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# Effect of Rhatannin on Glutamine Metabolism in Rat Liver

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The present investigation was undertaken to evaluate the effect of rhatannin (condensed tannin purified from Rhei rhizoma) on transamination from glutamine by measuring the individual amino acids formed from α-keto analogues of amino acids with glutamine or glutamate in the supernate of rat liver homogenate. Increased glycine formation was observed 2 to 4 h after the intraperitoneal administration of rhatannin (12.5 mg/kg body weight). Glutamate (derived from glutamine *via* glutaminase) may not participate in the rhatannin-induced elevation of glyoxylate amination by glutamine. This effect by rhatannin was completely abolished by cycloheximide treatment 30 min prior to rhatannin treatment, while actinomycin D had no effect. With 20 mM glutamine as the amino-donor, the order of effectiveness of 20 mM keto analogues of amino acids was glyoxylate > pyruvate > hydroxypyruvate > phenylpyruvate > α-ketoisocaproate, and little activity was observed with α-ketoisovalerate. Phenylalanine and leucine production rates were enhanced with either glutamine or glutamate in rhatannin-treated rats.

The results of the present investigation suggest that the hepatic glutamine transaminase pathway might be enhanced in rhatannin-treated rats. This enhancement might be associated with a protein synthesis process.

**Keywords**——condensed tannin; rhatannin; rhubarb; rat liver; glutamine metabolism; α-ketoacid amination

It is well known that the liver appears to function as either a glutamine-synthesizing or a glutamine-utilizing tissue, depending on the physiological state of the rat.[1-3]

It was shown previously[4] that glutamine synthetase activity in the liver was increased 2 to 4 h after the intraperitoneal administration of rhatannin[5] and that the free ammonia level in the hepatic vein was reduced 4 to 8 h after the treatment. In addition, the concentrations of glutamine and glutamate in the liver were decreased at 2 h and had returned to the control level at 4 h after the treatment. These results suggested that the treatment might enhance glutamine synthesis. However, the concentrations of plasma glutamine and glutamate were decreased till 8 h after the treatment. Therefore, it remains to be examined whether rhatannin affects a glutamine-utilizing process in the liver.

In the present investigation, the amination of α-ketoacids by glutamine was evaluated by measuring amino acids formed in the supernate of liver homogenate prepared from postabsorptive rats. The results indicated that hepatic glutamine transamination might be enhanced in rhatannin-treated rats and that a protein synthesis process might be associated with the increased transamination involving glyoxylate.

## Materials and Methods

**Materials**——Condensed tannin was isolated from commercial rhubarb by the method described previously.[5] The following compounds were purchased: sodium glyoxylate, sodium pyruvate, lithium β-hydroxypyruvate, sodium

α-ketoisovalerate, sodium α-ketoisocaproate, and sodium β-phenylpyruvate (Sigma Chemical Co., U.S.A.); glutamine, sodium glutamate, and cycloheximide (Wako Pure Chem. Ind., Japan); actinomycin D (P-L Biochemicals, Inc., U.S.A.); disodium ethylenediamine tetraacetate (EDTA) (Dojin Laboratories, Japan).

**Animals**——Male Wistar rats weighing 160—180 g were employed throughout the experiment. They were housed in air-conditioned quarters at 25 °C and 60% relative humidity. Animals were fed on laboratory pellet chow (CE-2, CLEA Japan Inc., Japan) and tap water freely, but were fasted for 24 h before experiment. Rhatannin (12.5 mg/kg body weight) was administered intraperitoneally to rats, and control rats were given an equal volume of saline. Cycloheximide (300 μg/100 g) and actinomycin D (100 μg/100 g), when used, were administered 30 min before rhatannin or saline treatment.

**Preparation of the Liver Extract**——Each animal was killed by decapitation, and the liver was removed and homogenized with 3 volumes of 0.05 M borate buffer (pH 8.5) in a Polytron homogenizer at full speed for 30 s. The homogenate was centrifuged at 15000 × *g* for 20 min and the supernate was assayed to determine the amino acid formed.

**Determination of Amino Acids Formed**——Assay mixture contained, unless otherwise specified, 20 mM glutamine or glutamate, 20 mM α-ketoacid, 0.05 M borate buffer (pH 8.5), 1 mM EDTA, and the liver extract. Incubation was done at 37 °C for 1 h. The reaction was terminated by adding 0.5 vol 20% trichloroacetic acid (TCA) and the protein precipitate was removed by centrifugation. The solution obtained by dilution with distilled water was applied directly to a Hitachi 835 amino acid analyzer (Hitachi Ltd., Japan). The protein concentration of the liver extract was estimated according to Lowry *et al.*,[6] with bovine serum albumin as a standard.

