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SELECTIVE ALTERATIONS IN HEPATIC ENZYME RESPONSE AFTER REDUCTION OF NUCLEAR TRIIODOTHYRONINE RECEPTOR SITES BY PARTIAL HEPATECTOMY AND STARVATION

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SUMMARY. Partial hepatectomy results in a decrease in the nuclear triiodothyronine binding capacity of rat liver. Although we confirmed reports that starvation causes a reduction in binding capacity, starvation alone did not fully account for the decrease after hepatectomy. Reduction in nuclear binding sites both by hepatectomy and starvation failed to impair the response to triiodothyronine administration as measured by an increase in the enzyme activity of mitochondrial α-glycerophosphate dehydrogenase, but significantly blocked the triiodothyronine response as measured by cytosolic malic enzyme. These findings indicate that triiodothyronine response is influenced by currently undefined metabolic factors and raises the possibility that such factors cause a selective decrease in receptors associated with the gene for malic enzyme.

Since the initial description of specific nuclear binding sites for T_3[1], a substantial body of evidence has accumulated to indicate that these sites are important in the initiation of thyroid hormone action at the cellular level[2]. Considerable interest has recently been expressed in the possibility that the number of receptor sites might be modified under various physiological and pathological conditions. Thus, Samuels et al.[3] have shown that in GH_1 tissue cultures, T_3 effects a prompt reduction in the number of its receptor sites with a corresponding decrease in the rate of growth hormone synthesis. In contrast, however, the number of receptor sites measured in the intact animals does not appear to change with varying thyroidal status[4,5] nor were there any indications that the level of receptor sites could be altered by other factors. We were therefore interested to find that partial hepatectomy caused a significant reduction in nuclear binding sites[6]. More recently, two reports have appeared that starvation causes a significant reduction in the number of nuclear receptor sites[7,8] Accordingly, we determined whether the reduction of binding sites after partial hep-

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ABBREVIATIONS USED. α-GPD, α-glycerophosphate dehydrogenase; malic enzyme, ME; L-T_3, L-3,5,3' triiodothyronine.

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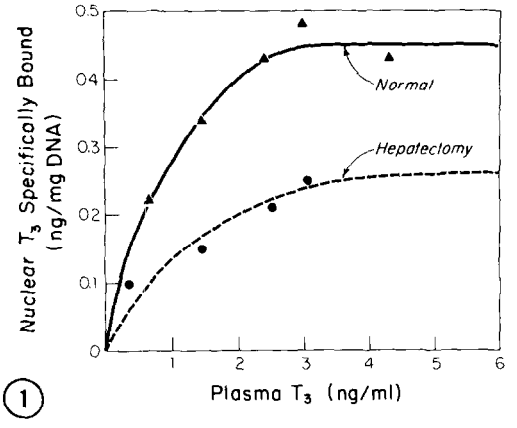
atectomy could be attributed to starvation. The reduction in binding sites both by hepatectomy and starvation also provided us with an opportunity to assess the relationship between the reduction in the number of receptor sites and the induc-duction of hepatic enzymes, α-GPD and ME by T_3.

METHODS. Male sprague-Dawley rats (150-200 g) were used in all experiments. Animals were rendered hypothyroid by surgical thyroidectomy and subsequently by administration of 100 μCi [^{131}I].

Partial hepatectomy was performed by the method of Higgins and Anderson[9]. All injections were carried out by the intravenous route through the tail vein. [^{125}I]T_3 (500 mCi/mg) was obtained from Abbott Pharmaceutical Laboratories. Nonradioactive T_3 was purchased from Sigma Chemical Incroporated, St. Louis, Missouri. Animals were starved for the period specified in the test but allowed tap water ad libitum during these periods. Plasma T_3 by radioimmunoassay was determined by the method of Surks et al.[10]. Hepatic cytoplasmic ME was measured by the method of Hsu and Lardy[11]. Hepatic mitochondrial α-GPD was determined by the method of Lee and Lardy[12]. Nuclear binding capacity was quantitated both by in vivo techniques[13] and by in vitro methods[14,15] as previously described. Stability of the receptor sites was assessed as follows. Nuclei were labeled by in vivo injection of tracer T_3. After preparation, the nuclei were incubated at 37° in the standard incubation medium in the presence of excess T_3 (2.0×10^{-7} M). Retention of radioactively labeled T_3 by the nuclei was measured in serial nuclear aliquots. The rate of decrease of nuclear radioactivity was considered to be the sum of the rate of dissociation of radioactively labeled T_3 from the receptor sites and the disappearance of receptor sites due either to proteolytic digestion or to leaching of such receptor sites from the nucleus into the medium[16]. DNA was determined by the method of Burton[17] and protein by the method of Lowry[18].

