Diabetes-induced Alterations in Liver Protein Synthesis

CHANGES IN THE RELATIVE ABUNDANCE OF mRNAs FOR ALBUMIN AND OTHER PLASMA PROTEINS

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Mechanisms responsible for diabetes-induced alterations in liver protein synthesis were investigated in vivo and in perfused liver using Bio-Breeding Worcester (BB/W) control rats, spontaneously diabetic BB/W rats maintained on insulin therapy, and diabetic BB/W rats withdrawn from insulin therapy for 48 h. Withdrawal of insulin therapy in the diabetic rats resulted in marked alterations in a number of parameters related to liver protein synthesis compared to BB/W control or insulin-maintained diabetic rats. Alterations seen in vivo following withdrawal of insulin included changes in the relative concentrations of several plasma proteins, a 40% reduction in total liver RNA relative to DNA, a 5-fold reduction in albumin synthesis relative to the synthesis of total liver proteins, a 5-fold reduction in albumin mRNA relative to total RNA, reductions in the relative abundance of mRNAs for at least four plasma proteins other than albumin, and a relative increase in mRNA for at least one plasma protein. Alterations observed in perfused liver included reductions in total liver protein synthesis (60% of control), albumin production (24% of control), and total secretory protein production (44% of control). All parameters studied were essentially unchanged from BB/W control values when the diabetic rats were maintained on insulin therapy. The results indicate that insulin deficiency leads to marked reductions in liver protein synthesis, particularly the synthesis of albumin and other plasma proteins. The mechanisms responsible for these alterations include changes in the relative abundance of specific mRNAs and a decrease in total cellular RNA.

Although the effects of diabetes on liver protein metabolism have been studied extensively in the past (revised in Refs. 13 and 40), the mechanisms responsible for alterations in protein synthesis are mainly unresolved. The diabetic models used in all previous studies were animals with experimentally induced diabetes. In most studies, diabetes was induced with either alloxan or streptozotocin, two chemicals with marked toxicity for the insulin-producing pancreatic ß-cells. These chemicals are not totally selective, however, and they are known to have cytotoxic effects on other tissues including the liver (42). Therefore, a question has always remained as to whether alterations observed in liver protein synthesis were the results of diabetes per se or a hepatotoxic effect of the agent used to induce diabetes.

The opportunity to resolve this question has been provided by the availability of a colony of spontaneously diabetic rats. The Bio-Breeding Worcester (BB/W) rat develops an insulin-dependent, nonobese diabetic state that closely approximates human insulin-dependent diabetes (24). Diabetes in the BB/W rat occurs between 60 and 120 days of age with an approximate 30% frequency in both sexes. Untreated animals are similar to human insulin-dependent diabetics (19), as both develop acute pancreatic insulitis with subsequent ketosis-prone insulin deficiency. Although the type of diabetes has been well defined, the biochemical basis of the syndrome remains largely unclear and little is known about alterations in metabolism in the BB/W diabetic rat. A recent report has characterized some alterations in hepatic carbohydrate metabolism in the BB/W rat (2), but no studies on liver protein metabolism have been reported.

In the present study, the BB/W rat has been used to investigate the mechanisms responsible for diabetes-induced alterations in liver protein synthesis. Results obtained in diabetic rats withdrawn from insulin therapy have been compared to those observed in diabetic rats maintained on insulin therapy and BB/W control animals. Since the liver may export up to one-third of the proteins it produces, synthesis rates for both secreted and nonexported proteins have been measured in vivo and in perfused liver. Special emphasis has been given to albumin, the major protein synthesized by the liver, and alterations in its rate of synthesis have been correlated with the albumin mRNA content of the liver.

