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# Carbohydrate Repression of Dietary Induction of Ornithine $\delta$-Transaminase in Rat Liver

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=국문초록=

## 식이에 의한 Ornithine $\delta$-Transaminase의 유도(induction)와 억제(repression)에 관한 연구

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단백질을 함유하지 않은 식이를 5일간 먹인 쥐에게 동량으로 혼합된 필수 아미노산 혼합물(amino acid mixture)을 투여하여 ornithine $\delta$-transaminase(OT)를 유도(induction)한 군과 또한 amino acid mixture를 먹인 후 6~9 $\frac{1}{2}$시간 후에 glucose를 동시에 먹인 군에서 OT의 유도 기전을 보기 위하여 방사성 동위원소인 L-methionine-$CH_3$-$C^{14}$ 또는 L-phenylalanine-$H^3$(uniformly labelled)을 사용하여 유도전 OT를 pulse label하여 준비된 항체로 각각 유리 분리시켜서 방사능을 측정하였다.

Amino acid mixture만을 투여한 군에서는 zero time에 비해 OT활성은 6시간 후에 1.5배, 12시간 후에는 약 3배, 18시간 후에는 13배가 증가되었다. 또한 OT에 incorporate된 표지 아미노산의 방사능은 6시간 후에 1.5배, 12시간 후에 약 3배가 증가되었음을 보였다. 그러나 이 기간동안 간장의 total soluble protein에 incorporate된 표지 아미노산의 방사능은 거의 증가하지 않았다. 그러므로, amino acid mixture에 의한 OT의 증가는 고유하게 유도되었으며 이때의 활성의 증가는 OT단백질의 분해률의 감소에 의한 것이 아니라 순수하게 OT단백질의 생합성에 의한 증가라고 볼 수 있다.

amino acid mixture를 투여한지 6시간 후에 glucose용액을 동시에 먹인 쥐에서는 OT활성은 증가를 하였으나 amino acid mixture만을 먹인 군보다는 약간 감소를 보였다. 또한 glucose용액이 투여된 후에는 OT에 표지 아미노산이 더 이상 incorporate되지는 않아 방사능의 증가는 보이지 않았으나 glucose가 투여될 당시보다 더 이상 감소되지는 않았다. 그러므로 glucose에 의한 유도에 대한 억제(repression)는 단백질의 분해률의 증가에서 온 것이라기 보다는 OT단백질의 생합성을 억제하여서 OT활성을 감소하지 않았는가 본다.

It has been established that in animal tissues a number of enzyme activities can be increased by the administration of hormones[1,2] or specific substrates[1,3] or by the alterations in the nutritional state of the animal[4~6].

Multiple intubations of enzymatically hydrolyzed casein or of an equimolar mixture of essential amino acids to rats that had been fed a protein-depleted diet for 5 days resulted in marked increases in the two amino acid catabolizing enzymes, threonine-dehydrase (serine dehydratase) and ornithine $\delta$-transaminase (OT). The induction of serine dehydratase, produced by the forced feeding of casein hydrolysate or a mixture of essential amino

Abbreviations: OT, ornithine $\delta$-transaminase

acids, was completelely repressed by the simultaneous or delayed adminstration of glucose or fructose, but not by an isocaloric amount of fat[7]. Although the induction of both OT and serine dehydratase by feeding casein hydrolysate were repressed by carbohydrates, OT induction was substantially less sensitive to repression by glucose than was the induction of serine dehydratase. A highly specific antibody against purifed hepatoma OT was prepared from the hepatomas was found to be antigenically indistinguishable from rat liver OT by immunodiffusion test[8].

OT was induced in rat liver by feeding amino acid mixture to determine whether the induced OT were repressible by delayed glucose feeding. The enzyme was pulse labelled *in vivo* by L-methionine-$CH_3$-$C^{14}$ or uniformly labelled L-phenylalanine-$H^3$. OT was then isolated by precipitation with its highly specific antibody and its radioactivity determined in oredr to find whether the increase or decrease in the enzyme level was controlled by the rate of enzyme synthesis or degradation.

