Table 2. Ratios of taurine to protein in stress-induced hypertensive male rats. The group size for brain is 3, and for other groups 5.

Group	Heart weight (g)	Systolic blood pressure (mm-Hg)	Taurine (μmole per gram of protein)		
			Heart	Muscle	Brain
Stress-induced hypertension	1.33 ± 0.06*	161 ± 3*	224 ± 10*†	41.1 ± 1.9	16.1 ± 2.3
Stress-induced mild hypertension	1.32 ± 0.05*	138 ± 2*	172 ± 12	45.5 ± 9.2	
Unstressed	1.15 ± 0.04	122 ± 2	166 ± 11	52.3 ± 9.3	18.2 ± 1.2

* $P < .05$ when compared to unstressed group. † $P < .025$ when compared to mildly hypertensive group.

age at death between group 1 and group 3.

Samples of aorta from patients with or without congestive heart failure showed no difference in taurine concentrations. In congestive heart failure, the mean value was 5.3 ± 0.8 μmole per gram of material precipitable by trichloroacetic acid ± standard error for six samples; the mean for the controls was 4.3 ± 0.3 μmole (six samples). This suggests that the increase in ventricular taurine found in congestive heart failure may be specific to the heart, and is not part of a general increase in body taurine.

Hypertensive rats show an increase in the ratio of taurine to protein in the heart similar to that observed in human congestive heart failure. Rats exposed to environmental stress (loud noises, flashing lights, and cage oscillation) develop hypertension (*6*). Table 2 shows that mild and marked degrees of hypertension produced in this way are associated with cardiac hypertrophy. In addition, the markedly hypertensive animals show an elevation of taurine relative to protein in the heart. There is no corresponding alteration in taurine in skeletal muscle or brain. The ratio of taurine to protein is also increased in the hearts of spontaneously hypertensive male rats of the Okamoto strain (*7*) by comparison with age-matched Wistar controls. In hypertensive rats the mean concentration of taurine in the heart was 351 ± 44 μmole per gram of protein (seven animals) compared to 163 ± 12 μmole in the controls (seven animals, Student's *t*-test between the two groups, $P < .001$). Taurine concentrations have also been shown to be increased in the right ventricles of dogs with experimentally induced right ventricular failure caused by constriction of the pulmonary artery (*8*).

The analyses for taurine was performed by homogenization of the tissue sample in five parts of water. A sample was taken for protein determination (*9*). One volume of 20 percent trichloroacetic acid was added to the remainder, and the precipitated protein was separated by centrifugation. The supernatant was extracted with ether to remove the trichloroacetic acid, and the aqueous layer was taken to dryness on a rotary evaporator. The residue was refluxed with 5 ml of 6*N* HCl for 15 minutes to hydrolyze phosphoethanolamine, which otherwise interferes with the taurine assay. The sample was evaporated to dryness and the residue was taken up in 1 ml of water and layered on an ion-exchange column containing AG 50 H^+ cation exchange resin (5.5 by 0.7 cm) layered over an equal quantity of AG 1 Cl^- anion exchange resin. The taurine was eluted from the column with water and assayed by a ninhydrin procedure (*10*). The loss of taurine was corrected as described (*11*) by the recovery of tracer amounts of [^{14}C]taurine added at the beginning of the procedure. Certain samples of human tissue were analyzed again to determine whether there were differences between sites. The repeated analyses fell into two groups. One group (six samples) differed by an average of 0.5 ± 0.1 μmole of taurine per gram (wet weight) from the first analysis, but another group (four samples) showed a variation of 3.9 ± 0.7 μmole. Analyses of samples frozen for as long as a year have shown no differences in taurine concentrations, demonstrating that taurine in autopsy samples is stable.

Nothing explicitly is known about the function or functions of taurine in the heart. The ultimate significance of our observation that taurine concentrations are elevated in human congestive heart failure cannot be assessed at present. It may be involved in the pathophysiology of heart disease or it may be a secondary and unimportant consequence of the disease. The observation is of interest, however, in that chemical changes have rarely been demonstrated in human heart failure.

