Biochem. J. (1987) 244, 345–350 (Printed in Great Britain)

Regulation by insulin of liver carbamoyl-phosphate synthase II (glutamine-hydrolysing)

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Evidence is provided that insulin controls the amount and synthetic rate of liver carbamoyl-phosphate synthase II (EC 6.3.5.5) (synthase II) in rat. In 3- and 6-day starvation, with low plasma insulin, synthase II specific activity decreased to 47 and 30%, respectively, of normal; on re-feeding and with concurrent insulin injections, liver synthase II activity increased to 2.5 and 3 times that of starved rats respectively. Treatment with anti-insulin serum during re-feeding prevented the rise in synthase II activity. In diabetic rats, synthase II activity decreased to 28% of normal and was increased by insulin treatment for 2 and 7 days to 4.8- and 5.6-fold of the activity in diabetic liver; this rise in activity was blocked by actinomycin. Immunotitration demonstrated that alterations in synthase II activity were due to changes in the enzyme amount. In starvation, the relative synthesis rate of synthase II decreased to 44%, with an increase in catabolic rate to 122%; re-feeding returned these to control values. In diabetes the synthase II synthesis rate decreased to 52% and the degradative rate was accelerated to 180%; insulin treatment induced synthesis and returned degradation to the control range. Thus the integrative action of insulin in liver pyrimidine metabolism entails regulation of the amount and turnover of synthase II.

INTRODUCTION

Carbamoyl-phosphate synthase II (glutamine-hydrolysing) (EC 6.3.5.5) catalyses the first and rate-limiting step in the '*de novo*' pyrimidine-biosynthetic pathway. The enzyme exists in the cytosol as a multi-enzyme complex (multi-functional protein) with aspartate carbamoyltransferase (EC 2.1.3.2) and dihydro-orotase (EC 3.5.2.3), the second and third enzymes of uridylate synthesis *de novo* (Jones, 1980). The complex regulatory properties of liver synthase II activity involve end-product inhibition by UDP and UTP and activation by 5-phosphoribosyl 1-pyrophosphate, as well as an absolute requirement for the cations Mg^{2+} and K^+ (Tatibana & Shigesada, 1972; Aoki *et al.*, 1982). Preliminary evidence was provided that insulin played a role in the maintenance of rat liver synthase II activity (Reardon & Weber, 1983).

The purpose of the present investigation was to elucidate the role of insulin in the regulation of hepatic synthase II and to test whether the adaptive responses of the enzyme in nutritional and hormonal regulatory conditions were due to changes in the amount and turnover of the synthase II protein. This paper provides direct evidence that in starvation and re-feeding, in diabetes and after insulin administration the synthase II activity, amount and synthetic rate change concurrently, demonstrating that insulin plays an essential role in the regulation of liver synthase II.

EXPERIMENTAL

Material

Protamine zinc insulin was purchased from Lilly Research Laboratories, Indianapolis, IN, U.S.A. and actinomycin from Merck, Sharp and Dohme, West Point, PA, U.S.A. $Na_2{}^{14}CO_3$ and OCS (organic counting scintillant) cocktail were from Amersham, Arlington Heights, IL, U.S.A. Ready-to-Use scintillation fluid III was from Eastman Kodak Co., Rochester, NY, U.S.A., and Protosol from New England Nuclear, Boston, MA, U.S.A. L-[4,5-^{3}H]Leucine and L-[U-^{14}C]leucine were purchased from ICN Radiochemicals, Irvine, CA, U.S.A. Sepharose 6B was from Pharmacia Biochemicals, Piscataway, NJ, U.S.A., and guinea-pig anti-insulin serum from Linco Research, Eureka, MO, U.S.A. Hydroxyapatite (Bio-Gel HTP) and reagents for protein assay were from Bio-Rad Laboratories, Richmond, CA, U.S.A. All other reagents, also of the highest available purity, were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A.

