ference was found in the spleen or the brain of these animals.

Although the conventional isolation techniques used in these studies (*7*) succeeded in removing detectable contaminating material from polysomes of euthyroid livers, our results show that free polysomes of thyroidectomized rats contain a large amount of nonpolysomal material which we have provisionally identified as ferritin. Furthermore, because this abnormality was mimicked by hypophysectomy and corrected in a dose-dependent fashion by exogenous T_3 administration, it is clearly a hormone-dependent phenomenon. Since the bound polysome profiles of thyroidectomized and euthyroid rats were identical, as were the free polysomes outside the 75*S* region, our studies call into question previous reports of differences in polysome structure and function in hypothyroidism (*3–6*).

To the best of our knowledge, this is the first report that concentrations of hepatic ferritin iron vary with thyroid hormone status. The mechanism by which this occurs is unclear. It is unlikely that existing ferritin apoprotein simply takes on more iron in hypothyroidism since the fivefold increase in ferritin iron observed would oversaturate the known ferritin apoprotein iron storage capacity (*16*). However, we cannot rigorously exclude this possibility because direct measurements of ferritin apoprotein were not made. A more probable explanation is that ferritin iron and apoprotein are elevated concomitantly, the result of either a primary increase in the synthesis or a primary decrease in the degradation of ferritin apoprotein. This phenomenon could be a direct effect of thyroid hormone or an indirect effect mediated by a primary increase in tissue iron in hypothyroidism (*17*).

However, it is interesting that ferritin iron increases in liver and kidney of hypothyroid animals but not in spleen and brain, an association that parallels the well-known thyroid hormone responsivity of these tissues as gauged by oxygen consumption (*18*). Of interest in this connection is the recent finding in our laboratory that thyroid hormone can inhibit the formation of specific mRNA species (*19*).

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Pineal *N*-Acetyltransferase Is Inactivated by Disulfide-Containing Peptides: Insulin Is the Most Potent

Abstract. *Pineal* N-*acetyltransferase can be inactivated in broken cell preparations by cystamine through a mechanism of thiol-disulfide exchange. Some, but not all, disulfide-containing peptides can inactivate this enzyme; the most potent inactivator is insulin. These findings suggest that a disulfide-containing peptide with high reactivity toward* N-*acetyltransferase may participate in the intracellular regulation of this enzyme.*

The activity of rat pineal *N*-acetyltransferase, the enzyme that controls large daily changes in melatonin production, is neurally stimulated 30- to 70-fold at night (*1, 2*). The stimulatory signals originate in another area of the brain, the suprachiasmatic nuclei. The pineal gland is stimulated for 10 to 14 hours every night through a neural circuit that includes central and peripheral structures. The suprachiasmatic nuclei appear to function as a biological clock. Light acting on this mechanism resets the clock every day, ensuring that it is entrained to environmental lighting, and it also prevents transmission of signals from the suprachiasmatic nucleus to the pineal gland. In the pineal gland, neural stimulation causes the release of norepinephrine, which elevates *N*-acetyltransferase activity through a β-adrenergic–cyclic AMP (adenosine 3′,5′-monophosphate) system; induction and activation mechanisms appear to be involved. When stimulation ceases, enzyme inactivation occurs and the activity of the enzyme decreases with a half-time ($t_{1/2}$) of about 3 to 4 minutes (*3, 4*).

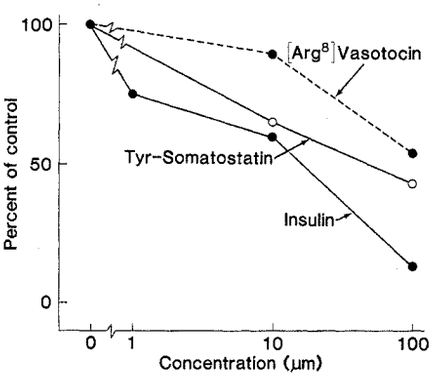

Fig. 1. Effects of disulfide-containing peptides on *N*-acetyltransferase activity. The peptides (*7, 8*) were dissolved in buffer (0.2*M* sodium citrate plus 0.02*M* barbital hydrochloride, *p*H 8.5) and 5-μl samples were added to 5 μl of a pineal gland supernatant. The solution was then incubated for 5 minutes. The enzyme activity was then measured in a total volume of 200 μl with acetyl coenzyme A (0.5 m*M*, 2 μCi/μmole) and tryptamine hydrochloride (10 m*M*) (*5*). Every value is based on duplicate runs, each of which was within 5 percent of the mean value. Insulin (100 μ*M*) did not go into solution completely at 0° to 2°C and was used as a suspension; lower concentrations were soluble.