EXPERIMENTAL PROCEDURES

Animals—Male diabetic BB/W rats and their nondiabetic sex-matched littermates of the fourth to sixth inbred generation were graciously provided by Dr. A. A. Like of the Department of Pathology at the University of Massachusetts Medical School, Worcester, MA. All animals were maintained in the animal facilities of that Medical School and were provided Purina Laboratory chow and water ad libitum; they weighed 300-500 g at the time of use. Diabetic rats were
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maintained for at least 1 month with insulin therapy consisting of 1.0 units of protamine zinc insulin/100 g of body weight/day. One group of diabetic rats was withdrawn from insulin therapy 48 h prior to experimentation. All animals were anesthetized prior to use with an intraperitoneal injection of pentobarbital (5.0 mg/kg of body weight).

Antibody Preparation—Antibodies to purified rat serum albumin and to pooled plasma from BB/W control, insulin-maintained diabetic, insulin-withdrawn diabetic, and diabetic BB/W diabetic rats were raised in rabbits, respectively, by serial injection with Freund’s complete and incomplete adjuvant. Purity of the goat anti-albumin antibodies was evaluated by crossed immunoelectrophoresis (47) which showed a single immunoprecipitation peak with normal adult rat plasma.

Protein Synthesis Determinations—Liver perfusion was performed on samples of plasma according to the method of Weeke (47), using 70 mM Tris buffer, pH 8.6, which contained 25 mM barbital, 0.3 mM calcium lactate, and 0.02% sodium azide. Agarose gels (1%, v/w) were prepared in the same buffer. Plasma samples (2.5 µl) were applied to wells 2.5 mm in diameter and electrophoresed at 100 V for 4.5 h in a water-cooled immunoelectrophoresis cell (15°C).

An agarose strip (2 × 10 cm) containing antigens that had been subjected to electrophoresis was transferred to a second glass plate (10 × 10 cm). Approximately 12 ml of agarose solution containing rabbit anti-plasma protein antibodies (5 mg/ml) was poured onto the remaining part of the glass plate. Electrophoresis in the second dimension was performed at 70 V for 18 h at 15°C. Gels were then pressed with filter paper, washed 4 times in 0.9% NaCl and H2O, air-dried, and stained for 3 min with 0.25% Coomassie brilliant blue R-250 (dissolved in 45% methanol, 7.5% acetic acid, and filtered). The gels were destained in 45% methanol, 7.5% acetic acid.

Liver Perfusion—Livers were perfused in situ under the conditions previously described for obtaining measurements of the rates of total liver protein synthesis, albumin production, and total secretory protein production (34). The liver was first flushed with 50 ml of perfusate, which was discarded, and a recirculating perfusion (75 ml/min) was then established with the remaining 100 ml of perfusion medium. A 30-min period of equilibration was followed by the addition to the perfusate of 500 µCi of [3H]leucine (1-4,5-[3H]leucine, Amersham Corp.). Samples of perfusate collected during the following 120 min of perfusion were concentrated to remove excess leucine. These concentrated samples were frozen and stored at -70°C for subsequent analyses. At the end of the perfusion period, livers were removed, weighed, and stored frozen at -70°C until analyzed.

Protein Synthesis Determinations—The relative rate of albumin synthesis in vivo was determined as previously described (6). Anesthetized rats were injected intraduodenally with 500 µCi of [3H]leucine and 10 min later livers were removed and analyzed for the amount of radioactivity in total protein and albumin, using trichloroacetic acid precipitation and immunoprecipitation with specific goat anti-albumin antibodies (47). The relative rate of [3H]albumin synthesis was expressed as the percentage of radioactivity in albumin compared to that in total liver protein.

Protein synthesis measurements in perfused liver were obtained under conditions where preliminary experiments had established that the specific activity of IRI-bound leucine was the same as that of the free leucine in the perfusate (3). Incorporation of [3H]leucine into total secreted proteins and albumin was determined on perfusate supernatants by trichloroacetic acid precipitation and immunoprecipitation, respectively, as previously described (6). Frozen liver samples obtained at the end of perfusion were analyzed for incorporation of [3H]leucine into protein as an estimate of the rate of synthesis of nonexported proteins. Rates of production of total secretory proteins and albumin, and the rate of synthesis of nonexported proteins, expressed as milligrams of protein/g of wet weight of liver/h, were calculated as described. The relative rate of incorporation of [3H]leucine into albumin was expressed as the percentage of radioactivity in albumin compared to that in total liver protein.