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Thyroid Hormone Action: In vitro Demonstration of Putative Receptors in Isolated Nuclei and Soluble Nuclear Extracts

Abstract. *Saturable binding activities for triiodothyronine were demonstrated in vitro with isolated nuclei and soluble nuclear extracts of rat liver, kidney, and cultured GH_1 cells. The binding activity can be extracted from nuclei in soluble form with no significant change in hormone affinity and has properties of a nonhistone protein.*

Thyroid hormones appear to regulate a wide variety of biological processes in higher organisms, varying from oxygen consumption to cell growth and differentiation. Studies of thyroid hormone effects on amphibian metamorphosis and RNA polymerase activity in rat liver suggest that the diverse biological effects of these hormones may result from a primary effect on the control of gene expression (*1*).

We have demonstrated that triiodo-

thyronine (T_3) and thyroxine (T_4) induce a maximal threefold increase in the growth rate of GH_1 cells (a rat pituitary tumor cell line) (*2*). The estimated free hormone concentrations in culture which induce a half-maximal biologic effect were $0.8 \times 10^{-11} M$ for T_3 and $1 \times 10^{-10} M$ for T_4 (*2*).

Studies on the binding of [^{125}I]T_3 and [^{125}I]T_4 after incubation of hormone with intact cells demonstrated high-affinity, saturable binding sites in the cell nucleus (*3*). The estimated equilibrium dissociation constants determined by Scatchard analysis were $2.9 \times 10^{-11} M$ for T_3 and $2.5 \times 10^{-10} M$ for T_4 (*3*). The good correlation between these affinities and the hormone concentrations inducing a half-maximal biologic effect in this system suggested that these high-affinity nuclear binding sites function as receptors for the thyroid hormones. This description of high-affinity, saturable nuclear binding after incubation of the thyroid hormones with intact cells in culture is similar to that made by Oppenheimer *et al.* (*4*) after injection of hormone into intact rats.

In both cases, after [^{125}I]T_3 was incubated with intact GH_1 cells (*3*) or injected into intact animals (*5, 6*), T_3 associated with a macromolecule could be extracted in soluble form by incubation of nuclei with $0.4 M$ KCl (*3, 5, 6*). This procedure extracts approximately 50 to 70 percent of the T_3 bound to saturable nuclear sites (*3, 5*). The binding activity extracted from rat liver was reported to be a nonhistone protein (*5*) as judged by the extractability at different *p*H values. Surks *et al.* (*5*) reported, however, that saturable binding for T_3 was not detected if the nuclear extract of rat liver was incubated with [^{125}I]T_3 in vitro. This suggested that nuclear structural and functional integrity was necessary for association of T_3 with the nuclear binding activity (*5*).

We first demonstrated that high-affinity, saturable nuclear binding for T_3 can be detected with isolated nuclei of GH_1 cells and rat liver by incubation of hormone directly with nuclei in vitro (*3*). We report here that saturable binding activities can also be demonstrated by incubating T_3 in vitro with soluble nuclear extracts as well as with isolated nuclei of rat liver, kidney, and GH_1 cells.

GH_1 cells were grown in an atmosphere of 95 percent air and 5 percent CO_2 in Ham's F-10 medium containing 15 percent horse serum and 2.5 percent fetal calf serum (GIBCO) (*2*). To prepare GH_1 cell nuclei, cells were harvested in the late logarithmic phase of growth with a rubber policeman and centrifuged at $500g$ for 5 minutes. The cell pellet was washed three times with 10 ml of serum-free Ham's F-10 medium by repeated dispersion and centrifugation. All further procedures were carried out at 4°C. The final cell pellet was homogenized in at least ten volumes of STM buffer [$0.25 M$ sucrose, 20 mM tris(hydroxymethyl)aminomethane, and 1.1 mM $MgCl_2$ adjusted to *p*H 7.85 at 25°C] by 15 strokes at 5000 rev/min with a motorized Teflon pestle (Tri-R Instruments).

