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The Influence of Fasting and the Thyroid State on the Activity of Thyroxine 5'-Monodeiodinase in Rat Liver: A Kinetic Analysis of Microsomal Formation of Triiodothyronine from Thyroxine*

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ABSTRACT. In the present studies, we have evaluated the effects of fasting, variations in thyroid state, and interactions thereof on the kinetic properties of the microsomal enzyme in rat liver that generates T_3 from T_4. The formation of T_3 from T_4 (T_3-neogenesis) in preparations of rat liver microsomes enriched with 10 mM dithiothreitol was monitored by assessing the formation of $[^{125}I]T_3$ from $[^{125}I]T_4$ in the presence of various concentrations of stable T_4. T_3-neogenesis in this system was a saturable process that obeyed the laws of first order enzyme kinetics. The effects of diverse *in vivo* manipulations on the kinetics of T_3-neogenesis were assessed. The V_{max} was markedly decreased in preparations from thyroidectomized animals, being, respectively, partially and completely restored to normal with daily maintenance therapy with T_4 (0.5 and 1.5 μg/100 g BW) and markedly increased after the administration of supraphysiological replacement doses of T_4 (5.0 μg/100 g·day). K_m was not affected by alterations in thyroid state.

In experiments concerning the effects of fasting, cognizance was taken of the fact that hypothyroidism regularly evolves as a consequence of fasting. The V_{max} was decreased markedly, and K_m was slightly decreased in preparations from intact rats fasted 72 h but not given physiological replacement with T_4 or T_3. In contrast, V_{max} and K_m were unchanged in hepatic microsomes from intact rats fasted 72 h but given daily parenteral replacement with T_4 (1.5 μg/100 g) or T_3 (0.5 μg/100 g).

These data demonstrate that hypothyroidism resulting from either thyroidectomy or fasting produces decreased intrinsic activity of the microsomal T_4 5'-monodeiodinase. In contrast, fasting without concurrent hypothyroidism does not influence the intrinsic activity of the hepatic T_3-neogenetic enzyme. (*Endocrinology* **108:** 472, 1981)

THE GENERATION of T_3 from T_4 in peripheral tissues takes place through the enzymatic substitution of a hydrogen for an iodine atom at the 5' position of the T_4 molecule. The hepatic enzyme that catalyzes this reaction is found within the microsomal fraction (1–5), and its activity is enhanced by supporting cofactors present in the cellular cytosol (1–3). Current evidence suggests that the principal cofactor for this reaction is reduced glutathione (GSH) (2, 3, 6, 7), which serves to maintain the T_4 5'-monodeiodinase in its active reduced form (8, 9) and as a hydrogen donor in the enzymic transhydrogenation of T_4 to yield T_3 (2, 8).

In man, the overall generation of T_3 from T_4 in peripheral tissues, a process that we have termed T_3-neogenesis, is markedly reduced by fasting (10–16). A similar effect of fasting occurs in rat liver, since we and others have shown that T_3-neogenesis is greatly decreased in slices and homogenates of liver from rats fasted for 48–72 h (17–21). In seeking the mechanism of this effect, we conducted experiments in which T_3-neogenesis was evaluated in diverse mixtures of microsomes and cytosols from fed and fasted animals, variously enriched with one cofactor or another (2). From the results, we concluded that the principal abnormality in the T_3-neogenesis system produced by fasting is a deficiency of cytosol cofactor, most likely GSH itself, the intrinsic activity of the enzyme itself being unchanged. Other investigators have concluded, however, that the decreased hepatic T_3-neogenesis that results from fasting is due not only to a cytosolic abnormality but also to a major decrease in the activity of the enzyme itself (3, 21).

In earlier studies we found that T_3-neogenesis was markedly decreased in preparations of rat liver from hypothyroid rats (22), a finding that has been confirmed by others (19, 23). Fasting in the rat results in decreased levels of total and free T_4 and T_3 (17, 18, 20, 24) and an

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augmented TSH response to the administration of TRH (24), findings which suggest the presence of hypothyroidism. Therefore, we questioned whether the decreased activity of the T_3-forming enzyme reported by others to result from fasting might be due to the associated hypothyroidism. The present studies were performed to clarify this question by examining the interaction of thyroid state and fasting on the kinetic properties of the T_4 5'-monodeiodinase in rat liver.