RNA Extraction—Total RNA was extracted essentially as described by Chirgwin et al. (4) from livers stored in liquid nitrogen. The average leucine content of liver protein was 9.6% (39).

Preparation of Poly(A)-containing RNA—Total RNA was dissolved in 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer at pH 7.2 containing 10 mM EDTA and 1% sodium dodecyl sulfate, heated at 65°C for 10 min, then quickly cooled to 25°C. The RNA solution was then diluted with an equal volume of 0.6 M NaCl. Samples containing RNA were mixed with poly(U)-Sepharose (Pharmacia), equilibrated in the above final buffer, and mixed gently at room temperature for 1.5 h. The affinity matrix was then collected by filtration and was washed with 70 ml of the above buffer. Poly(A)-containing RNA was eluted with 70% formamide containing 1 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer (pH 7.2) at 500 ml/min. The eluate was adjusted to 0.24 M NaCl and the RNA was precipitated by addition of 2.5 volumes of ethanol at -20°C. Eluted RNA was re-precipitated before use in the cell-free translation system.

Cell-free Translation and Immunoprecipitation—Micrococcal nuclease-treated lysate from rabbit reticulocytes was prepared as described by Pelham and Jackson (36) with the modifications described by Tse et al. (46). This lysate was employed as a mRNA-dependent protein-synthesizing system. Immunoprecipitation of translation products was performed by an initial binding reaction with antiplasma protein antibodies followed by adsorption to a staphylococcal protein A (IgG Sorb, The Enzyme Center, Inc., Boston, MA) antibody adsorbent (17). Electrophoresis of translation products and immunoprecipitates was performed essentially as described by Lammli (19) on 10-18% gradient polyacrylamide gels containing 0.1% sodium dodecyl sulfate. To prepare fluorograms, the gels were impregnated with EN'HANCE (New England Nuclear), dried under vacuum, and exposed to Kodak XRP film at -70°C.

Other Analyses—Plasma glucose concentrations were determined with the Technicon AutoAnalyzer using the ferricyanide method (12). Plasma albumin concentrations were quantitated by rocket immunoelectrophoresis as described by Laurell (20) using rat serum albumin as a standard (Sigma, fraction V) and goat anti-rat albumin antibody. Previously described methods were used for determining the liver content of protein (21), RNA (9), and DNA (9).

Statistical Analyses—Comparisons of two sample means were made using Student's t test. p values less than 0.05 were considered to be significant.

RESULTS

The animals used in this study were evaluated on the basis of a number of parameters related to control of diabetes and liver protein metabolism (Table I). Compared to BB/W controls, insulin-withdrawn diabetic rats exhibited a marked elevation in plasma glucose concentration and a tendency toward a loss of body weight and liver weight. These changes are similar to those observed previously in BB/W diabetic rats (2). Plasma albumin concentrations were quantitated by rocket immunoelectrophoresis and were characteristic of uncontrolled diabetes. Insulin-withdrawn diabetic rats also lost significant amounts of liver protein and RNA when expressed on the basis of DNA, a reflection of protein and RNA content per cell. However, on the basis of tissue weight, liver protein content was unchanged.

1 The abbreviations used are: Rd, the product of RNA concentration in moles of nucleotides per liter and the time in seconds; Rdat, Rd values at 50% hybridization.
TABLE I

Comparison of parameters related to control of diabetes and liver protein metabolism in the BB/W rat

Blood samples were obtained from the abdominal aorta of anesthetized rats and were immediately chilled on ice. After blood cells were separated by centrifugation, plasma samples were kept frozen until time of analysis. Livers were also removed from anesthetized rats and were immediately frozen between aluminum blocks precooled to the temperature of liquid nitrogen. Livers were weighed and then pulverized, and the pulverized powder was analyzed for protein, RNA, and DNA as indicated under “Experimental Procedures.” Results expressed on the basis of liver weight are given per g wet weight. Values presented are means ± S.E. for determinations derived from seven animals in each group.