## Results

### Time Course of Effect of Rhatannin on Glycine or Alanine Production from Glutamine with Glyoxylate or Pyruvate

The time course of the effect of rhatannin on the transamination from glutamine to glyoxylate or pyruvate was tested in postabsorptive rats. The experimental conditions were selected on the basis of preliminary experiments. As illustrated in Fig. 1, the administration of rhatannin resulted in increased glycine formation 2 to 4 h after the treatment. The increments were 18% and 23%, respectively, as compared with the control. Glycine production was not affected by the addition of 1 mM pyridoxal 5′-phosphate to the reaction mixture (data not shown). In the case of alanine, rhatannin did not significantly affect the production rate during 8 h after the treatment.

### Effect of Rhatannin on Glycine Production under Various Conditions

The amination of glyoxylate is not specific to glutamine under the present experimental

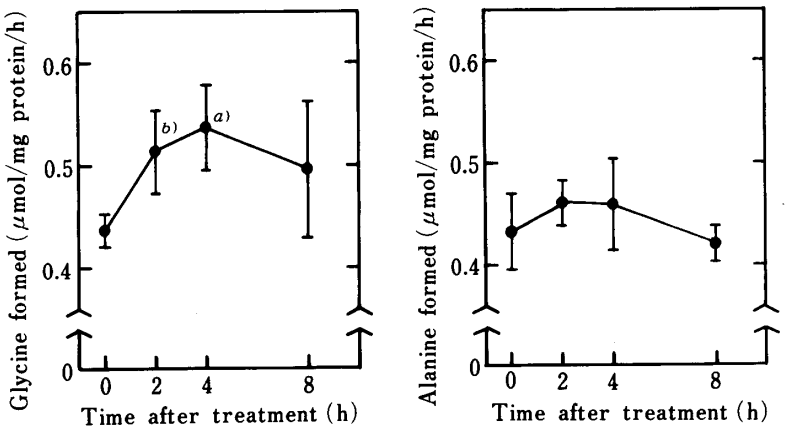


Fig. 1. Time Course of the Effect of Rhatannin on the Transamination from Glutamine to Glyoxylate or Pyruvate

Rats were fasted for 24 h before the injection of rhatannin (12.5 mg/kg body weight). The amino acids formed were estimated as described in Materials and Methods. The data are given as mean ± S.D. of 4 rats. The control is shown as 0 h, though in fact it is the mean ± S.D. of the data at each period after saline treatment.

Left-hand panel, glycine production; right-hand panel, alanine formation. *a*) $p<0.01$, *b*) $p<0.05$, student's *t* test.

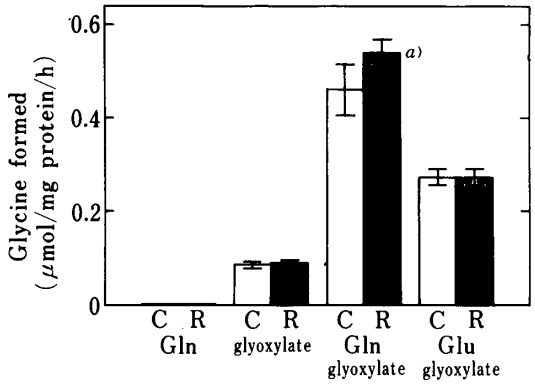


Fig. 2. Effect of Reaction Mixture Components on Glycine Formation, and the Influence of Rhatannin

The reaction mixture initially contained liver extract, 50 mM borate buffer (pH 8.5), 1 mM EDTA, and either 20 mM glutamine (Gln), 20 mM glyoxylate, Gln plus glyoxylate, or 20 mM glutamate (Glu) plus glyoxylate. Rhatannin was injected intraperitoneally into rats fasted for 24 h. Rats were sacrificed 2 h after rhatannin treatment.

Data are expressed as means ± S.D. of 4 rats. *a*) $p<0.05$, student's *t* test.

□ C, control rats; ■ R, rhatannin-treated rats.