Biological systems

Male Wistar rats were maintained in individual cages with water and food *ad libitum* unless otherwise specified. Diabetes was induced, in animals starved for 30 h, by a single intraperitoneal injection of 175 mg of alloxan monohydrate/kg body wt. Protamine zinc insulin (4 units/100 g body wt.) was injected subcutaneously every 12 h. Actinomycin (8 μg/100 g body wt.) was injected intraperitoneally every 12 h, always 30 min before insulin administration. Four New Zealand White rabbits, 3–4 kg in weight, were used for antibody production.

Assays

Synthase II activity was assayed with $KH^{14}CO_3$ as substrate by measuring the formation of L-[*carbamoyl*-^{14}C]citrulline in the presence of excess L-ornithine and

Abbreviation used: synthase II, carbamoyl-phosphate synthase II.

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ornithine carbamoyltransferase from *Streptococcus faecalis* (Aoki *et al.*, 1982). The protein concentration was measured directly by the method of Bradford (1976), by using the commercially available Bio-Rad reagent dye. Bovine serum albumin was used as the standard. Blood sugar was determined by Nelson's adaptation of the Somogyi method (Nelson, 1944). Plasma insulin concentrations were measured by the ^{125}I-insulin immunoassay (Helding, 1972; Kuzuya *et al.*, 1977).

Enzyme purification

Synthase II was purified to apparent homogeneity from hepatoma 3924A and antiserum was produced and extensively characterized as previously reported (Reardon & Weber, 1985, 1986). Unless otherwise specified, all potassium phosphate buffers, pH 7.8, contained a final concentration of 5% (w/v) glycerol, 30% (v/v) dimethyl sulphoxide, 4 mM-L-glutamine, 4 mM-L-aspartic acid and 10 mM-dithiothreitol.

Synthase II immunotitration

To achieve a precise and readily repeatable enzyme assay and immunotitration methods, it was necessary to separate the liver cytosolic glutamine-utilizing synthase II from contamination by liver mitochondrial carbamoylphosphate synthase I (EC 6.3.4.16), the ammonia-utilizing enzyme responsible for carbamoyl phosphate formation in the urea cycle. To measure cytosolic hepatic synthase II activity and amount, the 105000 *g* supernatant fractions from 20% (w/v) tissue homogenates were first subjected to a 30%-satd.-$(NH_4)_2SO_4$ fractionation and then washed in twice the volume of a hydroxyapatite slurry equilibrated in 30 mM-potassium phosphate buffer (Aoki *et al.*, 1982). Samples were assayed in the presence of NH_4Cl and NH_4Cl plus *N*-acetyl-L-glutamic acid, an essential allosteric effector of synthase I. When no activation of synthase I activity was observed on the addition of this activator, the liver fractions were considered free of this interfering activity.

Immunological neutralization of hepatic synthase II was conducted by incubation of a fixed volume of antigen (100 μl) with increasing amounts of antibody (20 mg/ml) in a final volume of 0.3 ml of sodium barbital buffer, pH 8.6, for 30 min at 37 °C, followed by a 4 h incubation at 0 °C. Goat anti-rabbit IgG was then added, and the incubation was continued for another 4 h at 0 °C. After centrifugation at 12000 *g* for 20 min at 4 °C, a precipitate was observed, and the synthase II activity remaining in the supernatant was assayed and compared with controls that were incubated with pre-immune serum.

The immunotitration data were plotted by using a linear-regression least-squares best-fit analysis for the straight portion of the titration curve, where the inhibition of the enzyme activity was directly proportional to the amount of antibody added.

Determination of the relative rates of synthesis *in vivo*

The incorporation of L-[4,5-^{3}H]leucine (sp. radioactivity 58 Ci/mmol) in synthase II was determined by pulse-labelling and specific immunoprecipitation. Each rat was injected intraperitoneally with 100 μCi of L-[4,5-^{3}H]leucine/100 g body wt. in a final volume of 0.4 ml of 0.9% NaCl; 4 h later the rats were killed, and the livers were immediately removed and homogenized in 4 vol. of 50 mM-potassium phosphate extraction buffer. After centrifugation at 105000 *g* for 30 min at 0 °C, 1 ml of the supernatant was precipitated with excess antibody. The immunoprecipitates were washed and collected three times by centrifugation at 7000 *g* for 20 min at 0 °C through a 1 M-sucrose cushion (2 ml). The pellet was then dissolved in 300 μl of Protosol and incubated for 12 h at 37 °C, after which 10 ml of OCS fluid was added and the radioactivity was counted.