Materials and Methods

Animals and treatment regimens

Intact and thyroidectomized male Sprague-Dawley rats, weighing 150–200 g, were purchased from Charles River Breeding Laboratories (Wilmington, MA). Animals were given a diet of pelleted chow containing 14% protein, 6% fat, and 54% carbohydrate and tap water *ad libitum* for 1 week before and during experiments. In studies of the effects of fasting, chow was withdrawn, but free access to drinking water was maintained for 3 days. During this period, some animals from the fed and fasted groups were injected sc daily with T_4 (1.5 μg/100 g BW) or T_3 (0.5 μg/100 g BW) to prevent the appearance of hypothyroidism associated with fasting, while controls received only diluent (0.1 N NaOH). In studies concerning the effects of variations in thyroid state, rats thyroidectomized 3 weeks before study were injected with diluent or varied daily doses of T_4, ranging from 0.5–5.0 μg/100 g BW, for 14 days before they were studied.

Chemicals, reagents, stable and isotopically labeled hormones, and diet

All chemical compounds and laboratory chow used in these studies were purchased from commercial sources.[1]

Preparation of microsomes from rat liver

Rats were killed by a blow to the head, and livers were rapidly removed. A 6-g portion of liver was homogenized in 15 ml 0.05 M phosphate-0.25 M sucrose buffer, pH 7.4, and the homogenate was centrifuged at 10,000 × *g* for 20 min. The pellet was discarded, and the supernatant was centrifuged at 105,000 × *g* for 1 h. The resulting supernatant was aspirated and discarded, and the pellet containing the crude microsomal membranes was washed twice, diluted in phosphate buffer, and divided into two portions that were frozen rapidly in a mixture of acetone and dry ice. Microsomal suspensions were stored at −70 C for, at most, 2 weeks before use, control studies having shown no loss of T_3-neogenetic activity under these conditions.

[1] L-T_4 labeled with ^{125}I in the phenolic ring ([^{125}I]T_4; SA, 50–70 μCi/μg) and 3,5,3'-L-triiodothyronine labeled with ^{125}I in the phenolic ring ([^{125}I]T_3) were purchased from Abbott Laboratories (North Chicago, IL). Crystalline T_4 and T_3 and dithiothreitol (DTT) were purchased from Sigma Chemical Co. (St. Louis, MO). Pelleted laboratory chow (RMH 1000) was purchased from Agway-Country Foods (Agway, Inc., Syracuse, NY).

Protein determinations

One portion of frozen microsomes was thawed and dispersed in a solution of 0.1% deoxycholate. The protein concentration was then measured with the Folin phenol reagent, according to the method of Lowry and coworkers (25).

Microsomal incubations

The other portion of frozen microsomes was thawed and diluted in phosphate buffer before incubation. The incubation mixtures comprised 2.0 ml 0.05 M phosphate buffer, pH 7.4, containing liver microsomes (1.0 mg protein) from individual rats; DTT (10 mM); either [^{125}I]T_4 (1.4 μCi; 1.4×10^{-8} M) or [^{125}I]T_3 (2.2 μCi; 2.7×10^{-8} M); stable T_4 (1.3–13 μM); and bovine serum albumin (BSA; 10 ng/ml). Albumin was added to solubilize T_4, control studies having shown that this concentration of albumin did not affect T_3-neogenesis in microsomal preparations. Incubation vessels were capped and incubated at 37 C for 2 h. T_3 formation in microsomal preparations was maximal in the presence of 10 mM DTT and increased linearly during the 2-h incubation. After incubation, vessels were placed in cracked ice, and a portion of the reaction mixture was combined with an equal volume of outdated blood bank plasma and stored frozen at −20 C for later analysis by paper chromatography.

Paper chromatography

The percent generation of reaction products and the percent degradation of substrate were assessed by paper chromatography in a descending solvent system employing hexane-tertiary amyl alcohol-2 N ammonia (1:10:11), as described previously (17). Values for the percent generation of labeled products during incubations were corrected for the percent contamination of these products found in control vessels incubated without microsomes. [^{125}I]T_4 was 96–97% pure, containing 0.3–0.8% [^{125}I]T_3 and 1–2% [^{125}I]iodide as contaminants. [^{125}I]T_3 was 95% pure and contained 2% [^{125}I]iodide as the only contaminant identified.