TABLE I. Effect of Glyoxylate on the Conversion of Glutamine to Glutamate and the Influence by Rhatannin

| Treatment | No. of rats | Glutamic acid formation (μmol/mg protein/h) | |
|---|---|---|---|
| | | Without glyoxylate | With glyoxylate |
| Control | 5 | 0.44 ± 0.09 | −0.03 |
| Rhatannin | 5 | 0.38 ± 0.08 | −0.04 |

The reaction mixture contained liver extract, 50 mM borate buffer (pH 8.5), 1 mM EDTA and 20 mM glutamine, with or without 20 mM glyoxylate. Rhatannin was injected intraperitoneally into rats starved for 24 h. Rats were sacrificed 2 h after the rhatannin treatment. Data are expressed as means ± S.D.

conditions. The net glycine formation might include the amination by and of endogenous substrates contained in the liver extract. Figure 2 shows the glycine production under various conditions, and the effect of rhatannin on it.

Glutamine alone caused no glycine formation. However, glycine production was observed after the addition of glyoxylate alone. The simultaneous additions of glutamine and glyoxylate had the greatest effect. Glutamate could partially substitute for glutamine. The extents of glycine production were 0.4%, 19%, and 59% using glutamine, glyoxylate, and glutamate plus glyoxylate, respectively, as glycine precursors, when the values are expressed relative to that for the formation from glutamine and glyoxylate, *i.e.*, 0.46 μmol/mg protein/h. In addition, rhatannin exhibited a more profound effect on glycine formation in the simultaneous presence of glutamine and glyoxylate. The increment induced by rhatannin was 17% as compared with the control value. Other glycine formations were not affected by rhatannin.

With respect to the conversion of glutamine to glutamic acid with or without glyoxylate, the effect of rhatannin administration on the glutamic acid production rate was examined 2 h after treatment. As shown in Table I, it was found that glutamic acid was formed from glutamine in the absence of glyoxylate and this production was abolished completely by the ketoacid. Moreover, rhatannin did not change the glutamic acid formation, which might derive from glutamine *via* glutaminase, regardless of the glyoxylate. This result is consistent with the previous observation that rhatannin did not alter the glutaminase activity in the liver.[4)]

The results of the present liver homogenate experiments suggested that the amination of glyoxylate by glutamine might involve a direct amination by glutamate. Nevertheless, it is possible that the increase of glycine formation might involve the amino acid derived from the transamination from glutamine *per se* to glyoxylate. Therefore, this transamination process

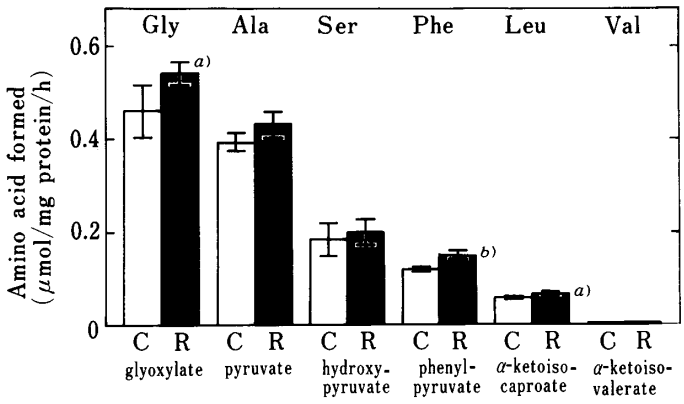


Fig. 3. Transamination from Glutamine to Various Keto Acids, and the Effect of Rhatannin

The reaction mixture initially contained liver extract, 50 mM borate buffer (pH 8.5), 1 mM EDTA, 20 mM glutamine, and either 20 mM glyoxylate, 20 mM pyruvate, 20 mM hydroxypyruvate, 20 mM phenylpyruvate, 20 mM α-ketoisocaproate, or 20 mM α-ketoisovalerate. Rhatannin was injected intraperitoneally into rats fasted for 24 h. Rats were sacrificed 2 h after rhatannin treatment.

Data are expressed as means ± S.D. of 4 rats. *a*) $p<0.05$, *b*) $p<0.01$, student's *t* test.

□ C, control rats; ■ R, rhatannin-treated rats.