| Parameter                  | BB/W control | Insulin-withdrawn diabetic | Insulin-maintained diabetic |
|---------------------------|--------------|----------------------------|----------------------------|
| Plasma glucose (mm)       | 9.67 ± 0.61  | 34.67 ± 2.28<sup>a</sup>  | 19.44 ± 3.50<sup>b</sup>  |
| Body wt (g)               | 420 ± 28     | 536 ± 17                   | 378 ± 14                   |
| Liver wt (g)              | 16.49 ± 1.12 | 13.21 ± 1.12<sup>b</sup>  | 18.40 ± 1.00               |
| Ratio of liver wt/body wt | 3.93 ± 0.17  | 3.71 ± 0.13<sup>b</sup>   | 4.87 ± 0.22                |
| Liver protein (mg/g)      | 237 ± 3      | 238 ± 7                    | 229 ± 6                    |
| Liver RNA (mg/g)          | 6.84 ± 0.19  | 5.43 ± 0.39<sup>b</sup>   | 6.83 ± 0.26                |
| Liver DNA (mg/g)          | 2.71 ± 0.11  | 3.47 ± 0.04<sup>b</sup>   | 2.72 ± 0.12                |
| Ratio of protein/DNA (mg/mg) | 87.5 ± 4.2  | 86.8 ± 2.4<sup>c</sup>   | 84.2 ± 3.8                 |
| Ratio of RNA/DNA (mg/mg)  | 2.52 ± 0.15  | 1.56 ± 0.10<sup>d</sup>   | 2.51 ± 0.07                |

<sup>a</sup>p < 0.001 versus control.
<sup>b</sup>p < 0.05 versus insulin-maintained diabetic.
<sup>c</sup>p < 0.05 versus control.
<sup>d</sup>p < 0.001 versus insulin-maintained diabetic.

in the diabetic rats while RNA content was decreased. These findings indicate that protein was lost to the same extent as other liver cell components following withdrawal of insulin therapy whereas RNA was lost to a greater extent. Loss of cellular components resulted in a higher DNA content when expressed on the basis of tissue weight. In contrast to these differences, insulin-maintained diabetic rats displayed normal values for all of the parameters studied with two exceptions. First, blood glucose concentration was higher than the control value. Second, the ratio of liver weight/body weight in the insulin-maintained group was significantly higher than the control value. An increase in liver weight in insulin-treated diabetic animals has been previously noted (45) and appears to result from an accumulation of glycogen and triglycerides.

When crossed immunoelectrophoresis was used to examine the protein composition of plasma samples obtained from the three groups of rats approximately 25-30 immunoprecipitin peaks were resolved (Fig. 1). Quantitative changes in each protein could be estimated by comparing the areas contained within the same peak in samples from the different groups of animals. Plasma samples obtained from insulin-withdrawn diabetic rats had similar concentrations of prealbumin and albumin compared to samples from the BB/W control group. In contrast, the relative concentrations of several proteins including peaks 1-6 and transferrin were substantially reduced, whereas peak 7 was increased in the insulin-withdrawn diabetic rats compared to the BB/W controls (compare B with A). The concentrations of individual plasma proteins in the insulin-maintained diabetic rats were essentially unchanged from those seen in the controls (compare C to A).

Hepatic protein synthesis in the three groups of rats was examined both in vivo and in perfused liver (Table II). A pulse-labeling technique was used to determine in vivo the rate of albumin synthesis relative to that of total liver protein. The validity of this technique was established in previous studies showing that newly synthesized secretory proteins including albumin are not secreted in less than 15 min (38) and that the leucine content of albumin is approximately the same as that of total liver protein (30). Furthermore, the technique avoided problems associated with differences in uptake of label and dilution of precursor specific activity. The results show that albumin synthesis in the BB/W control group accounted for approximately 13% of total liver protein synthesis in vivo (Table II), a value which agrees well with previous determinations in normal Sprague-Dawley rats using different methods to estimate the relative rate of albumin synthesis (6). Diabetic rats maintained on insulin therapy exhibited a similar relative rate of albumin synthesis; however, within 48 h of insulin withdrawal their rate of albumin synthesis accounted for less than 3% of total protein synthesis, representing approximately a 5-fold drop in this parameter.