The biochemical nature of this inactivation has been a mystery. We proposed that inactivation might occur by thiol-disulfide exchange (*5*)

$$\text{ENZ–SH}_{(\text{active})} + \text{X–S–S–X} \rightleftarrows \text{ENZ–S–S–X}_{(\text{inactive})} + \text{XSH}$$

This was primarily based on studies in which cystamine $[NH_2CH_2CH_2S–]_2$ was used (*5*). We now report that, of the nine disulfide-containing peptides we tested, the most potent inactivator was insulin. This is of some added interest because immunoreactive insulin reportedly oc-

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curs in brain cells (*6*), perhaps as a result of endogenous synthesis or transport from blood and subsequent uptake.

We used a pineal supernatant preparation [1 mg (wet weight) per 10 μl of 0.25*M* sucrose] (*5*). The glands were obtained from rats (100- to 125-g male Sprague-Dawley; Zivic-Miller Laboratories, Allison Park, Pennsylvania) treated with isoproterenol (15 mg/kg in two injections at a 2-hour interval) (*7*); animals were killed 2 hours after the second injection. This treatment elevates *N*-acetyltransferase activity about 60-fold through a mechanism essentially identical to that involved in the neural stimulation of the enzyme (*1*).

To examine the effects of each peptide, we mixed 5 μl of pineal supernatant with 5 μl of buffer containing the peptides (*7*); the mixture was then incubated for 5 minutes at 37°C. [1-^{14}C]Acetyl coenzyme A and tryptamine were then added to measure enzyme activity (*5*).

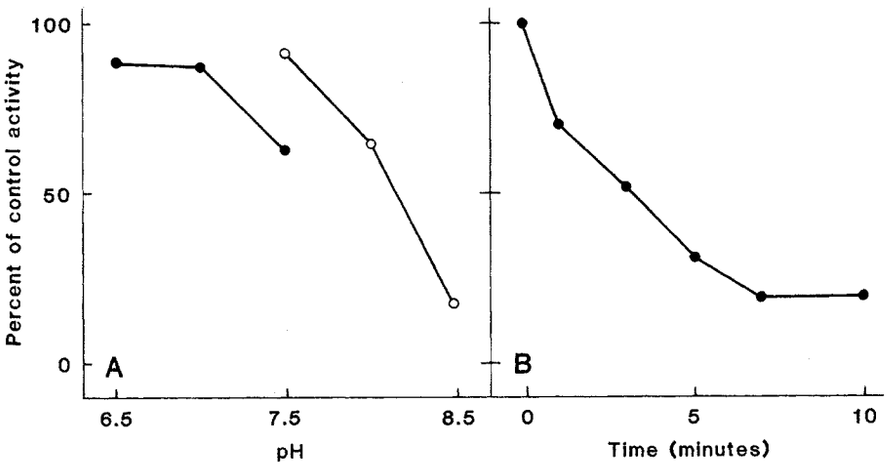

Fig. 2. Characteristics of the insulin inactivation of pineal *N*-acetyltransferase activity. *N*-Acetyltransferase activity is given as percent of control activity values. The inactivation period was 5 minutes. The assay was done in a total volume of 100 μl. For further details, refer to the legend to Fig. 1. (A). The solid circles represent observations obtained when 10 m*M* sodium phosphate buffer was used. The points represented by open circles were obtained with 10 m*M* barbital hydrochloride buffer. In all cases, the buffer solutions contained 100 m*M* sodium citrate (*19*). Measurements of *p*H were made at 0° to 2°C. Therefore, the actual *p*H during incubation (37°C) may have been slightly lower than those indicated. (B) Insulin (100 μ*M*) and pineal homogenates were incubated for the indicated periods before *N*-acetyltransferase assay reagents were added.

Table 1. Effect of DTT treatment on inactivation of *N*-acetyltransferase by insulin. Inactivation was done in the presence of 0.1 *M* sodium citrate plus 0.01*M* barbital hydrochloride, *p*H 8.5, in experiment 1 and 0.1*M* sodium citrate plus 0.01*M* sodium phosphate, *p*H 7.5, in experiment 2. The total volume during the treatment that preceded the assay was 20 μl in experiment 1 and 10 μl in experiment 2. Details of the assay are given in the legend to Fig. 2. The times given in parenthesis refer to periods of incubation (37°C) that preceded the assay. The DTT-treated insulin (experiment 1) was prepared by incubating (10 minutes, 37°C) a 5-μl sample of insulin solution (0.4 m*M*) with 5 μl of DTT (40 m*M*); this DTT-treated insulin preparation was then mixed with 5 μl of enzyme solution and 5 μl of 0.2*M* sodium citrate plus 0.02*M* barbital hydrochloride buffer, *p*H 8.5, and incubated (5 minutes, 37°C). The assay solution was then added. Insulin plus DTT (experiment 1) was prepared by mixing 5-μl samples of insulin (0.4 mM), DTT (40 m*M*), enzyme solution, and 0.2*M* sodium citrate plus 0.02*M* barbital hydrochloride buffer, *p*H 8.5 (total volume, 20 μl), and incubating the mixture (5 minutes, 37°C). The assay solution was then added. For preparation of buffer plus DTT or insulin plus DTT (experiment 2), a 10-μl volume of DTT solution (20 m*M*, 0.1*M* sodium citrate, *p*H 6.2) was incubated (37°C) with 10 μl of the insulin- or buffer-treated enzyme preparation. The assay solution was then added.