The homogenate was centrifuged at $800g$ for 10 minutes. The homogenate pellet was used to prepare nuclei by two successive suspensions and centrifugations in 10 ml of STM buffer containing 0.5 percent Triton X-100 (Packard) (*3, 7*). In nuclei isolated by this procedure, the ratio of protein to DNA was approximately 2.0 and that of RNA to DNA was 0.22. Protein, DNA, and RNA were determined as described (*8, 9*).

To prepare rat liver and kidney nuclei, euthyroid male Sprague-Dawley rats were killed by subluxation of the cervical spine. Approximately 0.3 to 0.5 g of tissue was excised, minced, and washed in STM buffer to remove serum protein. Nuclei from kidney and liver were prepared by using Triton X-100 as described for GH_1 cells.

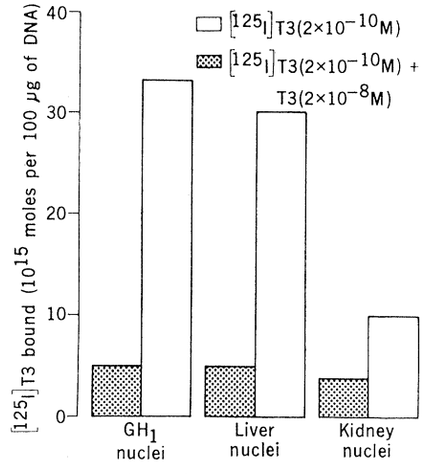

Fig. 1. In vitro binding of [^{125}I]T_3 to isolated nuclei of GH_1 cells, rat liver, and rat kidney. Isolated nuclei containing 20 to 50 μg of DNA were incubated with [^{125}I]T_3 (final concentration, $2 \times 10^{-10} M$) and with [^{125}I]T_3 plus a 100-fold excess of nonradioactive T_3. Nuclear binding was determined as described in the text. The "specific" saturable binding with $2 \times 10^{-10} M$ [^{125}I]T_3 is measured by the extent of inhibition by nonradioactive T_3 (open bar minus the shaded bar). Each point represents the mean of three determinations; each determination was within 10 percent of the mean.

Binding of T_3 to isolated nuclei was determined with an incubation mixture, prepared at 0°C, which contained a suspension of nuclei equivalent to 20 to 50 μg of DNA and STM buffer containing 2.0 mM ethylenediaminetetraacetic acid (EDTA), and 5.0 mM dithiothreitol in a volume of 0.9 ml. [^{125}I]T_3 (final concentration, $2 \times 10^{-10} M$), with and without a 100-fold molar excess of nonradioactive T_3, was added to the incubation mixture in 0.1 ml of STM buffer, and the preparation was incubated at 37°C for 45 minutes. [We had shown that the binding reaction of [^{125}I]T_3 with isolated nuclei attains an equilibrium within 30 to 40 minutes at 37°C (*3*).] After incubation, the samples were chilled in an ice bath and then centrifuged at $1000g$ for 8 minutes. The nuclear pellet was suspended in 1.0 ml of STM buffer containing 0.5 percent Triton and centrifuged again. [^{125}I]T_3 binding was determined in the resultant pellet with a gamma spectrometer. "Specific" saturable [^{125}I]T_3 binding in the nuclear pellet was considered to be that which was inhibited by a 100-fold excess of nonradioactive T_3.

The [^{125}I]T_3 (320 c/mole (Abbott) was examined by chromatographic analysis (*2, 3*) and found to be 98 percent [^{125}I]T_3 and 2.0 percent [^{125}I]iodide. The nonradioactive T_3 (Sigma) was also subjected to chromatography and found to be greater than 98 percent pure.

The nuclear binding activity was dissociated from isolated nuclei by incubation with 2.0 to 5.0 ml of extraction buffer (STM buffer containing 5.0 mM dithiothreitol and $0.4 M$ KCl). The suspension was gently agitated every 5 minutes for 15 minutes and then centrifuged at $6000g$ for 10 minutes. Approximately 25 to 30 percent of the total nuclear protein and less than 2 percent of the DNA was extracted by this procedure.