Radioactivity incorporated into total cytosolic protein was measured from the 105000 *g* supernatant fraction by the method of Mans & Novelli (1961) as modified by Dunaway & Weber (1974). The relative rates of synthesis were expressed as the ratios of [^{3}H]leucine incorporated into synthase II relative to that incorporated into total cytosolic protein (Hopgood & Ballard, 1973). This method allowed for the possible variations in the amount of label injected and for changes in the precursor leucine pool.

Determination of apparent rates of degradation *in vivo*

The apparent degradation rate for synthase II was determined by the double-isotope method of Glass & Doyle (1972). Each rat was injected intraperitoneally with 25 μCi of L-[U-^{14}C]leucine (sp. radioactivity 300 mCi/mmol)/100 g body wt. in a final volume of 0.4 ml of physiological saline at zero time. A second intraperitoneal injection of 100 μCi of L-[4,5-^{3}H]leucine (sp. radioactivity 60 Ci/mmol)/100 g was given at 0, 23, 47 and 71 h. The animals were killed 1 h after the [^{3}H]leucine injection. Therefore the rats were exposed to the [^{14}C]leucine for 1, 24, 48 and 72 h and to the ^{3}H label for 1 h. The apparent degradation rates were calculated from the ^{3}H/^{14}C leucine ratios obtained from the immunoprecipitates.

As proposed by Block *et al.* (1971), a control experiment was conducted where the animals were given both radioactive leucine labels at the same time (zero time) and killed 1 h later. This ^{3}H/^{14}C ratio obtained provided a correction factor for the isotopic leucine ratio expected without significant degradation.

Statistical evaluation of results

Results were statistically evaluated by Student's *t* test for small samples. Differences between the means yielding a probability of less than 5% were considered as significant. There were four or more rats per experimental group.

RESULTS AND DISCUSSION

Effect of starvation and re-feeding on liver synthase II activity and plasma insulin concentration

To assess the responsiveness of synthase II activity to insulin regulation, the effects of starvation and re-feeding were examined (Table 1). In control fed rats, liver synthase II activity was 9.2 ± 0.1 nmol/h per mg of protein and circulating plasma insulin concentration was 39 ± 2 μunits/ml. In 3- and 6-day-starved animals, liver synthase II specific activity decreased to 47 and 30% of control respectively. This decline in synthase II activity paralleled the plasma insulin concentrations, which fell to 28 and 18% of the normal value. Re-feeding the 3-day-starved rats resulted in the return to control values of the body and liver weights, plasma insulin and

Table 1. Effect of starvation and re-feeding on liver synthase II activity

Rats starved for 3 days were re-fed for 2 days. Insulin (4 units/100 g body wt.) was injected subcutaneously every 12 h. Actinomycin (8 μg/100 g body wt.) was injected intraperitoneally every 12 h during the re-feeding period. Anti-insulin serum (AIS; 2 ml; titre 2.5 munits/μl) was injected intraperitoneally in the starved rats 4 h before and every 6 h during the re-feeding period. Means $\pm$ S.E.M. for four rats per group are given: *significantly different from control fed rats ($P < 0.05$). Values in parentheses are percentages of controls.

Condition	Body wt. (g)	Liver wt. (g)	Blood sugar (mg/100 ml)	Plasma insulin (μunits/ml)	Synthase II activity (nmol/h per mg of protein)
Control fed	237±3 (100)	9.5±0.3 (100)	115±2 (100)	39±2 (100)	9.2±0.1 (100)
Starved 3 days	171±5 (72)*	4.5±0.2 (47)*	84±2 (73)*	11±1 (28)*	4.3±0.2 (47)*
Starved 6 days	157±3 (66)*	4.0±0.1 (42)*	70±1 (61)*	7±0.1 (18)*	2.8±0.1 (30)*
Re-fed	210±1 (89)*	9.1±0.2 (96)	122±2 (106)	38±3 (97)	10.9±0.2 (118)*
Re-fed+ insulin	220±2 (93)	10.4±0.1 (109)*	39±5 (34)*	153±17 (392)*	12.8±0.2 (139)*
Re-fed+ actinomycin	182±3 (77)*	5.3±0.1 (56)*	112±3 (97)	40±3 (103)	4.6±0.1 (50)*
Re-fed+ AIS	188±2 (79)*	4.3±0.1 (45)*	607±74 (528)*	5±2 (13)*	4.7±0.1 (51)*