Treatment of enzyme preparation	Enzyme activity (nmole/min-mg)
Experiment 1	
Buffer (0 minutes)	3.7
Buffer (5 minutes)	3.5
Insulin (5 minutes)	0.2
DTT-treated insulin (5 minutes)	2.4
Insulin + DTT (5 minutes)	2.0
Experiment 2	
Buffer (0 minutes)	3.8
Buffer (0 minutes) + DTT (10 minutes)	4.7
Buffer (10 minutes)	3.1
Buffer (10 minutes) + DTT (10 minutes)	3.3
Insulin (10 minutes)	1.6
Insulin (10 minutes) + DTT (10 minutes)	1.9
Buffer (20 minutes)	1.7
Buffer (20 minutes) + DTT (10 minutes)	2.0
Insulin (20 minutes)	0.8
Insulin (20 minutes) + DTT (10 minutes)	1.0
Buffer (30 minutes)	1.3
Buffer (30 minutes) + DTT (10 minutes)	1.5
Insulin (30 minutes)	0.4
Insulin (30 minutes) + DTT (10 minutes)	0.6

Of the three peptides with a substantial effect on inactivation, insulin was clearly the most potent (Fig. 1). With insulin, significant inactivation was observed at 1 μ*M*, and about 50 percent inactivation occurred within 5 minutes in the presence of 10 μ*M*. Other peptides tested were insulin A, insulin B, somatostatin, [Tyr^1]somatostatin (Tyr, tyrosine), vasopressin, pressinoic acid, nerve growth factor, oxytocin, [Asu^{1,6}]-oxytocin (Asu, aminosuberic acid), and [Asu^{1,6}, Arg^8]vasopressin (Arg, arginine) (*8*). They had either a very small effect or none at all at concentrations of 10 and 100 μ*M* (data not given). We believe the differential effect of the S–S-containing peptides is due to differences in the local environment of the S–S bond, which could alter both the affinity of the peptide for *N*-acetyltransferase, and the reactivity of the S–S bond. The aminosuberic acid peptides and the insulin A and insulin B preparations were probably ineffective because they lack S–S bonds (*8*).

To further characterize the insulin inactivation of pineal *N*-acetyltransferase, we determined its dependence on *p*H and its time course. Increasing the *p*H from 6.5 to 8.5, which accelerates thioldisulfide exchange reactions (*9*), increased inactivation (Fig. 2A). This supports the suggestion that thiol-disulfide exchange is involved in the insulin inactivation of pineal *N*-acetyltransferase. The $t_{1/2}$ of inactivation with a concentration of 100 μ*M* insulin was about 3 minutes (Fig. 2B).

We then tested the effects of insulin in the presence of dithiothreitol (DTT). If insulin was acting by thiol-disulfide exchange, DTT treatment might reduce the disulfide bonds in insulin, thus preventing thiol-disulfide exchange between insulin and *N*-acetyltransferase molecules. We found that DTT treatment of insulin substantially prevented insulin inactivation of *N*-acetyltransferase; treatment of the enzyme with DTT and insulin simultaneously produced less inactivation (Table 1, experiment 1). These findings provide additional support for the conclusion that insulin is acting by way of thiol-disulfide exchange. In other experiments we attempted to reverse the insulin inactivation of *N*-acetyltransferase by treating insulin-inactivated enzyme preparations with DTT. This was not successful (Table 1, experiment 2).

One explanation for the unsuccessful attempts to reactivate insulin-inactivated *N*-acetyltransferase is that insulin reacts with the enzyme to form an inactive mixed disulfide and that the disulfide bond is buried and inaccessible to DTT. The inability to reduce mixed disulfides formed by disulfide exchange reactions is well known in other systems (*10*). Failure to reactivate the insulin-inactivated *N*-acetyltransferase is consistent with the observations that it is not possible to reactivate physiologically inactivated *N*-acetyltransferase by any in vivo or in vitro treatment (*4, 11, 12*) and that stimulation of enzyme activity appears to require new protein synthesis (*4*).