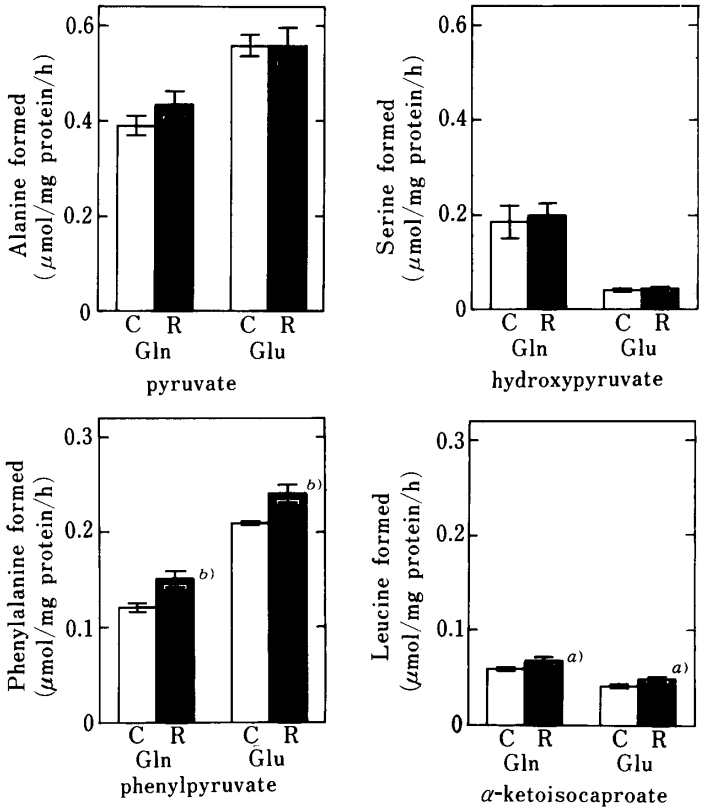


Fig. 4. Effects of Glutamine and Glutamate on the Transamination of Keto Acids, and the Influence of Rhatannin

The reaction mixture initially contained liver extract, 50 mM borate buffer (pH 8.5), 1 mM EDTA, 20 mM glutamine (Gln) or 20 mM glutamate (Glu) and either 20 mM pyruvate, 20 mM hydroxypyruvate, 20 mM phenylpyruvate, or 20 mM α-ketoisocaproate. Rhatannin was injected intraperitoneally into rats fasted for 24 h. Rats were sacrificed 2 h after rhatannin treatment.

Data are expressed as means ± S.D. of 4 rats. *a*) $p<0.05$, *b*) $p<0.01$, student's *t* test. Upper left-hand panel, alanine formation; upper right-hand panel, serine formation; lower left-hand panel, phenylalanine formation; lower right-hand panel, leucine formation.

□ C, control rats; ■ R, rhatannin-treated rats.

might be enhanced in rhatannin-treated rat.

## Characterization of the Effect of Rhatannin on Aminations by Glutamine of Various Keto Analogues of Amino Acids

It seemed interesting to determine whether rhatannin enhanced aminations by glutamine of a number of keto analogues of amino acids (α-ketoacids). Figure 3 shows the production rates of amino acids examined, and the effect of rhatannin at 2 h after the treatment. As

presented in Fig. 3, the relative rates of amination of α-ketoacids were 85%, 40%, 26%, 13%, and 2% using pyruvate, hydroxypyruvate, phenylpyruvate, α-ketoisocaproate, and α-ketoisovalerate, respectively; these values are expressed relative to the glycine formation rate observed in the amination of glyoxylate with glutamine, *i.e.*, 0.46 μmol/mg protein/h. In addition, rhatannin had a stimulatory effect on phenylalanine and leucine productions. The increments were 24% and 13%, respectively, as compared with the individual control values. Alanine, serine, and valine formations were not affected by rhatannin treatment. These results made it necessary to clarify whether or not direct amination from glutamate to keto analogues of these amino acids participated in the amination by glutamine. Figure 4 shows the individual amino acid production rates when glutamate was substituted for glutamine, and the effect of rhatannin on these amination processes at 2 h after the treatment. Glutamate was a more powerful amino-donor to both pyruvate and phenylpyruvate than glutamine. The relative rates of these aminations by glutamate were 143% and 173%, using pyruvate and phenylpyruvate as amino-acceptors, respectively, when expressed relative to those observed in the amination by glutamine, *i.e.*, 0.39 and 0.12 μmol/mg protein/h, respectively. Therefore, alanine and phenylalanine formations might significantly involve the amino acids derived from preferential transamination from glutamate to pyruvate and phenylpyruvate. On the other hand, glutamine was a more effective amino-donor than glutamate in the aminations of hydroxypyruvate and α-ketoisocaproate. The relative rates of the amination by glutamine are 440% in the former case and 144% in the latter, when the values are expressed relative to those observed in the amination by glutamate, *i.e.*, 0.04 μmol/mg protein/h in both, using the keto analogues of serine and leucine. These results indicate that serine and leucine formed might include in part the amino acids produced by transamination from glutamine to their keto analogues.