## Materials and Methods

**Chemicals** :

L-methioine-$CH_3$-$C^{14}$ (specific activity, 11.0 mc/mmole) and uniformly labelled L-phenylalanine-$H^3$ (specific activity, 5.3 c/mmole) were purchased from New England Nuclear Corporation. The amino acid mixtures were prepared by grinding the amino acids in a ball mill at a speed of 260 rpm for one day.

**Immunological, Procedure** :

An antiserum specific to hepatoma OT was prepard as described in previous paper[8]. The enzyme preparation used for immunological analyses was prepared as follows: 0.2% cetyltrimethylammonium bromide supernatant was pre prepared as described in previous work[8] and fractionated with powdered ammonium sulfate (0~60%). This fraction was dissolved in 0.05M $KH_2PO_4$-KOH buffer (pH 8.0) and then heated at 55°C for 2 minutes. The denatured protein was centrifuged down at 16,000 rpm and the supernatant was fractionated again with powdered ammonium sulfate (0~30%). This fraction was dissolved in a minimal volume of the phosphate buffer and this solution centrifuged before placing on a G-25 Sephadex column (1×12 cm). One milliliter of clear solution was introduced on a column and 2ml eluate was collected after the void volume. The Sephadex column eluate sdid not produce nonspecific precipitation with antibody and were used for the quantitative percipitin studies and for immunochemical isola-tion of OT protein for isotopic studies.

Quantitative precipitan reactions of partially purified OT from hepatomas were performed as follows: Varying amounts of enzyme preparation having the enzyme activity indicated in quantitative precipitan curve added to 0.1 ml of OT antiserum and to control serum. The final volume of each reaction tube was made up to 1.0 ml with 0.85% sodium chloride, and the tubes were stored at 4°C overnight. No precipitation was observed in any of the tubes containing control serum. The precipitates were collected by centrifugation at 2000 rpm and washed twice with chilled 0.85% (w/v) sodium chloride. All samples were run in two sets of duplicates. The supernatant fluids were assayed for OT activity as described before[8] and protein determinations were performed on the washed precipitates. The enzyme activity of the precipitates was determined on aliquots of suspensions of the washed precipitates.

**Animal Experiments** :

For the experiments *in vivo*, male albino rats weighing 130~150 g (Holtzman) were fed a commerically prepared protein-free diet ad libitum for 5 days. At the end of this period, the rats were fasted overnight (12 hrs) and the experiments were begun at 6 : 00 a.m., at which time the animals weighed from 100 to 110 g. In order to induce the enzyme under controlled conditions, protein-depl-

eted rats were force-fed by stomach tube an equimolar mixture of eight amino acids(lysine, leucine, valine, isoleucine, threonine, histidine, arginine and tryptophan, all in the L-form) in 4ml of water at a level of 0.49 gm (372 umoles of each amino acid). The rats were fed at the indicated time. To see the delayed effect of glucose on this induction, 2 g of glucose in 5 ml of water was given by stomach tube to each rat at 9.5 hr and 15 hr after initiation of induction by amino acids. Each mixture was adjusted to pH 7.4 with sodium hydroxide prior to feeding. Four ml of water were given to the zero time control rats.