Table 2. Effect of diabetes and insulin on liver synthase II activity

Insulin (4 units/100 g body wt.) was injected subcutaneously every 12 h. Actinomycin (8 μg/100 g body wt.) was injected intraperitoneally every 12 h, always 30 min before insulin administration. Means $\pm$ S.E.M. for four rats per group are given: *significantly different from control normal ($P < 0.05$).

Condition	Body wt. (g)	Liver wt. (g)	Blood sugar (mg/100 ml)	Plasma insulin (μunits/ml)	Synthase II activity (nmol/h per mg of protein)
Control normal	145±3 (100)	5.9±0.4 (100)	109±2 (100)	46±2 (100)	9.4±0.1 (100)
Diabetic	115±2 (79)*	3.6±0.1 (61)*	811±60 (744)*	8±4 (17)*	2.6±0.1 (28)*
Diabetic+ 2 days insulin	141±2 (97)	9.9±0.7 (168)*	46±5 (42)*	139±6 (302)*	12.5±0.3 (133)*
Diabetic+ 7 days insulin	186±3 (128)*	13.9±0.3 (236)*	31±2 (28)*	> 200 > (435)*	14.5±0.2 (154)*
Diabetic+ 2 days insulin +actinomycin	122±4 (84)*	4.3±0.2 (73)*	44±3 (40)*	157±8 (341)*	3.2±0.1 (34)*

synthase II activity. Thus the endogenous insulin released owing to the re-feeding stimulus, a reported 2–3 units/day per 100 g body wt. (Armin *et al.*, 1960), was enough to increase synthase II activity to 10.9±0.2 nmol/h per mg of protein, a 2.5-fold rise from that in 3-day-starved rats. When during the 2-day re-feeding period the animals were also treated with insulin, hepatic synthase II activity increased to 12.8±0.2 nmol/h per mg of protein, a 3-fold rise compared with activities in starved rats. Administration of actinomycin during the 2-day re-feeding prevented the re-feeding-induced rise in synthase II activity, without altering the return of plasma insulin concentrations to normal.

Effect of anti-insulin serum during re-feeding

To provide direct evidence for the role of insulin in the induction of liver synthase II, anti-insulin serum was injected to neutralize endogenously secreted insulin released in response to the re-feeding signal (Table 1). When the starved rats were re-fed and given concurrent injections of anti-insulin serum, the free plasma insulin was only 5±2 μunits/ml, 10% of the insulin concentration in the untreated re-fed rats. This severe lack of biologically active insulin was manifested in the serum-treated rats in a diabetic syndrome that resulted in hyperglycaemia, blood sugars averaging 581±74 mg/100 ml, polydypsia, polyuria, glucosuria and ketonuria.

In the animals given injections of anti-insulin serum during the re-feeding period, the synthase II activity failed to rise and remained at the value for the starved rats (51% of control). The stomach and intestines of the serum-treated rats were full of food at the time of killing, indicating that food was consumed. Therefore the re-feeding-induced metabolic adaptation was attributed primarily to insulin.