FIG. 1. Crossed immunoelectrophoresis patterns of plasma samples obtained from BB/W control rats (A), diabetic rats 48 h following cessation of insulin therapy (B), and diabetic rats maintained on insulin therapy (C). Plasma samples (2.5 µl) were placed in the appropriate well in the lower right corner of each panel. Electrophoresis in the first dimension was performed from right to left and in the second dimension from bottom to top. The second dimension gel (antibody-containing) contained an immunoglobulin fraction (5 mg of protein/ml) prepared from rabbit antisera by ammonium sulfate precipitation. The antiserum was obtained from rabbits immunized with a mixed plasma sample prepared from the three groups of rats. The amount of each plasma protein in the gel is reflected by the area of its respective peak. Numbered loops have not been identified. The known antigens were identified by the electrophoretic mobilities of purified standards. PA, prealbumin; Alb, albumin; Tf, transferrin.
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Table II

| Type of experiment | BB/W control | Insulin-withdrawn diabetic | Insulin-maintained diabetic |
|--------------------|--------------|---------------------------|---------------------------|
| In vivo            |              |                           |                           |
| Relative rate of albumin synthesis (% total protein synthesis) | 13.2 ± 0.4 | 2.6 ± 1.0<sup>a,b</sup> | 10.9 ± 1.6 |
| Plasma protein concentration (mg/ml) | 69.1 ± 1.7 | 69.0 ± 2.1 | 67.6 ± 0.9 |
| Plasma albumin concentration (mg/ml) | 21.3 ± 1.0 | 20.7 ± 0.8 | 19.0 ± 1.5 |
| Perfused liver |              |                           |                           |
| Albumin production (mg/g.h⁻¹) | 9.193 ± 0.016 | 6.047 ± 0.019<sup>a</sup> | 0.184 ± 0.017 |
| Total secretory protein production (mg/g.h⁻¹) | 0.469 ± 0.042 | 0.207 ± 0.049<sup>a</sup> | 0.476 ± 0.041 |
| Total production of non-albumin secretory proteins (mg/g.h⁻¹) | 0.276 ± 0.024 | 0.100 ± 0.038<sup>a</sup> | 0.282 ± 0.025 |
| Ratio of albumin/total secreted protein X 100 | 41.2 ± 1.0 | 22.7 ± 0.5<sup>a</sup> | 38.8 ± 1.0 |
| Synthesis of nonexported protein (mg/g.h⁻¹) | 2.08 ± 0.10 | 1.30 ± 0.10<sup>a</sup> | 2.04 ± 0.10 |
| Synthesis of total liver protein (mg/g.h⁻¹) | 2.55 ± 0.15 | 1.51 ± 0.15<sup>a</sup> | 2.52 ± 0.13 |

<sup>a</sup> <i>p < 0.01</i> versus BB/W control.
<sup>b</sup> <i>p < 0.01</i> versus insulin-maintained diabetic.

Summation of individual values for total secretory protein production and synthesis of nonexported proteins for each liver.

In spite of this large decrease in the relative rate of albumin synthesis, the plasma albumin concentrations were unchanged in either the insulin-withdrawn or insulin-treated diabetic groups compared to the BB/W control group (in agreement with the data shown in Fig. 1). The total plasma protein concentrations were also similar in the three groups of rats (Table II).