Binding of [^{125}I]T_3 to the nuclear extract was determined with an incubation mixture containing 0.3 ml of extract (50 to 100 μg of protein), 0.1 ml of STM buffer, 2.0 mM EDTA, and 5 mM dithiothreitol in a volume of 0.4 ml. Extraction buffer containing no nuclear extract and buffer containing 80 μg of bovine serum albumin served as controls. [^{125}I]T_3 (final concentration, $2 \times 10^{-10} M$) with and without a 100-fold excess of nonradioactive T_3 was added in 0.05 ml of STM buffer. The mixtures were incubated at 37°C

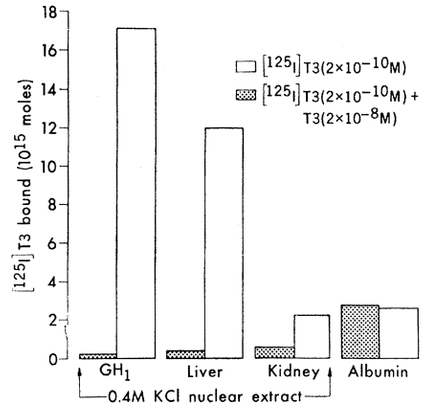

Fig. 2. In vitro binding of [^{125}I]T_3 to soluble nuclear extracts of GH_1 cells, rat liver, rat kidney, and bovine serum albumin. Nuclei containing 100 μg of DNA were extracted with 0.4*M* KCl as described in the text. The extracts, as well as buffer and bovine serum albumin controls, were incubated with [^{125}I]T_3 (final concentration, $2 \times 10^{-10}M$) alone or plus a 100-fold excess of nonradioactive T_3. The radioactivities eluted with the buffer control with both concentrations of T_3 were identical; this value was subtracted from the radioactivities eluted with the nuclear extracts and the albumin controls. The saturable binding with $2 \times 10^{-10}M$ [^{125}I]T_3 is measured by the extent of inhibition by nonradioactive T_3 (open bar minus the shaded bar). The results reflect the saturable binding extracted from nuclei containing 100 μg of DNA. The total protein values in the extracts were 55, 60, and 67 μg for GH_1 cells, rat liver, and kidney, respectively. The albumin control contained 80 μg of protein. Each point represents the mean of three determinations. Each determination was within 12 percent of the mean.

for 30 minutes and then transferred to an ice bath for an additional 40 minutes.

Bound and free [^{125}I]T_3 was determined by separation at 4°C on a 0.9 by 4.0 cm column (2.5 ml) prepared with Sephadex G-25 (fine) that had been swollen with STM buffer at 37°C. The entire sample was applied to the column and eluted with 1.5 ml of STM buffer to separate the bound and free forms. The eluted bound T_3 and the free T_3 that remained on the column were quantitated with a gamma spectrometer. "Specific" saturable [^{125}I]T_3 binding to the eluted fraction was considered to be that which was inhibited by a 100-fold excess of nonradioactive T_3. Trichloroacetic acid (3.0*M*, 0.5 ml) was added to the eluted bound T_3 to precipitate protein. After quantitation of the eluted [^{125}I]T_3, the sample was centrifuged at 8000*g* for 20 minutes to collect the protein precipitate. Protein was quantitated as described (*8*).

With control samples containing only extraction buffer, less than 0.5 percent of the total [^{125}I]T_3 radioactivity was eluted by 1.5 ml of STM buffer if the columns were prepared at least 2 to 4 hours before use and the flow rate was no greater than 1.5 ml/min. Occasionally, for unknown reasons, a higher percentage of radioactivity was eluted when extraction buffer was incubated with [^{125}I]T_3 compared to buffer also containing a 100-fold excess of nonradioactive T_3. Therefore, the elution of radioactivity of the extraction buffer controls was examined before a binding experiment, and the Sephadex was used only if the eluted counts were identical.