**Isotope Incorporation :**

Animals which received the experimental diets were killed at 0, 6, and 12 hours after the beginning of the experiment. Forty min. prior to killing each animals, 10 $\mu$c of L-methionine-$CH_3$-$C^{14}$ or 50uc of L-phenylalanine-$H^3$ were dilute to a volume of 0.4 ml in 0.85% NaCl, and administered intraperitoneally to each animal. At the times indicated, animals were killed by decapitation. Livers were quickly removed and homogenized in 4 molumes of 0.05 M $KH_2PO_4$-KOH buffer (pH 8.0) containing $10^{-4}$M pyridoxal phosphate and $5\times10^{-3}$M mercaptoethanol. Homogenates were sonicated for 6 min at 20 kilocycles to ensure maximum release of OT. The sonicated homogenates were centrifuged at 105,000$\times$g for 90 min. in a Spinco model L centrifuge. The supernatant was carefully removed and centrifuged again at 105,000 g for 60 min. to remove lipid. Failure to carry out the second centrifugation resulated in high radioactivity. The clear suppernatants were then used for the assay of OT and for isolation of the enzyme through quantitative immunochemical precipitation. A sample of the high speed (105,000 g) supernatant was also used for determination of total radioactivity present in the form of trichloroacetic acid-precipitable protein. For the precipitation of OT by its specific antibody, identical aliquots of each clear supernatant (usually 0.5 ml) were divided into 3 equal portions, A, B, C in duplicate. To the first portion (A) was added a sufficient amount of OT antibody (determined from the quantitative precipitin curve) to precipitate all of the OT present in the sample including added unlabelled carrier enzyme. Since each sample contained varying amounts of OT, varying amounts of unlabelled carrier enzyme were added so that an equal amount of total enzyme activity was always precipitated from each sample. To correct for any nonspecific adsorption of radioactivity to the antigen-antibody precipitate, the second portion(B) was prepared in such a way that the total enzyme activity in each tube was twice that in A tube. A volume of antiserum equal to that in A was added to each sample of B and sufficient unlabelled carrier enzyme was added to bring the toal precipitate formed in this tube to twice that formed in A tube. To the third portion(C), the same volume of control serum and carrier enzyme as in B tube were added. The volumes of A, B and C were made equal with 0.85% NaCl. If no nonspecific adsorption occurred in the precipitate, the radioactivity found in the antigen-antibody precipitate should be equal in A and B. If the radioactivity of the precipitate from B was greater than that from A, then nonspecific adsorption of radioactivity had occurred. This could be corrected for by plotting the counts of A and B versus the enzyme units of A and B of each tube, corrected for the counts in tube C, and hence extrapolating the counts to zero enzyme units, thus correcting for nonspecific adsorption of the precipitate. This method assumes that nonspecific adsorption is proportional to the amount of the antigenantibody precipitate. No precipitation had occurred in the the control tubes containing control serum, but these control samples still gave significant counts.

All tubes were stored 20~24 hours at 4°C. Precipitates were collected by centrifugation at 2000 rpm and washed twice with 3 ml of chilled 0.85% NaCl. The total soluble protein from the supernatant fraction was precipitated with 10% (w/v) tricholoroacetic acid and washed twice in the same

solution. Each antigen-antibody precipitate and the precipitate of soluble proteins were dissolved in 0.25 ml of 15% tetramethyl ammonium hydroxide and incubated at 37°C for 30 min. to ensure dissolution of the precipitate. The radioactivity of all protein precipitates, including controls, was determined in ANPO in a Packard Lipuid Scintillation Counter. Correction for quenching was made using automatic external standardization.

## Results and Discussion

Fig. 1 demonstrates the quantitative precipitin titration curve of partially purifed OT preparation from hepatomas(the Morris 5123 and 7800). As can be seen from Fig. 1, the titration curve was symmetrical and did not show a second peak indicating the purity of the antibody. The suspended antigen-antibody precipitates exhibited about 70 % of the OT activity of that added at that titer. The total enzyme activity of the antigen-antibody precipitate plus that of the supernatant fluid approached 100% of that originally added to tube. This result suggests that the antibody is not binding to the active site of the enzyme antigen or that the antigen-antibody complex is easily dissociated while it is being assayed. The lack of complete recovery of enzyme activity from the precipitate may be due to the incomplete dissociation of antigen from antibody or to a partial blockage of the active site of enzyme by antibody or to both.

Fig 2. shows the increase in OT activity produced by the repeated administration of the amino acid mixtures as well as inhibition produced by glucose feeding Expressing the results per liver per grams body wight partly compens-ates for the change in liver size since the livers of the glucose-treated rats were larger than those of the untreated rats due to a considerable increase in glycogen content. There was a 13-fold increase in the enzyme level in 18 hr by feeding the