Kinetic analyses

The rate of T_3 formation from T_4 by liver microsomes (micromoles per mg protein/h) was calculated as the product of twice the fractional generation of [^{125}I]T_3 (percent of added [^{125}I]T_4 per mg microsomal protein/h) and the total concentration of substrate T_4 (micromoles).[2] T_3 formation was measured in the presence of five different concentrations of total T_4, ranging from 1.3–13 μM. The data obtained were analyzed by the method of Lineweaver and Burk to obtain estimates of V_{max} and K_m for T_3-neogenesis.[3] The best fitting straight lines for data points were determined by the least squares method.

[2] In the calculation of the fractional generation of T_3 from T_4, the fractional generation of [^{125}I]T_3 from [^{125}I]T_4 was multiplied by 2, since 5'-monodeiodination of phenolic ring labeled [^{125}I]T_4 yields either labeled or unlabeled T_3.

[3] Since proteins other than the T_4 5'-monodeiodinase in microsomal preparations may bind T_4, thus limiting the availability of substrate for the reaction, values for the K_m and V_{max} of T_3-neogenesis are only estimates.

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Statistical analyses

Statistical significance of differences was assessed using Student's t test when two experimental groups were studied or analysis of variance, followed by Duncan's multiple range test (26), when three or more groups were studied.

Results

Reaction products

Under the conditions of study, rat liver microsomes formed two principal ^{125}I-labeled metabolites from [^{125}I]T_4, [^{125}I]T_3, and [^{125}I]iodide. No other iodothyronines and only small amounts of chromatographically immobile origin material were detected. [^{125}I]T_3 generated from [^{125}I]T_4 should have been exceedingly stable in the incubation system, since in parallel incubations with [^{125}I]T_3, approximately 96% of the added labeled substrate remained after 2 h.

Effect of thyroid state

As shown in our earlier studies (27), thyroidectomy did not influence the concentration of microsomal protein. Values (milligrams per g liver; mean ± SE) in preparations from eight intact control rats and eight thyroidectomized rats, respectively, were 14.3 ± 0.8 and 11.9 ± 1.5 (P = NS). As seen previously in studies with slices (22), homogenates (19, 23), and microsomes (27, 28) of rat liver, T_3-neogenesis was markedly impaired in hepatic microsomes from hypothyroid rats, even in the presence of 10 mM DTT. [^{125}I]T_3 formations (percent of added [^{125}I]T_4) in hepatic microsomes from control and thyroidectomized rats, respectively, were 8.3 ± 0.4% *vs.* 3.5 ± 0.4% ($P < 0.01$) in the presence of 1.3 μM T_4 and 3.7 ± 0.6% *vs.* 1.5 ± 0.2% in the presence of 13 μM T_4 ($P < 0.01$).[4] Values for [^{125}I]T_3 formation were inversely related to the concentrations of stable T_4 employed, and at all intermediate concentrations, preparations from control animals were more active than those from thyroidectomized animals. The mean V_{max} for the reaction in microsomes from thyroidectomized animals was reduced to 40% of that found in microsomes from euthyroid rats, but K_m values were essentially the same (Fig. 1).

To assess the effect of differing thyroid states, groups of three thyroidectomized rats were injected for 14 days with either diluent or T_4 in doses of 05, 1.5, or 5.0 μg/100 g BW daily. The concentration of microsomal protein in livers of thyroidectomized animals was not influenced by hormone replacement (data not shown). As seen before, the mean V_{max} of T_3-neogenesis was markedly reduced in liver microsomes from thyroidectomized rats and in-