Rhatannin enhanced phenylalanine and leucine formations in the amination by glutamate, as also observed in the amination by glutamine. The increments are 24% in phenylalanine and 17% in leucine, using glutamate as the amino-donor.

The results presented here indicate that the stimulatory effect of rhatannin on the aminations of phenylpyruvate and α-ketoisocaproate by glutamine might involve direct amination of these ketoacids by glutamate.

**Effects of Administration of Actinomycin D or Cycloheximide on the Enhanced Transamination Induced by Rhatannin**

In order to analyze the mechanism by which rhatannin enhances the amination of glyoxylate by glutamine, the effects of actinomycin D or cycloheximide on the increased glycine formation induced by rhatannin were tested. Actinomycin D, cycloheximide or saline was injected 30 min before the administration of rhatannin. Glycine formation rates were estimated 4 h after rhatannin treatment. The results are illustrated in Fig. 5. Actinomycin D and cycloheximide alone had no significant effect on the control level of glycine production. Cycloheximide completely abolished the elevated level of glycine formation induced by

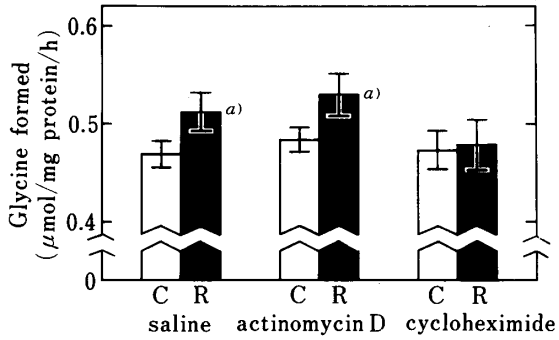


Fig. 5. Effect of Actinomycin D or Cycloheximide on the Rhatannin-Stimulated Increase in Glycine Formation

Rats fasted for 24 h were administered actinomycin D, cycloheximide, or saline 30 min prior to rhatannin treatment, and the glycine formation rate was estimated 4 h after the rhatannin treatment.

Data are expressed as means ± S.D. of 5 rats. *a)* $p < 0.05$, student's *t* test.

□ C, control rats; ■ R, rhatannin-treated rats.

rhatannin, while actinomycin D did not. The present results appear to indicate that new protein synthesis is required for the stimulatory effect of rhatannin.

The lack of inhibition by actinomycin D suggested that the administration of rhatannin might exert its effect at the level of translation. However, the sensitivity to actinomycin D inhibition of the amination from glutamine to glyoxylate is obscure. Therefore, these results suggest that the increase in glycine production induced by rhatannin depends on protein synthesis.

## Discussion

It is well known that glutamine transaminase is involved in the amination of α-ketoacid analogues of amino acids. Cooper and Meister indicated that it may function under normal physiological conditions to re-aminate small amounts of α-ketoacids formed during normal metabolism of essential amino acids.[7]

The present work was undertaken to evaluate the effect of rhatannin on the glutamine transaminase pathway in rat liver. The results of the investigation give a definitive picture with respect to the effect of rhatannin on the transamination of glutamine to glyoxylate. The administration of rhatannin brought about an increase in the glycine production rate 2 to 4 h after the treatment.

The data in the present investigation strongly suggested that we were not dealing with a single transamination process under the present experimental conditions. Several aminations of the keto analogues of amino acids are known, including those catalyzed by (a) glutamine-α-ketoacid transaminase,[8-10] (b) glyoxylate aminotransferase,[11-13] (c) serine–pyruvate transaminase,[14] and (d) leucine aminotransferase.[15]

For example, glutamine-α-ketoacid transaminase occurs in liver.[16] Gorden has shown that added glutamine increases the conversion of α-keto-β-methylthiobutyrate to methionine in the supernate of rat liver homogenate.[17] Cooper and Meister have examined the specificity of the enzyme towards a number of ketoacids: the relative activities are glyoxylate > hydroxypyruvate > pyruvate > phenylpyruvate > α-ketoisocaproate, and α-ketoisovalerate does not react.[18]

The results of the present liver homogenate experiments indicated that the order of effectiveness of ketoacids (20 mM) as co-substrates was glyoxylate > pyruvate > hydroxypyruvate > phenylpyruvate > α-ketoisocaproate, and little activity was observed with α-ketoisovalerate in the presence of 20 mM glutamine (Fig. 3). With glutamate (20 mM), the order was pyruvate > glyoxylate > phenylpyruvate > hydroxypyruvate, α-ketoisocaproate, and little activity was observed with α-ketoisovalerate (Figs. 2 and 4). Therefore, glutamine-α-ketoacid transaminase might participate in part in the glutamine transamination reaction involving these ketoacids.