RESULTS. Nuclear binding capacities were performed using both in vivo as well as in vitro techniques. Figure 1 illustrates the maximal binding capacity in control and partially hepatectomized animals determined by in vivo techniques 24 hours after the surgical procedure. A 45% decrease in maximal binding capacity was noted, from baseline values of approximately 0.22 ng/mg DNA to 0.05 ng/mg DNA. In confirmation of a recent report by Leffert and Alexander[19], we noted that the plasma concentration of T_3 in these animals fell from a baseline value of 0.69 ± 0.139 ng/ml to 0.32 ± 0.143 ng/ml 24 hours after hepatectomy. From the nuclear/plasma ratio of tracer T_3, we could calculate that the nuclear concentration of T_3 fell from baseline values of 0.45 ng/mg DNA to 0.25 ng/mg DNA and resulted in a reduction in the percent of saturation from 48% under baseline conditions to 20% after hepatectomy.

Scatchard analysis of in vitro nuclear binding indicated a signficant reduction in the binding capacity without alteration in the association constant. Al-



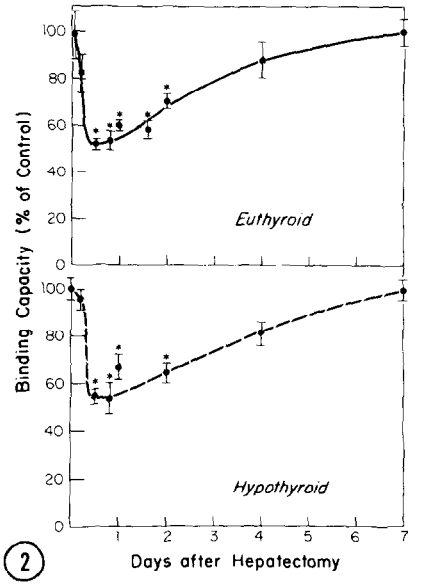


Figure 1

Nuclear binding capacity was determined by in vivo techniques in euthyroid rats 24 hours after hepatectomy and in control animals. The specifically bound nuclear T_3 was determined from the product of the nuclear/plasma ratio, after the correction for nonspecific binding, and the plasma T_3 concentration. The nuclear binding capacity is decreased by 50% in hepatectomized animals. The values represent the means from 3 animals.

Figure 2

Nuclear binding capacity was determined by in vitro techniques in euthyroid and hypothyroid animals at different times after hepatectomy. The asterisks indicate a statistically significant difference from control values ($p < 0.005$, or less); bars $\pm$ S.D. Each group consisted of 4 animals.

ready 12 hours after hepatectomy, a 50% loss in nuclear receptor sites occurred (Figure 2). A statistically significant decrease in receptors persisted until 2 days after hepatectomy and by 7 days maximal binding capacity had returned to normal baseline values. Sham operation did not lead to a statistically significant decrease in nuclear binding capacity at corresponding time intervals. The stability of nuclear receptors as defined under "Methods" was the same for nuclei derived from control and hepatectomized animals. The decrease in binding capacity/mg DNA after hepatectomy cannot be explained by induction of DNA synthesis which is know to follow hepatectomy. Increases in DNA synthesis start to

occur 16-20 hours after hepatectomy[20] and a 50% decrease in nuclear binding sites was already apparent 12 hours after hepatectomy. Moreover, the decrease in nuclear binding capacity at later time points was due to a net loss of binding sites in the regenerating liver remnant and not simply to an increase in DNA synthesis.