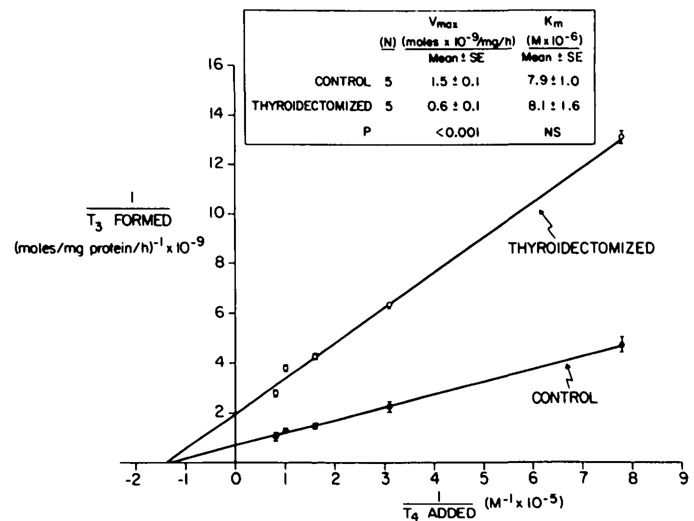

FIG. 1. Microsomes from livers of individual intact and thyroidectomized rats were incubated with [^{125}I]T_4 (1.4 μCi; 1.4 × 10^{-8} M), stable T_4 (1.3–13 μM), DTT (10 mM), and BSA (10 ng/ml) in single vessels for 2 h. Data *points* and *brackets*, respectively, denote the mean ± SE obtained in the number of rats indicated by N. Values for V_{max} (moles × 10^{-9} per mg microsomal protein/h) and K_m (M × 10^{-6}) of T_3-neogenesis were determined by the method of Lineweaver and Burk and are displayed in the *inset.*

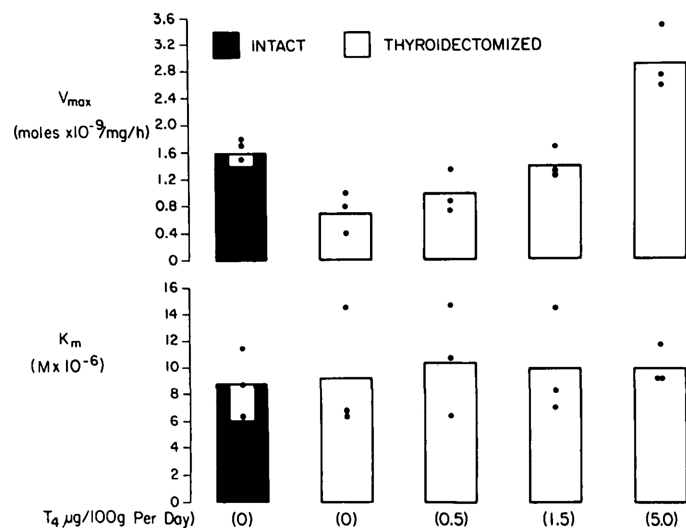

FIG. 2. Intact and thyroidectomized rats were injected daily for 14 days with the doses of T_4 indicated in parentheses. Microsomes from livers of intact and thyroidectomized rats were incubated as described in Fig. 1. *Vertical bars* denote the means of values for V_{max} (moles × 10^{-9} per mg microsomal protein/h) and K_m (M × 10^{-6}) obtained in microsomes from three animals (indicated by *circles*).

creased progressively in preparations from thyroidectomized animals given increasing doses of T_4 (Fig. 2). As a result, microsomes from animals given the physiological dose of T_4 (1.5 μg/100 g) displayed a V_{max} that was approximately equal to that seen in preparations from intact controls, while microsomes from animals given the supraphysiological dose of T_4 (5 μg/100 g) displayed a V_{max} that was approximately 80% above the control value.

[4] The percentage of [^{125}I]T_3 found in incubated buffer controls (~0.5%) was similar to that contaminating the substrate [^{125}I]T_4, indicating no net formation of [^{125}I]T_3 in the absence of microsomes.

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In none of the experimental groups did values for the K_m differ significantly from values in the other groups.

Effect of fasting

Over the entire range of T_4 concentrations tested, liver microsomes from animals fasted for 3 days but not given T_4 replacement formed markedly less $[^{125}I]T_3$ from $[^{125}I]T_4$ than those from fed control animals. The formations of $[^{125}I]T_3$ (percent of added $[^{125}I]T_4$) in hepatic microsomes from fed and fasted animals, respectively, were 7.7 ± 0.5% *vs.* 5.3 ± 0.3% ($P < 0.005$) in the presence of 1.3 μM T_4, and 3.4 ± 0.3% *vs.* 2.1 ± 0.1% ($P < 0.005$) in the presence of 13 μM T_4. Kinetic analysis of these data (Fig. 3) revealed that fasting produced a 43% decrease in the mean V_{max} of microsomal T_3-neogenesis ($P < 0.005$). An attendant decrease in K_m, small but significant ($P < 0.02$), was also seen.

