Synthesis of rat liver histidase is under multihor- 
omonal control; estrogen, glucocorticoid, and glucagon, 
via cAMP, induce this enzyme. By means of in vitro 
[3H]leucine incorporation into immunoprecipitated his-
idase, relative to that incorporated into total soluble 
protein, we have now demonstrated that de novo he-
aptic histidase synthesis, as well as catalytic activity, 
is selectively increased following hypophysectomy. 
Treatment of hypophysectomized rats with physiolog-
ical doses of T3 diminished histidase 
synthetic rates and catalytic activities to normal levels, 
despite concomitant elevation in total soluble protein 
synthesis. Thyrotoxic doses of T3 further reduced his-
idase synthesis to barely measurable rates. Since thy-
roid hormone is under pituitary regulation, this hor-
mon e may be primary in the hypophyseal suppression of 
histidase.

Estrogen does not induce hepatic histidase in the 
hypophysectomized rat. However, administration of 
the histidase suppressor, T4, or prolactin, which in itself 
has no effect on this enzyme, was ineffective in rein-
stating estrogen inducibility of histidase in the hypo-
physectomized animal. Thus, some as yet unknown 
hypophyseal agent is required for estrogenic inducib-
ility of this liver enzyme.

Hepatic histidase (L-histidine ammonia lyase, EC 4.3.1.3) 
of the rat is under multihormonal regulation. Previous studies 
have demonstrated that estrogen, glucocorticoid, glucagon, 
and cAMP each evoke increased de novo synthesis of histidase 
in liver, resulting in increased cellular content of this enzyme 
protein and enhanced catalytic activity (1–6). Furthermore, 
selective increases in hepatic histidase catalytic activity are 
observed following hypophysectomy (7), indicating that the 
pituitary secretes a factor(s) which is suppressive to this 
enzyme. The inhibitory action of this putative hypophyseal 
factor(s) has been found to be supplementary to and distinct 
from trophic influences of the pituitary on the testes and 
adrenal (7), which in themselves contribute to suppression of 

dhistidase activity under certain conditions (5, 8). ACTH, 
acting extra-adrenally, and growth hormone each decrease 
liver histidase activity to a minor extent and may be partially 
responsible for the hypophyseal suppression of hepatic histi-
dase. Since thyroxine has been reported to depress histidase 
catalytic activity (9–11), thyrotropic stimulation of thyroid 
hormone secretion may account for an additional portion of 
the pituitary suppression of this enzyme. These responses 
of histidase to hypophysectomy and thyroid hormone are atyp-
ical. The presence of the pituitary (12–17) and administration 
of thyroid hormone (17–25) are both known to enhance the 
activities and syntheses of many hepatic enzymes and indeed 
to promote generalized protein synthetic activity in liver. The 
present studies explore the mechanisms by which hypophy-
sectomy elevates and 3,5,3'-triiodothyronine (T3) diminishes 
hepatic histidase activity. We have inquired as to whether 
these endocrinologically evoked alterations in catalytic activ-
ity are consequent to corresponding changes in rates of de 
novo synthesis of hepatic histidase relative to total protein 
synthesis.

The histidase inducer, estrogen, in contrast to other in-
ducers of this enzyme, is unable to enhance hepatic histidase 
activity in hypophysectomized animals (26). We have exam-
ined the possibility that T3 or prolactin, the latter of which is 
under positive estrogenic regulation, may be capable of re-
storing estrogenic enhancement of liver histidase in the hypo-
physectomized animal and thereby serve as essential re-
quirements or mediators for this induction.

MATERIALS AND METHODS

Animals—Intact (300 to 350 g) and hypophysectomized (200 to 250 
g) male Sprague-Dawley rats were purchased from Taconic 
Farms, Germantown, NY. Intact, thyroidectomized, and hypophyse-
tomized 25-day-old female Sprague-Dawley rats were purchased 
from Charles River Breeding Laboratories, Wilmington, MA. Female rats 
remained intact or were thyroidectomized or hypophysectomized at 
21 days of age by the vendor; all were bilaterally ovarieectomized at 26 
days in our laboratory. Hypophysectomized and thyroidectomized 
rats were maintained on drinking water containing 5% glucose and 1% 
CaCl2, respectively, in addition to standard laboratory chow. Rats 
were killed by a blow to the head followed by decapitation. Complete-
ness of hypophysectomy was confirmed by significant reduction in 
adrenal, testes, prostate, and seminal vesicle weights.