Effects of diabetes on liver synthase II activity

In the insulin-deficient state of diabetes, hepatic synthase II activity markedly decreased (to 28%) and insulin administration returned it to above the control values (Table 2). In the control normal group, liver synthase II activity was 9.4 ± 0.1 nmol/h per mg of protein; plasma insulin and blood glucose concentrations were 46 ± 2 μunits/ml and 109 ± 2 mg/100 ml respectively. In the diabetic rats, plasma insulin concentration decreased to 17% (8 ± 4 μunits/ml) and the enzyme activity to 28% (2.6 ± 0.1 nmol/h per mg of protein) of the control normal values; the blood sugar increased to 811 ± 60 mg/100 ml. Insulin treatment of the diabetic animals resulted in a marked rise in liver synthase II activity, to 12.5 ± 0.3 nmol/h per mg of protein after 2 days and to 14.5 ± 0.2 nmol/h per mg after 1 week, a 4.8- and 5.6-fold rise from the activity of the diabetic rats. When the diabetic animals were given actinomycin injections (30 min before insulin injections), the insulin-induced rise in liver synthase II activity was blocked; the decrease in blood sugar was unaffected by this drug. This study provided presumptive evidence that the insulin-induced rise in hepatic synthase II activity was due to synthesis of new enzyme protein. To test this possible mechanism of insulin action, the enzyme was purified and antibody was produced for immunotitration and turnover studies.

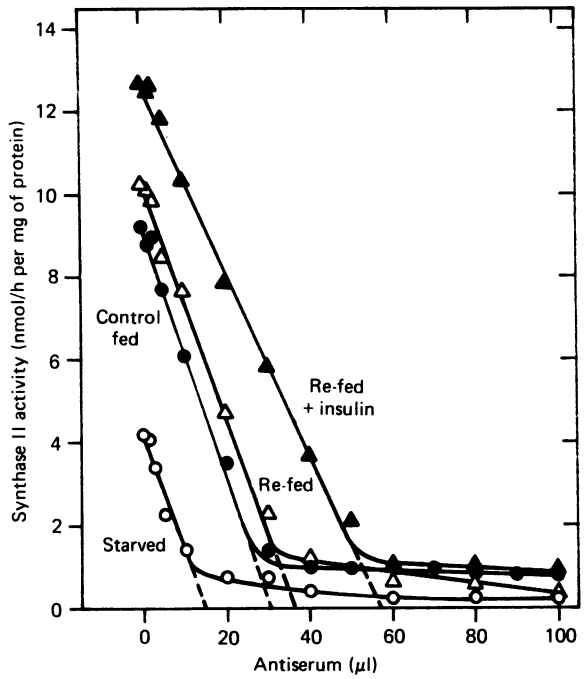

Fig. 1. Immunotitration of liver synthase II in starvation and re-feeding

Before immunotitration, the liver supernatant fractions [prepared from 20% (w/v) homogenates] were subjected to a 30%-satd.-$(NH_4)_2SO_4$ precipitation and then washed in twice the volume of hydroxyapatite slurry, equilibrated in 30 mM-potassium phosphate buffer, followed by assay in the presence of NH_4Cl and NH_4Cl plus *N*-acetyl-L-glutamic acid, an essential allosteric effector of mitochondrial synthase I activity. Liver samples from the various experimental groups were immunotitrated with increasing amounts of antibody in a final volume of 0.3 ml of sodium barbital buffer, pH 8.6, for 30 min at 37 °C and then for 4 h at 0 °C. Goat anti-rabbit IgG was added and the incubation was continued for another 4 h at 0 °C. After centrifugation, synthase II activity remaining in the supernatant was assayed.

Immunotitration of liver synthase II in starvation and re-feeding

The quantity of antiserum required to neutralize synthase II activity from livers of normal fed rats was 31 ± 3 μl, and in the hypo-insulin state of starvation this decreased to 15 ± 1 μl, or 48% of the control value. When the starved rats were re-fed, or re-fed with concurrent insulin therapy, the antiserum amount required for titration of synthase II was 36 ± 1 and 57 ± 2 μl, a 2.4- and 3.8-fold rise respectively from that for starved rats (Fig. 1).

Immunotitration analysis of liver synthase II in diabetes and insulin treatment

The mechanism of the alterations in hepatic synthase II activity and amount was studied further by immunotitration of the enzyme obtained from cytosolic fractions of livers. Fig. 2 shows that the quantity of antiserum required to titrate the liver synthase II protein from normal animals was 34 ± 2 μl; in diabetes this amount decreased to 9 ± 1 μl, or 26% of the control normal value. When diabetic rats were given insulin for 2 days, the amount of immunoreactive enzyme increased 6.2-fold above that observed in the diabetic rats, requiring 56 ± 2 μl of antiserum for neutralization. Actinomycin blocked the rise in synthase II specific activity and the enzyme protein amount, with both parameters remaining in the range of diabetic rats.