The rhatannin-induced elevation of glycine formation was indicated to be accounted for by the increased transamination from glutamine. The rhatannin treatment may exert its effect at the level of translation, since actinomycin D failed to prevent the increased glycine formation, whereas cycloheximide prevented it. However, it is not clear what kind of glutamine transaminase contributed to the increased transamination reaction involving glyoxylate.

Cooper and Meister suggested that transamination of glutamine may provide a means for the reamination of α-ketoacids formed by other transaminases, and this might serve as a salvage function for α-keto analogues of amino acids.[19] The present results indicate that the increased glycine formation might be mediated by glutamine transaminase in rhatannin-treated rats. This is possible only when glutamine and glyoxylic acid are preferentially usable for glycine formation under the physiological conditions of the rat. If this is a case, it is

reasonable to consider that rhatannin might enhance the salvage function.

Thus, the present work suggests that the administration of rhatannin might enhance the transaminase pathways for glutamine degradation in rat liver, although the underlying mechanism of the enhancement induced by rhatannin is obscure. This might be in part related to the decreased plasma glutamine level observed following rhatannin treatment.

## References


1) J. D. McGivan, N. M. Bradford, A. J. Verhoven, and A. J. Meijer, "Glutamine Metabolism in Mammalian Tissues," ed. by D. Häussinger and H. Sies, Springer-Verlag, Berlin, Heidelberg, 1984, p. 122.
2) A. Meister, "Glutamine Metabolism in Mammalian Tissues," ed. by D. Häussinger and H. Sies, Springer-Verlag, Berlin, Heidelberg, 1984, p. 3.
3) P. Lund, *FEBS Lett.*, **117**, K86 (1980).
4) S. Shibutani, T. Nagasawa, H. Oura, G. Nonaka, and I. Nishioka, *Chem. Pharm. Bull.*, **31**, 2378 (1983).
5) G. Nonaka, I. Nishioka, T. Nagasawa, and H. Oura, *Chem. Pharm. Bull.*, **29**, 2862 (1981).
6) O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).
7) A. J. L. Cooper and A. Meister, *J. Biol. Chem.*, **249**, 2554 (1974).
8) A. Meister, "The Enzymes," Vol. 6, ed. by P. D. Boyer, H. Lardy, and K. Myrbaeck, Academic Press, New York, 1962, p. 247.
9) A. J. L. Cooper and A. Meister, *Comp. Biochem. Physiol.*, **69B**, 137 (1981).
10) A. J. L. Cooper and A. Meister, *Prog. Clin. Biol. Res.*, **144B** (Chem. Biol. Aspects Vitam. $B_6$ Catal., part B), 3 (1984).
11) T. Noguchi, Y. Minatogawa, Y. Takada, Y. Okuno, and R. Kido, *Biochem. J.*, **169**, 113 (1978).
12) T. Noguchi, Y. Minatogawa, Y. Takada, Y. Okuno, and R. Kido, *Biochem. J.*, **170**, 173 (1978).
13) B. Hsieh and N. E. Tolbert, *J. Biol. Chem.*, **251**, 4408 (1976).
14) T. Noguchi, Y. Takada, and R. Kido, *Biochem. J.*, **161**, 609 (1977).
15) K. Aki, K. Ogawa, and A. Ichihara, *Biochim. Biophys. Acta*, **159**, 276 (1968).
16) B. J. Langer, Jr., *Biochem. J.*, **95**, 683 (1965).
17) R. S. Gorden, *Ann. N. Y. Acad. Sci.*, **119**, 927 (1965).
18) A. J. L. Cooper and A. Meister, *Biochemistry*, **11**, 661 (1972).
19) M. Walser, P. Lund, N. B. Ruderman, and A. W. Coulter, *J. Clin. Invest.*, **52**, 2865 (1973).