Insulin is known to activate phosphoprotein phosphatase (*13*), and a phosphorylation-dephosphorylation mechanism has been speculated to regulate *N*-acetyltransferase activity (*14, 15*). Thus, it is possible that insulin or closely related peptides could regulate *N*-acetyltransferase activity indirectly via a phosphorylation-dephosphorylation mechanism. Insulin appears to undergo thiol-disulfide exchange with a number of systems including the insulin receptor and thioredoxin, a dithiol peptide (molecular weight, 12,000) (*16*).

The finding that disulfides can inactivate *N*-acetyltransferase (*5*) leads to the formulation of an attractive hypothesis to explain the inactivation of *N*-acetyltransferase by a regulatory disulfide compound. A rapid reduced-oxidized shift would cause an immediate increase in the concentration of the inactivating (S–S) form of this compound. A shift of this sort might result from the specific action of an oxidase (*17*) or from a shift in the general redox state of the cell. The hypothetical shift in the redox state of the cell could be related to the change in membrane potential required for the adrenergic control of *N*-acetyltransferase activity (*18*).

Although it is unreasonable at this time to conclude that insulin is the active S–S compound regulating *N*-acetyltransferase activity, our findings raise the possibility that an S–S peptide participates in the physiological inactivation of this enzyme. Such a peptide could have far greater reactivity toward *N*-acetyltransferase than insulin, yet share a somewhat similar primary structure.

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8. Insulin A and B are the two chains of insulin joined by disulfide bonds in the native molecule. The preparations used were the periodic acid–oxidized forms and hence should not contain any SH or S–S bond; sulfur should exist primarily in the SO_3H form. [Tyr^1]Somatostatin is a modified form of somatostatin in which the alanine residue is replaced by tyrosine. Tyr-Somatostatin has an additional tyrosine added to the NH_2-terminal alanine residue. Pressinoic acid is a disulfide-containing peptide with six amino acids, Cys-Tyr-Phe-Gln-Asn-Cys (Cys, cysteine; Phe, phenylalanine; Gln, glutamine; Asn, asparagine). [$Asu^{1,6}$]Oxytocin is a modified oxytocin containing aminosuberic acid [$NH_2(COOH)CH(CH_2)_5COOH$]. Aminosuberic acid and tyrosine are linked by a peptide bond; the molecule does not have an S–S bond (Tyr-Ile-Gln-Asn-Asu-Pro-Leu-Gly-NH_2) (Ile, isoleucine; Pro, proline; Leu, leucine; Gly, glycine). [$Asu^{1,6}$, Arg^8]Vasopressin is a similar compound (Tyr-Phe-Gln-Asn-Asu-Pro-Arg-Gly-NH_2).
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Early Removal of One Eye Reduces Normally Occurring Cell Death in the Remaining Eye

Abstract. *During normal development of the hamster eye, there is a substantial loss of cells from the retinal ganglion cell layer in the first two postnatal weeks. If one eye is lost at birth, this cell death is reduced in the remaining eye. This may account for the increased ipsilateral projection from this eye to the thalamus and midbrain observed in these animals.*

Damage to the brain early in development can result in a variety of reorganized patterns of connectivity (*1*, *2*). Relatively little is known about the qualities of the growing brain that permit this pronounced plasticity. We have investigated one possible source of plasticity, the ubiquitous overproduction and subsequent death of neurons during normal development (*3*).

When one eye is removed at birth from hamsters or rats, the remaining eye shows at maturity a markedly increased ipsilateral projection to a variety of retinal targets (*2*, *4*). Several mechanisms could produce this effect: failure to retract axons that initially projected ipsilaterally; diversion of contralaterally going fibers; increased bilateral branching of optic nerve axons at the optic chiasm; or hypertrophy of the normal ipsilateral projection. Of these mechanisms, increased bilateral branching has been demonstrated (*5*). The demonstration that early in development retinal axons distribute more broadly ipsilaterally than they do at maturity supports the hypothesis of failure of retraction but does not prove it (*6*).

In chicks and anurans, enlargement of the terminal field available to a population of growing neurons markedly reduces neuron death in that population. For example, if an additional leg is grafted onto a developing chicken embryo, cell death is substantially reduced in the anterior horn of the spinal cord projecting to that limb (*7*). Removing one eye of a growing mammal may be an equivalent experiment, since it results in an increased terminal field available to the remaining eye. Reduced ganglion cell death in the remaining eye might thus be expected. If so, this result could account for failure of axon retraction or hypertrophy of the normal ipsilateral pathway.

Degenerating cells, which can be identified by their liquefied, darkly staining and pycnotic nuclei (*8*), can be seen in the retinal ganglion cell layer of the hamster eye in the first ten postnatal days of normal development (*9*). In this period, retinal axons are establishing their central connectivity (*10*). Studies in chick

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