Alterations in hepatic protein synthesis were investigated further using the isolated perfused liver. Following a lag period of approximately 30 min, which represented the time required for synthesis, processing, and secretion into the perfusion medium, accumulation of label in albumin and total secreted protein fractions increased linearly with duration of perfusion for all three groups of rats (Fig. 2). However, in livers of insulin-withdrawn diabetic rats the rates of production of both albumin and total secretory proteins were depressed markedly compared to the values obtained in livers of BB/W control and insulin-maintained diabetic rats. These differences, as well as data for the synthesis of nonexported proteins in perfused liver, are shown in Table II. Livers of insulin-withdrawn diabetic rats produced albumin and total secretory proteins 24 and 44% of the control rates, respectively. Thus, diabetes resulted in a 40% reduction in the rate of production of non-albumin secretory proteins, whereas the reduction in the rate of albumin production was 76%. This preferential effect on albumin production is reflected by the fall in the percentage of albumin in the total fraction from 41.2 and 22.7%.

Synthesis of nonexported proteins by livers of insulin-withdrawn diabetic rats as well as total liver protein (i.e. secreted plus nonexported) was reduced to approximately 60% of the control rate. Livers of diabetic rats maintained on insulin therapy synthesized all fractions of proteins at rates similar to those of the controls.

The biochemical lesion responsible for the defect in albumin synthesis in livers of insulin-withdrawn diabetic rats was investigated by quantitating the amount of albumin mRNA in extracts of total tissue RNA. To quantitate albumin mRNA, labeled cDNA copies of purified albumin mRNA were used in RNA-excess cDNA hybridization reactions (Fig. 3). From the observed E<sub>0</sub>/E<sub>0</sub>' values, albumin mRNA was calculated to comprise approximately 0.28, 0.06, and 0.20% of the total RNA from livers of BB/W control, insulin-withdrawn diabetic, and insulin-maintained diabetic rats, respectively (Table III). Assuming that messenger RNA accounts for approximately 2% of total RNA (15, 29), then albumin mRNA would represent 13.9, 2.8, and 10.0% of the total messenger populations in the three groups of animals. These estimates of the amount of albumin mRNA are in excellent agreement with the values obtained for the relative rates of albumin synthesis in vivo in the three groups of rats (compare Tables II and III).
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More general studies were directed toward the effects of diabetes on the relative abundance of mRNAs for other liver proteins. Poly(A)-containing RNA fractions isolated from livers of the three groups of rats were translated in a mRNA-dependent, cell-free, protein-synthesizing system. Total translation products, and the translation products immunoprecipitated with anti-plasma protein antibodies, were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Fig. 4). When the electrophoretic patterns of translation products were compared, there was essentially no difference between the control group and the insulin-maintained diabetic group (Fig. 4, compare lane c to lane a). In contrast, the results obtained with the poly(A)-containing RNA isolated from livers of insulin-withdrawn diabetic rats differed significantly from the other two groups (Fig. 4, lane b). The electrophoretic band showing the most striking change was the $M_r$ = 68,000 protein, which corresponds to albumin and is in agreement with the hybridization data for albumin mRNA presented in Fig. 3. Changes in the translation products of mRNAs coding for the synthesis of the major plasma proteins are seen more clearly in Fig. 4B. These data show that withdrawal of insulin therapy resulted in decreases in at least four proteins other than albumin (bands 5, 4, 5, and 6) and an increase in a $M_r$ = 48,000 protein (band 2).

**Fig. 4.** Electrophoretic patterns in a sodium dodecyl sulfate-polyacrylamide gel of the cell-free translation products directed by poly(A)-containing RNA fractions obtained from livers of BB/W control rats (lanes a and d), diabetic rats 48 h following cessation of insulin therapy (lanes b and e), and diabetic rats maintained on insulin therapy (lanes c and f). A represents total translation products while B depicts the translation products immunoprecipitated by anti-plasma protein antibodies. The numbers on the right of the panels correspond to molecular weight markers run in a parallel lane on the gel. The numbers on the left indicate sequences that change in amount following withdrawal of insulin therapy in the diabetic group.

