in certain rat liver hepatomas (6–8). In livers from chicks treated with β-estradiol, a larger than normal portion of newly synthesized purines will be incorporated into RNA and DNA during the rapid liver growth instead of being excreted as uric acid. Thus, this tissue can be used as a model for study of regulation of purine biosynthesis as requirements for nucleotides vary.

The results of this study indicate that purine metabolism in rapidly growing uricotelic liver is regulated in a manner different than observed in mammalian uricotelic liver or in high amino acid feeding in the chick where nitrogen excretion is elevated. Enzyme levels in the chick do not change in response to the increased metabolic flux, in contrast to rat liver. Regulation appears to be primarily at the level of P-Rib-PP1 concentration which serves as the precursor for de novo purine biosynthesis.

**EXPERIMENTAL PROCEDURES**

*The abbreviations used are: P-Rib-PP, 5-phospho-a-D-ribopyrano-ose; PEI, polyethyleneimine; HPLC, high performance liquid chromatography; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; PPD, 3,5-diphenyloxazole; POPOP, 1,4-bis[2-(5-phenyloxazolyl)]benzene.

1 The "Experimental Procedures" are presented in miniprint as prepared by the authors. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. 82M-1170, cite the authors, and include a check or money order for $3.60 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.

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The chick liver homogenate makes the assay of adenylosuccinate difficult.

A radioactive assay developed by Dr. R. B. Hoppe (personal communication) was employed to measure the level of adenylosuccinate synthetase in the chick liver. The radioactive incorporation into purines of this enzyme was determined in 0.1 M potassium chloride, 2.5 M phosphoglyceric acid, and 0.3 M dihydroxyacetone. The homogenate was added to a 0.1 M potassium chloride, 2.5 M phosphoglyceric acid, and 0.3 M dihydroxyacetone (pH 7.0) and the reaction was stopped by adding 0.01 M perchloric acid. The mixture was homogenized and centrifuged at 10,000 g for 10 minutes and the supernatant was run on a high pressure liquid chromatography column and monitored at 254 n.

the chick liver homogenate was used as a source of this enzyme and it was found to be very inactive.

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interconversion pathway, as shown by the adenine-guanine pool sizes being channelled into the GMP branch of the purine biosynthetic pathway. Some regulation changes also occur as the IMP pool size increases during the initial 24 h of this increase in de novo synthesis but not under normal growth conditions.

The overall rate of purine biosynthesis was determined by the incorporation of formate into cellular purines (both acid- and base-soluble and those incorporated into RNA and DNA). The rate of de novo synthesis of purine nucleotides from 1C formate is increased by 7- to 8-fold in the liver of chickens treated with β-estradiol, showing that the rapid growth of the liver induced by the treatment with β-estradiol is supported by an increase in the rate of purine synthesis de novo. The branches of the purine interconversion pathways are equally expressed during the rapid growth situation. Some regulation changes also occur as the IMP pool size increases during the initial 24 h of this increase in de novo synthesis but not under normal growth conditions.

AMP synthesis has returned almost to normal, even though the overall rate of synthesis remains high. Thus, it appears that the rapid growth of the liver induced by the treatment with β-estradiol is supported by an increase in the rate of purine synthesis de novo. The branches of the purine interconversion pathways are equally expressed during the initial 24 h of this increase in de novo synthesis than under normal growth conditions.

| Enzyme levels in livers from β-estradiol-treated chickens |
|-----------------------------------------------------------|
| **Enzyme** | Activity |
| 24 h after injection (n = 6) | 48 h after injection (n = 6) | Control (n = 12) |
| Amido phosphoribosyltransferase | 4.7 ± 1.2 | 5.9 ± 0.4 | 5.6 ± 0.9 |
| Adenylosuccinate synthetase | 0.21 ± 0.10 | 0.17 ± 0.02 | 0.17 ± 0.03 |
| GMP synthetase | 0.062 ± 0.003 | 0.042 ± 0.002 | 0.044 ± 0.007 |
| Adenylosuccinate lyase | 4.02 ± 0.7 | 4.14 ± 0.04 | 3.46 ± 1.12 |
| AMP deaminase | 5.9 ± 0.9 | 5.8 ± 0.3 | 7.7 ± 2.2 |
| IMP dehydrogenase | 1.31 ± 0.31 | 1.58 ± 0.41 | 1.74 ± 0.87 |
| Xanthine dehydrogenase | 12.9 ± 0.3 | 14.1 ± 2.1 | 14.8 ± 5.9 |

* Each enzyme was assayed as described in the text. The levels were treated as described under "Experimental Procedures."
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