These immunotitration studies demonstrated that alterations in liver synthase II activity in starvation and re-feeding and in diabetes and insulin treatments were accompanied by changes in the amounts of immuno-

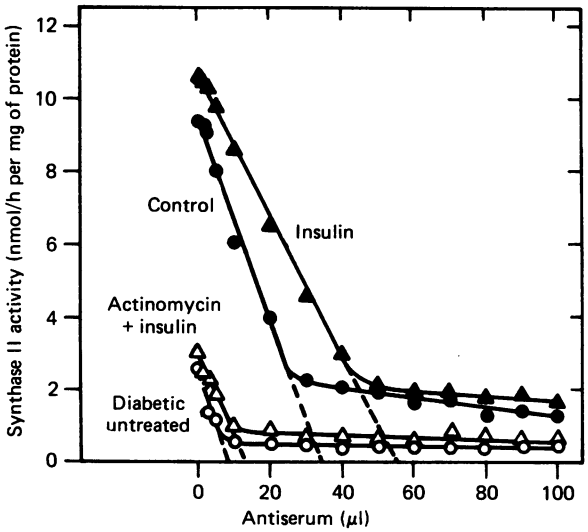

Fig. 2. Immunotitration of liver synthase II in diabetes and therapy

Sample preparation and procedures were as described in Fig. 1.

Table 3. Determination *in vivo* of the synthesis and degradation rates of hepatic synthase II

Relative rates of synthesis and apparent degradation rates were determined as described in the Experimental section; 3-day-starved rats were re-fed for 2 days. Means ± S.E.M. for four rats per group are given: *significantly different from the controls ($P < 0.05$).

Condition	[^{3}H]Leucine incorporation (d.p.m./g of tissue) into:		$10^3 \times {}^3$H incorporation ratio (synthase II cytosolic protein)	Apparent degradation rate ($t_{\frac{1}{2}}$, h)
	Synthase II	Cytosolic protein		
Control, normal fed	9159 ± 1077 (100)	527100 ± 9399 (100)	17.4 ± 0.4 (100)	69.3
Starved 3 days	2837 ± 277 (31)*	375575 ± 16062 (71)*	7.6 ± 0.1 (44)*	56.8
Re-fed	7852 ± 1075 (86)	472825 ± 10530 (90)	16.6 ± 0.3 (95)	70.1
Control, normal fed	10980 ± 1367 (100)	637463 ± 8940 (100)	17.2 ± 0.2 (100)	69.3
Diabetic	3507 ± 944 (32)*	394000 ± 3830 (62)*	8.9 ± 0.1 (52)*	38.5
Diabetic + insulin	15310 ± 1737 (139)*	642410 ± 2910 (101)	23.8 ± 0.4 (138)*	71.5

reactive enzyme. No inhibitory effect was observed on addition of control pre-immune serum (results not shown). The slopes of the titration curves were approximately the same or parallel, 0.28–0.31 for the experimental groups, except where exogenous insulin was administered (Figs. 1 and 2). For the insulin-treated animals, the slopes were somewhat steeper, 0.20–0.21, indicating that the increase in synthase II activity was not perfectly proportional to the amount of immuno-identified enzyme protein.

Relative synthesis and apparent degradation rates of liver synthase II in starvation and re-feeding

The pulse-labelling studies showed that in the control fed group the ratio of [^{3}H]leucine incorporation into hepatic synthase II to total cytosolic protein was $17.4 (\pm 0.4) \times 10^{-3}$. As a result of a 3-day starvation, the enzyme synthesis rate decreased to 44% of the rates observed in the fed animals. On re-feeding, the synthesis rate returned to normal range, $16.6 (\pm 0.3) \times 10^{-3}$ (Table 3).