Hormone Injection Schedule—Adult hypophysectomized male rats 
were administered eight daily intraperitoneal injections of the indi-
cated dosages of T3 (Sigma Chemical Company) dissolved in 0.9% 
NaCl beginning on the fourth day after hypophysectomy. Intact and 
hypophysectomized control rats received equivalent volumes of saline 
over the same time schedule.

Twenty-six-day-old ovarieectomized hypophysectomized female 

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1 The abbreviations used are: ACTH, corticotropin; T3, 3,5,3'-triio-
dothyronine; SDS, sodium dodecyl sulfate.
rate received eight daily subcutaneous injections of the following hormones per kg of body weight: 100 μg of estradiol-17β benzoate (Steraloids, Inc.) in propylene glycol, 50 μg of T3 in 0.9% NaCl, 5 mg of prolactin (bovine, NIH-P-135, 32.2 units/mg) in 0.9% NaCl. Control animals received appropriate volumes of diluents.

Histidase Assay—Histidase activity was measured by the method of Tubergen and Mehler (27), as modified in our laboratory (6). In experiments in which histidase activities were low (young animals of Table I) 0.1 mM dithiothreitol (Sigma Chemical Co.) was substituted for glutathione in the reaction mixture.

Rates of Histidase Biosynthesis Relative to Total Soluble Protein Synthesis—Two hours after the last hormone injection, animals were injected intraperitoneally with 5 mCi of [3H]leucine (40 to 60 Ci/mmol, Amersham/Searle) per kg of body weight. Following a 40-min incorporation period, the animals were killed and 25% liver homogenates were prepared in 10 mM Tris-Cl, pH 7.2, containing 14 mM MgCl₂ and 0.6 M KCl.

A portion of each homogenate was centrifuged at 122,000 × g for 60 min at 4°C, and the cytosol was used to determine incorporation of radioactivity into total soluble protein. One-half milliliter of these high speed supernatants was precipitated with 3 ml of ice-cold 10% trichloroacetic acid; the precipitates were washed three times with 3 ml of 10% trichloroacetic acid and then resuspended in 1 ml of 0.5% NaOH for determination of radioactivity in Aquasol (New England Nuclear). Total soluble protein synthetic rates were computed as the ratio of counts per min in trichloroacetic acid-precipitable protein and counts per min in equal volumes of unprecipitated cytosol, in order to adjust for variations in amounts of isotope reaching the liver.

Rates of isotope incorporation into immunoprecipitated histidase were determined as follows. A portion of liver homogenate was incubated at 55°C for 20 min, cooled to 4°C, and centrifuged at 66,000 × g for 60 min. This heat treatment removes many nonhistidase proteins, yet results in negligible loss of histidase activity (6). Histidase activities of the 66,000 × g supernatants were assayed. Mono-specific goat antibodies against rat liver histidase, prepared as described by Larmannet and Peigell (6), were added to aliquots of supernatants in quantities which were 125% of the amounts required to neutralize enzyme activity, as determined by prior immunotitration of the antibody. Radiolabeled histidase was immunoprecipitated in Buffer A (100 mM Tris-Cl, pH 7.5, 10 mM EDTA, 50 mM NaCl, 50 mM nonradioactive leucine, 1% Triton X-100 (Sigma Chemical Co.) and 0.5% sodium deoxycholate). Samples were brought to room temperature for 30 min, and immunoprecipitation was allowed to go to completion by remaining overnight at 4°C; the immunoprecipitates were collected by centrifugation at 2,000 × g for 20 min on a 2-mL layer of 1 M sucrose in Buffer A. The supernatants remaining on top of the sucrose were removed, and the immunoprecipitates were washed six times by resuspension and centrifugation in 2 ml of Buffer A. Quantitative immunoprecipitation of histidase was confirmed by determining that no enzyme activity remained in the supernatant and that incubation of the supernatants with a second portion of antihistidase resulted in no further precipitation of radioactivity.