Since it was noted that hepatectomized animals lost 5-10% of their total body weight 1 day after hepatectomy, the possibility arose that the reduction in binding capacity observed after hepatectomy might simply represent the effects of starvation noted by Burman et al.[7] and DeGroot et al.[8]. In confirmation of the reports cited, a significant decrease in binding capacity was noted. Nevertheless, 17 hours after starvation was started the reduction in binding capacity amounted to only 31% $\pm$ 3 S.D. of control values, whereas, after hepatectomy, a 50% $\pm$ 2.5 S.D. decrease in binding capacity was already noted 12 hours after the surgical procedure. Differences between starvation and hepatectomy at 17 hours were significant at $p < 0.01$ level using analysis of variance. The stability of receptors as well as their affinity constants were identical in starved and control animals. These findings made it unlikely that the rapid decrease in binding capacity observed after hepatectomy could be only attributed to starvation.

The effects of hepatectomy and starvation on receptor number provided us an opportunity to assess the physiological consequences of the reduction in nuclear binding capacity. We therefore examined the T_3 response by measuring the activity of mitochondrial α-GPD and cytoplasmic ME. Euthyroid animals were injected iv with 0.5 mg T_3/100 g body weight 17 hours after hepatectomy. This dose was sufficient to saturate all the nuclear receptors for the duration of the experiment[4]. Twenty-four hours after T_3 injection and 41 hours after hepatectomy, levels of the enzymes were determined (Figure 3). Simultaneous binding capacities were determined and a 50% depression in binding capacity persisted during the time of T_3 action. No significant differences in α-GPD levels was noted between control (0.894 $\pm$ 0.027 S.D.) and hepatectomized animals (0.867 $\pm$ 0.0149 S.D.) and the response of α-GPD levels to T_3 was indistinguishable in both groups (Figure 3).

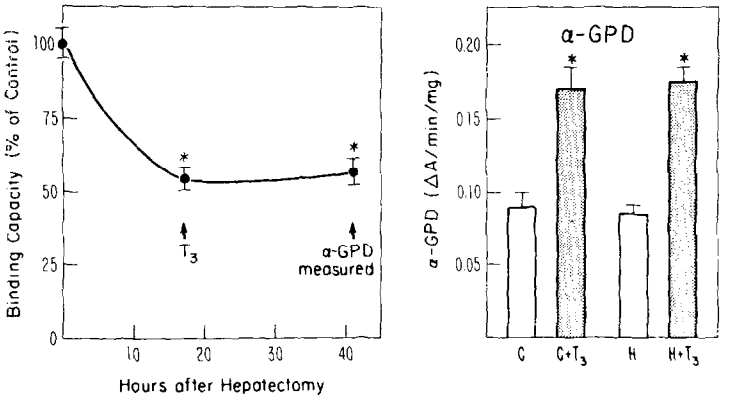


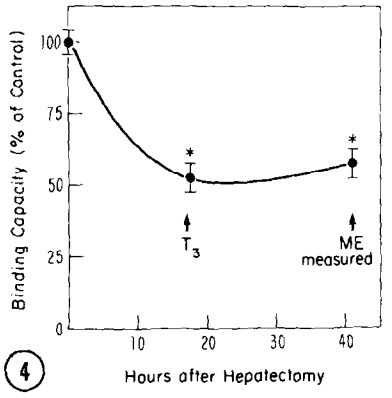
Figure 3

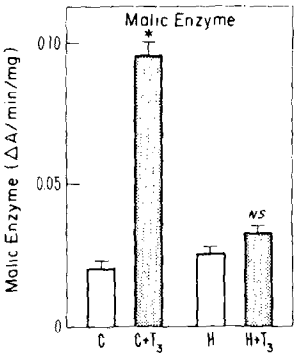
Euthyroid control animals (C) and rats hepatectomized (H) 17 hours earlier were injected with 0.5 mg T_3/100 g BW. The α-GPD response was measured 24 hours after T_3 injection. The asterisks indicate a statistically significant difference from control values ($p < 0.005$, or less); bars ± S.D. Each group consisted of 5 animals. Two additional experiments yielded identical results.