In the fed rats, the apparent degradation rate (K_d) of liver synthase II was 0.0100 h^{-1}. When the animals were starved over a 3-day period, the degradation rate increased to 0.0122 h^{-1}; re-feeding returned the rate to normal range. The corresponding half-lives ($t_{\frac{1}{2}}$) were 69.3, 56.8 and 70.1 h. Plots of these results indicated that the decay of liver synthase II followed a first-order reaction, with linear correlation coefficients of 0.928, 0.872 and 0.964 for the fed, starved and re-fed groups respectively (results not shown).

Relative synthesis and apparent degradation rates of liver synthase II in diabetes and in insulin treatment

In the control normal rats, the ratio of [^{3}H]leucine incorporation was $17.2 (\pm 0.2) \times 10^{-3}$, and in diabetes the relative rate of synthesis decreased to $8.9 (\pm 0.1) \times 10^{-3}$, or 52%. Insulin injections to the diabetic rats induced enzyme synthesis to $23.8 (\pm 0.4) \times 10^{-3}$, overshooting control normal rates by 38% (Table 3).

For liver synthase II in the normal rats the apparent K_d was 0.0100 h^{-1}, and in the diabetic rats this value increased to 0.0180 h^{-1}. Insulin treatment of the diabetic rats resulted in an apparent K_d of 0.0097 h^{-1}, thus returning the rate to the normal range. These apparent K_d values corresponded to half-lives ($t_{\frac{1}{2}}$) of 69.3, 38.5 and 71.5 h, which showed that there was an accelerated breakdown of liver synthase II in diabetes compared with the control and the insulin-treated groups. The plots of these results yielded straight lines, suggesting that in each case the enzyme degradation followed a first-order decay process. The linear correlation coefficients calculated for the lines for the normal, diabetic and insulin-injected groups were 0.970, 0.920 and 0.981 respectively (results not shown).

Integrative action of insulin in liver pyrimidine metabolism

Evidence was provided that insulin has a role in the maintenance of rat liver synthase II amount and turnover. The requirement for insulin in maintaining the activity of this rate-limiting pyrimidine-synthetic enzyme was shown by the rapid decrease in synthase II activity in metabolic alterations where plasma insulin decreased. With restoration of circulating insulin concentration, the enzymic activity returned to normal range. This correlation between plasma insulin concentration and liver synthase II activity was shown in starvation and diabetes and in re-feeding and insulin treatment. Studies with actinomycin suggested that insulin acted through enzyme synthesis involving production of mRNA. The anti-insulin-serum treatment of starved rats undergoing re-feeding indicated that insulin was a major, if not the sole, factor responsible for the rise of synthase II activity. Immunotitration provided evidence showing that insulin acted as an inducer of liver synthase II activity and the alterations caused by insulin action were due to changes in the amount of hepatic synthase II. Turnover studies demonstrated that insulin achieved the induction of liver synthase II activity primarily through an increased rate of synthesis.

Novel aspects of this study include the evidence that the integrative action of insulin on rat liver entails a regulation of pyrimidine metabolism through the control of the rate-limiting enzyme of uridylate biosynthesis *de novo*. These new observations on the regulation of synthase II by insulin agrees well with the fact that, along with the decreased insulin concentrations in diabetic rats, the hepatic UTP concentration significantly declined and insulin treatment restored UTP pools in a dose-dependent

manner (Weber *et al.*, 1980). Furthermore, this decrease in liver synthase II amount and UTP concentration in diabetic rats corresponded with the metabolic depletion in glycogen stores, and the restoration by insulin of synthase II activity and UTP pools is in accord with the utilization of this nucleotide for the action of UTP–glucose-1-phosphate uridylyltransferase (EC 2.7.7.9) activity for glycogen synthesis. Thus this investigation provides direct evidence that insulin is a major, if not critical, factor in the regulation of liver synthase II amount and turnover.

This investigation was supported by grants CA-42510 and CA-13526 from the National Cancer Institute for the United States (G.W.) and by a Fellowship Award from the Ladies Auxiliary of the Veterans of Foreign Wars (M.A.R.).

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Received 18 November 1986/26 January 1987; accepted 12 February 1987