To assure the specificity of the newly synthesized enzyme present in the immunoprecipitates and its identity with histidase, radioactive profiles of dissociated immunoprecipitates were assessed by sodium dodecyl sulfate acrylamide gel electrophoresis. The radioactive immunoprecipitates were suspended in 62.5 mM Tris-Cl, pH 6.8, containing 2% SDS, 5% mercaptoethanol, and 10% glycerol and solubilized by placing in a boiling water bath for 3 min. Samples were added to aliquots of supernatant  and  biosynthetic rates were elevated 2- and 3-fold, respectively (Fig. 2). During the same period liver weights and total liver protein content each decreased more than 40%, reflecting a marked reduction in bulk tissue components. Thus, hypophysectomy results in an increased bio-

Effects of Hypophysectomy on Hepatic Histidase Catalytic Activity and Synthesis—Eleven days following hypophysectomy, adult male hepatic histidase catalytic activities, expressed per g of liver, and biosynthetic rates were elevated 2- and 3-fold, respectively (Fig. 2). During the same period liver weights and total liver protein content each decreased more than 40%, reflecting a marked reduction in bulk tissue components. Thus, hypophysectomy results in an increased bio-

Effects of Increasing Dosages of T₃ on Hepatic Histidase Catalytic Activity and Synthesis in Hypophysectomized Rates—In order to ascertain whether hypophyseal suppression of hepatic histidase activity and biosynthesis could be the result of thyroid hormone action, histidase catalytic activities

Fig. 1. Typical profile of radioactive newly synthesized histidase. The histidase was immunoprecipitated from heat-treated liver cytosol and electrophoresed on a 10% sodium dodecyl sulfate polyacrylamide gel at 3 mA for 4 h as described under "Materials and Methods." The Coomassie blue stained marker gels at the top are: unlabeled similarly prepared histidase immunoprecipitate of liver cytosol (A) and purified hepatic histidase (B).
and rates of synthesis, relative to total soluble protein synthesis, were determined in livers of hypophysectomized adult males which had received eight daily injections of increasing doses of T₃ (Fig. 3A). An approximately 30% decrease in catalytic activity and biosynthesis was observed following daily injections of 5 μg of T₃/kg, both parameters were reduced to essentially negligible values following thyrotoxic doses of T₃ (150 μg/kg). The levels of histidase catalytic activity and synthesis observed in hypophysectomized rats administered 15 μg of T₃/kg are quite similar to those intact animals (cf. Figs. 2 and 3A), implying this T₃ dose is a replacement dose with respect to its effects on liver histidase. These data demonstrate that the T₃-induced reduction in histidase catalytic activity is due principally to a diminished rate of its biosynthesis. In contrast to its suppressive effects on histidase synthesis, thyroid hormone stimulated total soluble protein synthesis in livers of the same animals (Fig. 3B), in agreement with the findings of others (17, 25). However, it is to be noted that the marked decreases in relative histidase synthesis (histidase synthesis relative to total soluble protein synthesis) cannot be accounted for by the modest increases in total soluble protein synthesis.

Possible Roles of Thyroid Hormone in Estrogen Induction of Hepatic Histidase—The possible participation of thyroid hormone in estrogen induction of hepatic histidase has been investigated in the experiment depicted in Table I. Immature rats were employed to ensure that histidase activities would be sufficiently low, despite hypophysectomy- and thyroidectomy-induced elevations, to allow further increases in activity in response to estrogen administration, if they were to occur. Rats were ovariectomized in order to eliminate endogenous pubertal secretion of estrogen during the course of the experiment. As shown in Table I and as previously observed (26), estrogen induction of hepatic histidase activity, which was observed in the young ovariectomized animals, did not occur

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action of thyroid hormone on hepatic histidase. Administration of prolactin to hypophysectomized animals, which in itself exerted no effect on histidase activity, also did not reinduce estrogens inducibility (data not shown), suggesting that this hormone likewise does not play a role in estrogenic induction of this enzyme.

**Discussion**

Previous studies have shown that increased hepatic histidase catalytic activities in response to estrogen, glucocorticoid, glucagon, and cyclic AMP are due to accelerated rates of de novo synthesis of the enzyme (6). This and earlier studies (7) have demonstrated that hypophysectomy likewise results in a 2-fold increase in histidase catalytic activity, expressed per unit of liver weight, with a lesser increase in total liver histidase activity due to marked reduction in liver mass in these animals. The present study further demonstrates a 3-fold increase in histidase biosynthetic rate. The resultant of this enhancement in the synthetic rate of this enzyme and the influence of hypophysectomy on its degradative rate, which is at present unknown, determines the total quantity of histidase in the livers of these animals. Although elevations in the activities of a few other hepatic amino acid metabolizing enzymes have been reported following hypophysectomy (11, 29, 30), total hepatic protein synthesis (15-17), as well as a good many other hepatic enzyme activities (12-14), is diminished in hypophysectomized animals. One or more pituitary factors, therefore, seem to exert an atypical suppressive effect on the synthesis of hepatic histidase. Since hypophysectomy is capable of elevating histidase at various postnatal stages, this suppressive effect of the pituitary on histidase synthesis exerts a long term "dampening" effect on the multiphasic progressive rise in hepatic histidase synthesis and activity observed in liver during normal postnatal development of the intact rat (4, 31-34).