Figure 1 illustrates the binding of [^{125}I]T_3 to isolated nuclei of rat liver, kidney, and GH_1 cells after 45 minutes of incubation at 37°C. "Specific" saturable binding activities for T_3 (open bar minus the shaded bar) of similar magnitude were observed in GH_1 cell nuclei and rat liver nuclei. The saturable binding in rat kidney nuclei was somewhat lower. We have consistently observed this difference with rat kidney nuclei. To determine whether this reflects a different affinity or total number of binding sites would require a careful analysis with varying hormone concentrations.

Figure 2 shows the magnitude of saturable binding for T_3 with 0.4*M* KCl extracts prepared from rat liver, kidney, and GH_1 cell nuclei, each containing 100 μg of DNA. The albumin control demonstrated no saturable binding at these hormone concentrations. When compared to saturable binding in the corresponding isolated nuclei (Fig. 1), the saturable binding in nuclear extracts was 60 percent for GH_1 cells, 48 percent for rat liver, and 33 percent for rat kidney. These in vitro values for GH_1 cells and rat liver are similar to the percentages of total dissociable saturable T_3 binding activities determined by extraction of nuclei prepared after incubation of intact GH_1 cells (*3*) or injection of intact rats (*5*) with [^{125}I]T_3. This suggests that the T_3 binding activity was dissociated from nuclei with no substantial change in T_3 binding affinity and that the binding activity remained stable when incubated with hormone at 37°C. In addition, the in vitro reassociation of T_3 with nuclear extract suggests that the binding observed with isolated nuclei does not result from a specific nuclear membrane transport system for T_3.

The possibility exists that the binding activity determined with nuclear extracts in vitro differs from that measured with intact GH_1 cells or in intact animals. To determine whether these binding activities are identical would require characterization after purification. Rat liver and GH_1 cell nuclear extracts demonstrate approximately a tenfold greater affinity for T_3 than T_4 in vitro (*10*). This difference in relative affinity is similar to that determined with intact GH_1 cells (*3*), and suggests that the binding activity determined in vivo and in vitro may be identical.

Our results contrast with those of Surks *et al.* (*5*), who reported that the binding activity in the nuclear extract was rapidly inactivated at 37°C and that specific reassociation of T_3 did not occur in vitro. This might result from differences in proteolytic activity in the extract (different methods were used to isolated nuclei), or from differences in the composition of the incubation mixture used in the binding assay.

Chromatography of the 0.4*M* KCl extract from GH_1 cell nuclei on Bio-Rex 70 (Bio-Rad) by the method of van den Broek *et al.* (*11*) suggested that the binding activity was not a histone protein (*10*). This, along with the fact that the binding activity was inactivated with trypsin and Pronase and not with deoxyribonuclease or ribonuclease, suggested that the binding activity is a nonhistone protein (*10*).

According to current concepts on the mechanism of steroid hormone action, the steroid enters the cell and binds to a specific cytosol receptor, and the hormone-receptor complex transfers after activation to sites in the nucleus (*12*). The interaction of the steroid-receptor complex with nuclear sites is thought to mediate in some way the biological action of the steroid hormones.

Several investigators have reported that cytosol from a variety of tissues contains saturable binding proteins for T_3 and T_4 (*13*). It is not clear, however, whether these binding proteins function as receptors for the thyroid hormones or mediate some other function such as deiodination or metabolism of the thyroid hormones. We reported that binding of [^{125}I]T_3 to isolated GH_1 cell nuclei appeared to be enhanced if the cells were first incubated with nonradioactive T_3 before nuclear isolation (*3*).

The question of whether this apparent increase in nuclear binding in vitro resulted from an increase in the affinity, stability, or synthesis of the nuclear "receptor" or from the transfer of a T_3-cytosol receptor complex remains to be defined.

The nuclear binding of $[^{125}I]T_3$ after incubation of hormone with intact GH_1 cells (*3*) is similar to that observed by Oppenheimer and colleagues (*4, 5*) after injection of $[^{125}I]T_3$ into rats. As we have demonstrated, putative receptors for T_3 detected in vitro are similar in isolated nuclei and soluble nuclear extracts of rat liver, kidney, and GH_1 cells. This suggests that our studies of thyroid hormone action in cell culture might serve as a valid model for studying the mechanisms of thyroid hormone–receptor interaction as well as thyroid hormone regulatory effects in vivo.