The effect of replacement with physiological doses of T_4 or T_3 in fasted animals was then evaluated (Table 1). Groups of intact rats were either given chow or fasted for 3 days. During this period, some animals from each group were injected daily with diluent only, T_4 (1.5 μg/100 g), or T_3 (0.5 μg/100 g). The concentrations of hepatic microsomal protein per unit wet weight did not differ among all of the experimental groups (data not shown). In liver microsomes from animals receiving no exogenous thyroid hormone, the mean V_{max} of T_3-neogenesis was reduced by approximately 50% ($P < 0.01$) in the fasted group. Replacement with physiological doses of either T_4 or T_3 restored the V_{max} in the hepatic microsomes of fasted rats to the level found in the preparations from fed controls. K_m values were decreased slightly in specimens from fasted animals, but this change was not observed when rats were injected with either T_4 or T_3.

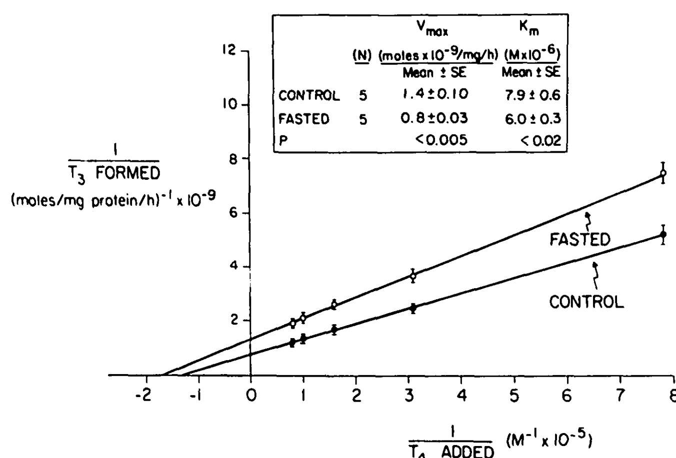

FIG. 3. Groups of animals were fed or fasted for 72 h and were not replaced with thyroid hormone. Microsomes from livers of individual animals were incubated, and kinetic analyses were performed as described in Fig. 1. Values for V_{max} (moles × 10^{-9} per mg microsomal protein/h) and K_m (M × 10^{-6}) are given in the *inset*.

Discussion

There is general agreement that T_3-neogenesis is decreased in preparations of livers of fasted rats, but there is no agreement as to how this change is brought about. Although Jennings *et al.* (29) concluded from data obtained in liver perfusion studies in rats that this change is due to decreased hepatic uptake of T_4 rather than to decreased conversion of T_4 to T_3, numerous studies have shown that decreased T_3-neogenesis persists in broken cell preparations of livers of fasted rats (2, 3, 17–21).

In our previous studies, in which various mixtures of hepatic microsomes and cytosols from fed and fasted rats were tested, we found no evidence of an enzymatic abnormality in microsomes from fasted animals (2). Rather, the defect appeared to reside in deficient cytosolic support of T_3-neogenesis, which could be overcome by the addition of GSH (2). Others, however, have presented data which led them to conclude that an abnormality in the enzyme is the major factor responsible for the decreased T_3-neogenesis observed in livers from fasted animals (3, 21). This conclusion was based mainly on the observation that the impaired T_3-neogenesis in whole homogenates or particulate fractions from livers of fasted animals could not be restored to normal by the addition of the mercaptan, DTT. We believe, however, that the present and previous observations serve to resolve this discrepancy.