These effects of hypophysectomy are reversed by the administration of T3. The finding that histidase synthetic rate and catalytic activity decrease in parallel in response to increasing dosages of T3 suggests that the observed decreased catalytic activity is a consequence of reduced enzyme synthesis. It seems unnecessary to propose specific involvement of additional mechanisms to account for thyroid hormone inhibition of histidase activity, e.g., alternations in levels or activities of enzymic activators or inhibitors, post-translational modifications which decrease catalytic efficiency or enhanced enzyme degradation. The mechanism by which T3 inhibits histidase synthesis remains to be explored. Possible mechanisms underlying thyroid hormone suppression of histidase synthesis are: 1) reduction in the levels of mature histidase mRNA by inhibition of the rates of transcription of the histidase gene or processing of the gene transcript or by stimulation of histidase mRNA degradation; 2) specific curtailment in the efficiency of translating histidase mRNA.

Histidase catalytic activities and synthetic rates of livers of hypophysectomized animals are lowered to normal levels following administration of eight doses of 15 µg of T3/kg of body weight/day. Conversely, thyroidectomy results in a rise in the level of hepatic histidase activity. Thus physiological levels of thyroid hormone exert a major control over the synthesis and level of this enzyme. Pituitary regulation of thyroid hormone secretion via thyrotropin may provide the major physiological basis for hypophyseal suppression of histidase synthesis. Administration of either gonadotropins (7) or prolactin has little effect on histidase activity. However, growth hormone and ACTH, acting extra-adrenally, have been found to exert slight suppressive actions on hepatic histidase activities (7). Thus hypophyseal suppression of hepatic histidase biosynthesis seems to be due to the concerted action of thyroid hormone, growth hormone, ACTH, and possibly other unknown pituitary-derived or regulated substances. The magnitude of the response of hepatic histidase to thyroid hormone suggests that this hypophyseally regulated hormone may be the principal agent involved in the pituitary suppression of histidase biosynthesis.

Thyroid hormone-induced decreases in the rate of histidase synthesis occur concomitantly with an accelerated rate of total soluble protein synthesis, as observed in this and other (17, 25) studies, emphasizing the specificity of the effect of T3 on the synthesis of this liver protein. Administration of thyroid hormone in low physiological doses produces multiple specific anabolic effects within the liver. Among these are increased RNA polymerase activities (18, 19), nuclear protein kinase activity (20), mitochondrial Na+,K+-ATPase activity (21), and induction of synthesis of specific proteins, including mitochondrial α-glycerophosphate dehydrogenase (22), fatty acid synthetase (17), malic enzyme (23), and α-globulin (35, 36). Thyroid hormone is also known to reduce catalytic activities of monoamine oxidase, kynurenine-3-hydroxylase, and retinoic acid-sensitive NADH-cytochrome c reductase in the outer mitochondrial membrane (37), and alcohol dehydrogenase (38), as well as histidase, in the cytosolic fraction of rat liver.

To our knowledge, inhibition of histidase synthesis is the first report of an example of suppression involved in the de novo synthesis of a specific enzyme by thyroid hormone.

Although capable of liver histidase induction at all stages beyond the neonatal period (4, 6, 34), estrogen is ineffective as an inducer of this enzyme in untreated hypophysectomized animals or in hypophysectomized rats whose depleted estrogen receptors (39, 40) are partially restored by prolactin (39) or whose rate of histidase synthesis is reduced to normal levels by thyroid hormone replacement. Thus neither thyrotropin acting via thyroid hormone, prolactin, nor a number of other pituitary hormones (26) interact with estrogen to induce the synthesis of this enzyme. The identity of the required pituitary factor and the nature of its participation in the estrogenic induction of hepatic histidase remain to be elucidated.

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**References**

Pituitary and Thyroid Effects on Histidase Synthesis