**DISCUSSION**

This study examines diabetes-induced alterations in liver protein synthesis using the spontaneously diabetic BB/W rat as an animal model of insulin-dependent diabetes. An advantage of this model is that an insulin-deficient condition is attained without the use of chemicals such as alloxan or streptozotocin to induce a diabetic state. The BB/W diabetic rat is dependent upon insulin replacement therapy and, if therapy is discontinued, the animal rapidly becomes insulin-deficient, displaying only trace levels of immunoassayable plasma insulin within 48 h of the last treatment (2). Because BB/W diabetic rats removed from insulin therapy for longer periods of time show a high mortality rate, animals used in the present study were investigated 48 h after their last insulin injection. The aim was to choose a time period that would result in the clearest effects on liver protein synthesis, but that would avoid complications associated with animals in a poor state of health. That the changes observed with respect to parameters relating to liver protein synthesis were due to the insulin deficiency per se is indicated by the findings that diabetic rats maintained on insulin therapy did not differ significantly from BB/W control animals.

Investigations of the protein content of plasma might be expected to reflect diabetes-induced alterations in liver protein synthesis since most of the plasma proteins are synthesized in

**Fig. 3.** Quantitation of albumin mRNA sequences in livers of BB/W control rats (O---O), diabetic rats 48 h following cessation of insulin therapy (-----), and diabetic rats maintained on insulin therapy (Δ--Δ). Total RNA was hybridized in excess (up to 2500-fold) to 200 pg of albumin cDNA. Hybrid formation was assayed by resistance to S1 nuclease. The curves presented are representative of three separate determinations on RNA samples obtained from pools of six livers for each condition.

**TABLE III**

Comparison of albumin mRNA data derived by hybridization analysis

| Type of animal       | Log $R_{1/2}$ | $R_{1/2}$ | Albumin mRNA$^a$ | % total RNA | mg/g tissue | $R_{1/2}$ | $R_{1/2}$ |
|----------------------|--------------|-----------|------------------|-------------|------------|-----------|-----------|
| BB/W control         | -0.40        | 0.398     | 0.28             | 19.2        | 137        | 13.9      |           |
| Insulin-withdrawn    | 0.25         | 1.778     | 0.06             | 3.3         | 109        | 2.8       |           |
| Insulin-maintained   | -0.25        | 0.562     | 0.20             | 13.7        | 137        | 10.0      |           |

$^a$ Based on the $R_{1/2}$ of 1.10 x $10^{-3}$ mol-s-liter for the hybridization between purified albumin mRNA and albumin cDNA (23).

$^b$ Derived from the product of albumin mRNA as a percentage of total RNA and the amount of total RNA per g of liver given in Table I.

$^c$ Based on the amount of total RNA per g of liver (Table I) and the estimation that mRNA represents approximately 2% of total RNA (15, 29).

$^d$ M. C. Appel and A. A. Like, unpublished results.
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and exported from the liver (28). However, other factors aside from hepatic synthesis and secretion can influence the concentration of proteins in plasma. These factors include plasma volume, the distribution of plasma components between vascular and extravascular compartments, and the rate of removal of proteins from the plasma either by degradative processes or loss in the urine. In the present study, most of the plasma proteins responding to an acute insulin deficiency declined in concentration although at least one unidentified protein increased relative to others. It is especially interesting to note that the plasma albumin concentration was unaffected by insulin deficiency of 48 h duration in spite of a greatly reduced rate of albumin synthesis and secretion by the liver. The failure to observe a change in plasma albumin concentration could be due to a decrease in plasma volume in the insulin-withdrawn diabetic rats. Dehydration is a common feature of untreated diabetes (10) and was observed in the insulin-withdrawn diabetic rats used in the present study. The decrease in plasma volume in effect concentrates the plasma proteins and diminishes the magnitude of the reduction which would be expected from the observed decrease in hepatic synthesis and secretion of plasma proteins. Whether alterations in the rate of degradation could partially account for the changes in concentrations of plasma proteins is presently unknown, although the half-lives of plasma proteins are thought to be sufficiently long to preclude this mechanism from contributing substantially to differences occurring over a 48-h period of insulin deficiency. A loss of plasma proteins in the urine could also account for some of the observed changes since BB/W diabetic rats have been observed to exhibit proteinuria.1 That the mechanisms responsible for alterations in plasma protein concentrations are probably also operative in man is suggested by several reports showing changes in the levels of plasma proteins in human diabetics (14, 25–27). The most frequently reported alterations involve a decline in total plasma proteins with a relatively greater depression in albumin concentrations in the absence of proteinuria. Of particular interest are reports of a relative increase, with respect to total plasma proteins, in the concentrations of several glycoproteins in diabetes (14, 25, 27). Some of these glycoproteins are typically classified as acute phase reactants (plasma proteins whose production is greatly stimulated by inflammatory agents). The relative increase in glycoproteins has been associated with an increase in serum viscosity (26), and efforts have been made to correlate the alterations in glycoproteins with the vascular complications of diabetes (14, 26).