It is generally agreed that hypothyroidism also results in decreased hepatic T_3-neogenesis *in vitro* (19, 22, 23) and that this effect is mainly due to the decreased intrinsic activity of the microsomal 5′-deiodinase for T_4 (27, 28). It is also the case, however, that even short periods of fasting induce a state of hypothyroidism in the rat (17, 18, 20, 24). Consequently, it appeared possible that hypothyroidism, rather than fasting, was reponsible for the enzymic abnormality found by others in the livers of fasted rats, particularly since replacement doses of thyroid hormone were not routinely administered in these studies.

In contrast, in our earlier studies with microsomes and cytosols, which appeared to exclude decreased activity of the microsomal enzyme as a cause of decreased T_3-neogenesis during fasting, all animals were treated with maintenance doses of T_4 (2). Moreover, Chopra (20) demonstrated that DTT could completely reverse the defect in liver homogenates from rats fasted for 48 h only when physiological T_4 replacement was performed. That this is the relevant variable is shown conclusively in the present studies. Here we have demonstrated that although the V_{max} of the microsomal enzyme is decreased in preparations from fasted animals, the abnormality is entirely reversed by treatment with maintenance doses of T_4 or T_3. For reasons that are not clear, the K_m of T_3-

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TABLE 1. Effect of fasting and thyroid hormone supplementation on the activity of T_4 5'-monodeiodinase in rat liver microsomes

Experimental group	No. of animals	V_{max} (mol × 10^{-9}/mg protein·h)		K_m (M × 10^{-6})	
		Mean ± SE	Significance	Mean ± SE	Significance
A. Control	7	1.49 ± 0.12	$P < 0.01$ for A *vs.* B, B *vs.* C, B *vs.* D, B *vs.* E, B *vs.* F	9.4 ± 1.2	$P < 0.05$ for A *vs.* D
B. Fasted	7	0.76 ± 0.03		6.0 ± 0.2	$P < 0.01$ for A *vs.* B
C. Control + T_4 (1.5 μg)	7	1.54 ± 0.16		7.9 ± 0.5	
D. Fasted + T_4 (1.5 μg)	7	1.21 ± 0.10		6.9 ± 0.5	
E. Control + T_3 (0.5 μg)	8	1.46 ± 0.10		7.7 ± 0.5	
F. Fasted + T_3 (0.5 μg)	8	1.41 ± 0.09		7.4 ± 0.9	

Rats were fasted or consumed chow *ad libitum* for 72 h and during this period were either given no hormone replacement or were injected daily with the indicated dose (micrograms per 100 g) of T_4 or T_3. Microsomes from the livers of these animals were incubated with $[^{125}I]T_4$ (1.4 μCi; 1.4 × 10^{-8} M), stable T_4 (1.3–13 μM), DTT (10 mM), and BSA (10 ng/ml) for 2 h. The V_{max} and K_m of T_3-neogenesis were determined by the method of Lineweaver and Burk.

neogenesis was slightly lower in microsomes from fasted than in those from fed animals, but the greater affinity of the enzyme for T_4 in fasted animals could not, of course, explain a lesser rate of T_3-neogenesis. Whatever its cause, the small change in K_m values seen in microsomes from fasted animals was also reversed by thyroid hormone replacement.

In the present studies we have confirmed and extended observations reported by Kaplan (28) that the principal effects of variations in thyroid state on the T_3-generating enzyme are to produce changes in its V_{max}. Data obtained in the present studies and in those we have reported earlier (27), taken together, clarify, to a certain extent, the nature of the enzymatic abnormality with respect to T_3-neogenesis that occurs in the hypothyroidism that follows thyroidectomy or fasting. In both cases, the principal abnormality was a decrease in the V_{max} of the reaction. It is not clear to what extent this abnormality reflects a decrease in enzyme concentration or reflects a decrease in the responsiveness of the enzyme to sulfhydryl cofactor. This differentiation is difficult because of the low activity of the normal enzyme in the absence of cofactor. Nevertheless, in our earlier studies, T_3-neogenesis was significantly lower than normal when hepatic microsomes from hypothryoid animals were incubated in buffer alone (27). In addition, the proportionate increase in T_3-neogenesis induced by the addition of cofactors and especially by replacement of buffer by cytosols was less in the case of the microsomes from hypothyroid animals (27). Together, these findings would suggest that in hypothyroidism, the hepatic 5'-monodeiodinase for T_4 is abnormal in both concentration and its response to cofactor.

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