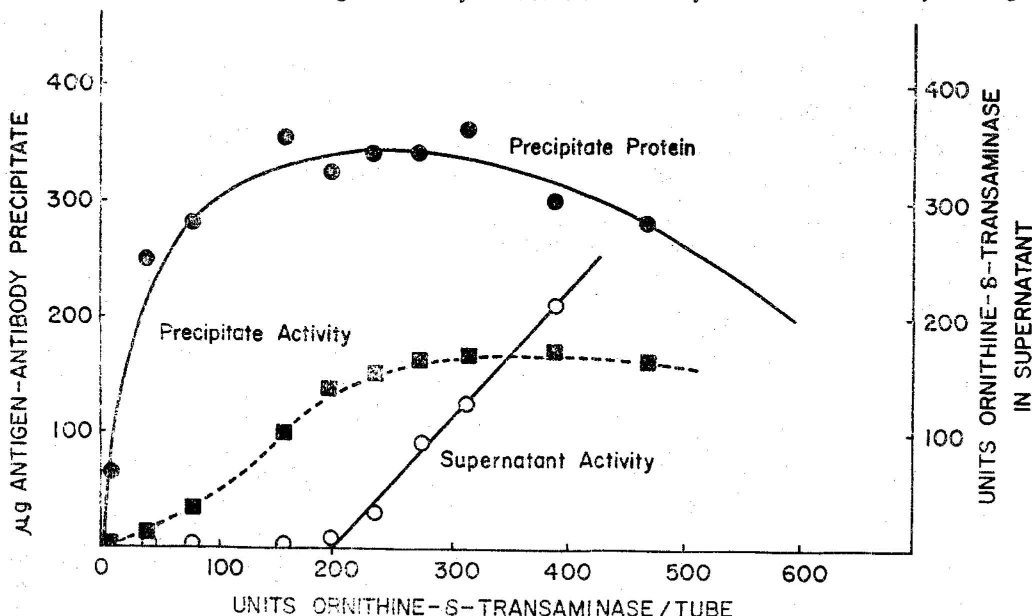


**Fig. 1.** Quantitative precipitin reactions of partially purified hematoma ornithine δ-transaminase.

To 0.1ml of ornithine δ-transase antiserum was added the amount of preparation containing the enzyme activity indicated. Following completion of precipitation as described under "Materials and Methods", the suppernatant fluids were assayed for enzyme activity. The precipitates were washed twice with chilled 0.85% NaCl. Enzyme activity was determined on suspensions of the washed precipitates and the protein content of the precipitates in the tube was determined. A similar experiment with nonimmune serum yielded no visible precipitate.

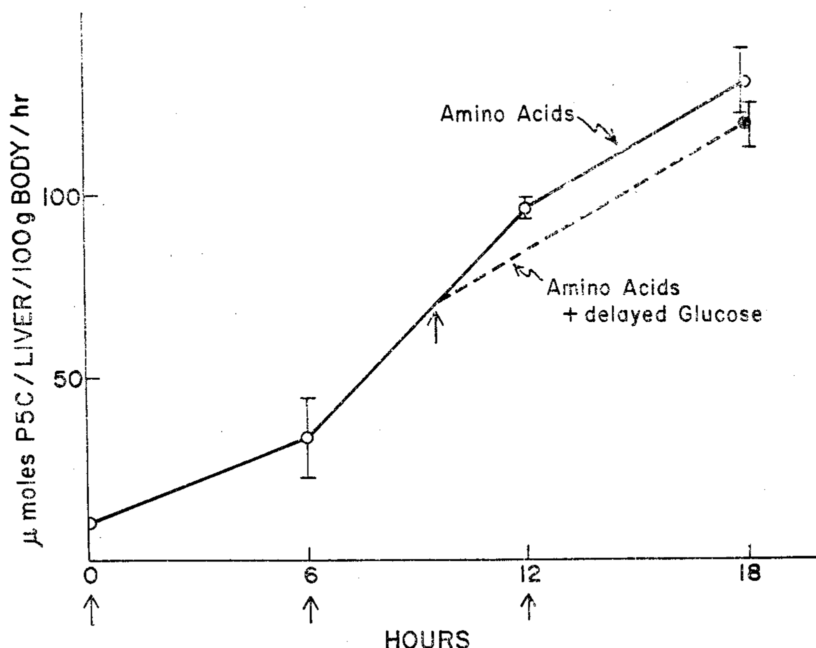


Fig. 2. Effect of delayed glucose feeding on the induction of ornithine δ-transaminase by free amino acids.