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Leprosy: Confirmation in the Armadillo

Abstract. *Bacteria isolated from lesions of lepromatoid leprosy in the armadillo were studied in comparison with* Mycobacterium leprae *isolated directly from human lepromatous leprosy lesions. Three methods were used to show that the bacteria from the lesions of the armadillo were identical to those of the human lesions: (i) extraction of the bacteria with pyridine and subsequent staining with various techniques, (ii) the competence in clearing bacilli (CCB) test, and (iii) the Mitsuda test.*

Leprosy is an important public health problem in many parts of the world. It is generally considered to be one of the oldest diseases known to afflict mankind and yet, ironically, it is one of the least understood. Only limited knowledge is available concerning the epidemiology of the disease (such as modes of transmission and susceptibility) as well as the most effective means to control and treat it. This is the result of not being able to grow the causative agent, *Mycobacterium leprae*, in culture and also, until recently, of not having a suitable animal system in which to study the disease. Although multiplication of the organism will occur in the mouse footpad, the lesions that result are microscopic and no gross evidence of disease can be seen in immunologically intact rodents.

Leprosy is a very unusual disease with respect to the different courses it takes in humans and the large variety of lesions that it presents. The organism *M. leprae* is an intracellular parasite and as such its growth is controlled by the cell-mediated side of the immune response. While many individuals are immunologically capable of localizing the disease and manifest the more benign type known as tuberculoid leprosy, some individuals are unable to eliminate the organism and thus develop the severe disseminated form known as lepromatous leprosy. In addition, there is a large group that falls somewhere between these two polar forms immunologically, and such patients develop a dimorphous form known as borderline leprosy. Because of these variations in host response, leprosy is considered by many to be the most complete model of the granulomatous diseases, and the study of it could therefore result in significant contributions to an understanding of cell-mediated immunity and its role in other infectious processes.

Storrs (*1*) and Kirchheimer and Storrs (*2*) were able to produce lesions in the nine-banded armadillo (*Dasypus novemcinctus*, Linn.) after inoculation of *M. leprae* isolated from a patient with lepromatous leprosy. Lesions containing large numbers of acid-fast bacilli were seen and histopathologic examination showed changes that were consistent with a diagnosis of lepromatous leprosy (*3*).

In order to ascertain that the organisms isolated from infected armadillos were indeed *M. leprae* and not some other closely related mycobacteria, we decided to compare them with *M. leprae* isolated from human cases. This comparison was done with the use of tests that had been shown to be valid for differentiating *M. leprae* from other mycobacteria.

The infected armadillo tissue which we studied had been taken from two infected animals (*4*): armadillo 5, which had been inoculated 31 months prior to its death with *M. leprae* from a human lepromatous case, and armadillo 18, which had been inoculated 26 months before its death with organisms obtained from a strain of human *M. leprae* that had been passaged through the footpads of mice.

For the identification of the mycobacteria isolated from armadillos 5 and 18 we used three methods; these methods confirmed that the bacilli found in infected armadillos are *M. leprae*.

1) Effect of pyridine treatment on acid-fastness, Baker's stain for phospholipids, and fluorochrome staining with the use of auramine-rhodamine: Of all other known mycobacteria, only *M. leprae* completely loses its ability to be stained by the above three methods after 2-hour treatment with pyridine (*5*). To determine the reaction of the mycobacteria from the armadillo, smears and sections were prepared from each of the armadillo specimens, from human lepromatous lesions, from murine leprosy lesions (*M. lepraemurium*), and BCG (bacillus Calmette-Guérin). The specimens were treated with pyridine for 2 hours and stained along with untreated control slides. All slides were read "blind," and it was found that the organisms from the armadillos behaved identically to human *M. leprae*; that is, they lost their acid-fastness, their ability to be stained with