In contrast, however, hepatectomy appeared to block the induction of ME (Figure 4). In an experiment of identical design to that used in the study of α-GPD response, administration of T_3 did not result in a significant increase in ME activity above the hepatectomized baseline control.

We next examined the effects of T_3 on enzyme induction in starved animals. After 24 hours of starvation, animals received 0.5 mg T_3/100 g body weight. Starvation was continued and the animals were killed 24 hours subsequently. During the time of T_3 action, starvation resulted in a 30% decrease in nuclear binding capacity. The rise in α-GPD was comparable in starved and control animals. In contrast, however, the ME response was markedly diminished in starved animals. These findings confirm the studies of Tarentino et al.[21] who showed a similar dissociation between the response of α-GPD and ME to T_3 in starved animals. In other experiments, animals received 0.5 mg T_3/100 g body weight after 3 days of starvation and the α-GPD response was measured 24 hours later. Again, the rise in α-GPD above baseline levels was identical in control and in starved animals (Figure 5).

DISCUSSION. The experiments described in this communication clearly indicate that





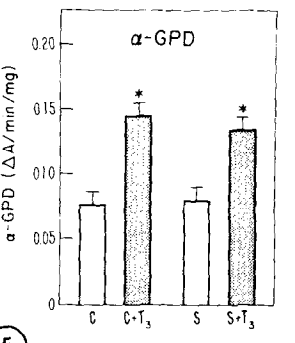


Figure 4

Euthyroid control animals (C) and rats hepatectomized (H) 17 hours earlier were injected with 0.5 mg T_3/100 g BW. The ME response was measured 24 hours after T_3 injection. The asterisks indicate a statistically significant difference from control values ($p < 0.005$, or less); bars $\pm$ S.D. Each group consisted of 5 animals. NS not statistically significant. Two additional experiments were performed with identical results.

Figure 5

Euthyroid control animals (C) and starved (S) rats were injected with 0.5 mg T_3/100 g BW after 3 days of starvation. The response was measured 24 hours after T_3 injection. The asterisks indicate a statistically significant difference from control values ($p < 0.025$, or less); bars $\pm$ S.D. EAch group consisted of 5 animals. One additional experiment yielded identical results.

hepatectomy can cause a major reduction in the number of hepatic receptor sites. Although our findings confirm the observation of others[7,8] that starvation can also result in a decrease in receptors, this effect by itself cannot completely explain the reduction in receptor sites observed after partial hepatectomy.

Of considerable interest to us was the sharp dissociation between the response of α-GPD and ME to the administration of T_3 both after partial hepatectomy and starvation. The induction of α-GPD appeared uninfluenced by the marked loss of nuclear receptors whereas in contrast, the induction of ME was inhibited to a major extent.

A number of explanations of this discrepancy can be advanced. First, one can argue that the response of α-GPD and ME is actually not under the control

of nuclear T_3 receptor sites. This would be most unlikely given the correlation between α-GPD, ME and nuclear occupancy[22] and the susceptibility of both to inhibitors of RNA formation[23]. Alternatively, one can postulate a number of schemes in which starvation and hepatectomy can modulate in a differential fashion the effect resulting from the binding of T_3 to a reduced number of T_3 receptors. Any model must take into account the fact that a 50% reduction in receptor sites leads to unimpaired α-GPD response and almost complete inhibition of ME induction. A speculative explanation which, however, is appealing on the basis of its simplicity is that hepatectomy or starvation in some way reduces the number of T_3 receptors associated with certain genes such as those of ME but preserves the number of T_3 receptors associated with other genes such as those for α-GPD. A selective reduction of receptor sites could be brought about by a modification of the T_3 receptor attachment site on the chromatin. A selective reduction of ME associated receptors could then lead to an impaired ME response but would allow a normal α-GPD induction This formulation would raise the general possibility of metabolic control through selective modulation of receptor subsets associated with specific portions of the genome. Regardless of the eventual explanation of this phenomenon, any comprehensive description of thyroid hormone action at the cellular level must take into account the disparate results with respect to the induction of these hepatic enzymes under the circumstances described in this report.

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