0.49 g. of an equimolar amino acid mixture (lysine, leucine, valine, isoleucine, threonine, histidine, arginine, tryptophan, all in the L-form) fed at 0, 6, a and 12 hours.

The above amino acid mixture fed at 0, 6, and 12 hours plus 2.0 gm of glucose at 9.5 and 15 hours.

The vertical line at each point on the curve shows the standard error of the mean for 3 rats.

amino acid mixture compared to those of control animals. In the present study it was assumed that a measurement of enzyme activity was a valid measure of the content of enzyme protein. However, this aminoacid-mediated increase of OT activity may be the result of either a decrease in the rate of enzyme degradation, i.e., enzyme stabilization, or an increase in the rate of enzyme synthesis or from both.

2 g. of glucose fed at 9.5 hrs gave a 10% inhibition of OT induction at the 18 hr point. Peraino & Pitot[9] reported that no inhibition of OT was observed when liver homogenates from carbohydraterepressed rats were added to those of untreated rats or when varying amounts of glucose were added to the homogenates. These results suggest that glucose repression does not involve an inhibition of the activity of newly formed enzyme.

**Pulse-labelling of Ornithine δ-transaminase *in vivo*:**

Studies on the incorporation of amino acids into OT were undertaken to obtain the direct evidence indicating whether the aminno acid mediated increases of enzyme level result from an increase in the rate of enzyme synthesis or a decrease of the rate of enzyme degradation and also to determine the effect of glucose-feeding on this phenomenon. As a measure of enzyme synthesis, the extent of L-methionine-$CH_3$-$C^{14}$ or uniformly labelled L-phenylalanine-$H^3$ incorporation in short time periods into the protein precipitated by the specific OT antiserum was determined. A 40-min period of isotope incorporation in vivo was chosen because at such a time the maximal amount of labelling of protein had usually occurred, and a minimum period of time had elapsed during which enzyme was being synthesized from

an amino acid pool with a low radioactivity[1]

Table 1 shows the results of experiments on the incorporation of L-methionine into OT of liver from rats that had received the amino acid mixture with or without glucose. The results were expressed in two different ways; i.e. enzyme activity or radioactivity (expressed in disintegration per min) per gm liver as well as per total liver. The first column shows the enzyme activity, the second radioactivity incorporated into OT which was precipitated by antibody and the last column the radioactivity of total soluble proteins which served as an indicator of individual differences in the size of the hepatic amino acid pools, as well as of changes with induction in the radioactivity. From the data of Table 1, it can be seen that there was approximatelya 1.5-fold increase in enzyme activity and a 1.8-fold increase in the incorporation of labelled amino acid into OT at the 6 hr point. 2.7-fold increase in the enzyme level occurred after a 12 hr period of induction. The total incorporation of $C^{14}$-amino acid into the soluble liver proteins increased slightly during the same period of time. When this is taken into consideration, the effect of animo acid administration is seen to result in a specific induction of OT during this 12 hr interval. If it is assumed that this induction of OT may be the result of an inhibition of enzyme degradation, then the dpm incorporated into OT should not appreciably be increased although the total amount of enzyme activity was increased during the induction period. However, there was an increase in the pulsed incorporation of radioactivity into the enzyme during the induction period. Hence this data suggested that OT induction by the amino acid mixture results from an increased rate of enzyme synthesis. Furthermore, a significant amoont of labelled amino acid was also incorporated into OT in the non-induced rat (0 time point). This implies that OT is being synthesized and degraded in the non-induced state.

In glucose-fed rats at 6 hr after the initiation of induction there was only a slight to variable inhibition in the resultant levels of enzyme activity at 12 hr although there was no increase in incorporation of radioactivity into OT at this time as compared to the 6 hr point. If glucose administration results in both a decrease in the rate of synthesis and an increase in the rate of degradation of the enzyme, then one might expect there may be a greater inhibition in the increase

**Table 1.** Pulse-Labelling of Oirnthine δ-Transaminase (OT) in vivo Using L-Methionine-$CH_3$-$C^{14}$*

| | No. of rats | OT units/g liver | L-methionine-$CH_3$-$C^{14}$ incorporation in dpm | |
|---|---|---|---|---|
| | | | OT/g liver | soluble prot./g liver |
| $H_2O$ (0 time) | 3 | 16.7± 1.1 | 213± 52 | 174,000±23,000 |
| Amino acids (6 hr) | 3 | 25.0± 3.2 | 387± 92 | 212,000± 7,000 |
| Amino acids (12 hr) | 3 | 42.4± 1.1 | 540±196 | 259,000± 5,000 |
| Amino acids+delayed Glucose (6 hr) | 3 | 37.3± 2.8 | 373±134 | 160,000± 5,000 |

| | No. of rats | OT units/liver | L-methionine-$CH_3$-$C^{14}$ incorporation in dpm | |
|---|---|---|---|---|
| | | | OT/liver | soluble prot./liver |
| $H_2O$ (0 time) | 3 | 53.7± 3.0 | 692±169 | 559,000±68,400 |
| Amino acids (6 hr) | 3 | 82.8±11.2 | 1273±303 | 700,000±21,100 |
| Amino acids (12 hr) | 3 | 143.0± 3.3 | 1826±657 | 908,000±86,300 |
| Amino acids+delayed Glucose (6 hr) | 3 | 44.0± 8.1 | 1395±451 | 620,000±17,400 |

*All values±standard error of the mean for three rats.

Table 2. Pulse-Labelling of Ornithine δ-Transaminase(OT) *in vivo* Using uniformly Labelled L-phenylalanine-$H^3$*

| | No. of rats | OT units/g liver | L-phenylalanine-$H^3$ incorporation in dpm | |
|---|---|---|---|---|
| | | | OT/gliver | soluble prot./g liver |
| $H_2O$(0 time) | 2 | 16.9± 0.8 | 270± 64 | 219,000± 7,000 |
| Amino acids(6 hr) | 3 | 20.6± 3.5 | 420± 29 | 254,000±19,500 |
| Amino acids(12 hr) | 4 | 28.3± 5.3 | 613±149 | 208,000±17,100 |
| Amino acids+delayed Glucose(6 hr) | 3 | 21.3± 1.8 | 307±104 | 160,000±11,700 |

| | No. of rats | OT units/liver | L-phenylalanine-$H^3$ incorporation in dpm | |
|---|---|---|---|---|
| | | | OT/liver | soluble prot./liver |
| $H_2O$(0 time) | 2 | 51.4± 2.6 | 821±195 | 666,400±33,300 |
| Amino acids(6 hr) | 3 | 79.0±17.3 | 1591±185 | 941,700±62,800 |
| Amino acids(12 hr) | 4 | 126.0±25.2 | 2598±568 | 900,300±59,600 |
| Amino acids+delayed Glucose(6 hr) | 3 | 98.2±11.1 | 1314±387 | 720,100±54,900 |

*All values±standard error of the mean for 2–4 rats.

in enzyme activity and in the incorporation of radioactivity into the enzyme at 12 hours. In the case of serine dehydratase glucose administration with or without amino acids results in a complete inhibition in radioactive amino acid incorporation into the serine dehydratase antigen[2]. However, the glucose effect was only partial with OT induction in that only the further incoporation of radioactivity after 6 hours was inhibited. If the assumption, that the amino acid-mediated induction results from an increased rate of enzyme synthesis, is correct, then glucose adminis-tration only partially in hibited the rate of enzyme synthesis. The glucose effect on the rate of degradation of OT, if any, is not very marked since the amount of the enzyme increased between 6 and 12 hours even though its rate of synthesis was inhibited.

Table 2 also shows the same results of this experiment when using uniformly labelled L-phenylalanine-$H_3$. There was 3.2-fold increase in the incorporation of radioactivity into the enzyme after a 12 hr period of induction. In glucose treated rats, there was also only slight inhibition in the levels of enzyme activity obtained at 12 hr and no greater incorporation of radioactivity into OT at 12 hr than at 6 hr indicating the reproducibility of the results.

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