The major factor contributing to the alterations in the absolute quantities as well as the concentrations of plasma proteins occurring in response to insulin withdrawal in the BB/W diabetic rats appears to be the greatly reduced capacity of the liver to synthesize and secrete plasma proteins. This altered function of the liver is evident from in vivo determinations as well as from measurements carried out in the isolated perfused liver. In vivo, an 80% decline in the synthesis of albumin relative to total liver protein following cessation of insulin treatment of BB/W diabetic rats is in excellent agreement with the results of a similar determination made in rats with alloxan-induced diabetes (33). The finding of a decline of similar magnitude in albumin production by perfused livers from insulin-withdrawn BB/W diabetic rats argues for a defect in synthetic mechanisms and an unaltered secretory process. The preferential effect of diabetes on albumin production compared to total secretory protein production and a 40% decline in the rate of synthesis of nonexported proteins by BB/W diabetic rat livers are findings in agreement with similar determinations made in perfused livers of rats with alloxan-induced diabetes (33). Whether diabetes affects the synthesis of nonexported liver proteins has been a controversial question on which some reports claim no effect (32) while others show results similar to those observed in the present study (11, 37). The similarity of results between BB/W diabetic rats and other animal models of diabetes indicates that previously observed alterations in liver protein synthesis in chemically induced diabetic animals are the result of diabetes per se and not a hepatotoxic effect of the diabetogenic agent.

One point to note with regard to the perfused liver data presented here is that the overall rate of protein synthesis is about 35% lower than values previously reported (33). Furthermore, production of albumin and total secretory proteins by perfused livers of BB/W control rats accounted for 7.6 and 18.4% of total protein synthesis, respectively, compared to previously reported values for these parameters of 13–14 and 36–40% (33). The value of 7.6% for albumin production compares with an in vivo relative rate of albumin synthesis of 12.2% (Table II), indicating that a fraction of newly synthesized albumin may be degraded intracellularly rather than secreted. A similar phenomenon has been observed to occur in perfused livers of thyroidectomized rats (35). No explanations are readily available for these differences in protein synthesis and secretion by perfused livers, although they most likely relate to the fact that the BB/W rats used in the present study were older and larger than the liver donors previously investigated (33).

Two apparent mechanisms account for the diabetes-induced alterations in liver protein synthesis observed in the present study. First, the fall in RNA content of the tissue following cessation of insulin therapy in the BB/W diabetic rat is sufficient to account for about one-half of the overall reduction in liver protein synthesis when the synthesis rate is expressed on the basis of RNA content. Since the bulk of total tissue RNA consists of ribosomal RNA, this presumably represents a proportionate reduction in the number of ribosomes and thus the capacity of the tissue for protein synthesis. Second, cessation of insulin therapy in the BB/W diabetic rats brought about rapid quantitative as well as qualitative changes in mRNA. In the case of albumin mRNA, its relative abundance dropped from 13.9 to 2.8% of the total mRNA within 48 h of withdrawal of insulin therapy. The extremely close correspondence between the relative abundance of albumin mRNA and the relative rate of albumin synthesis in the three groups of animals investigated here and in previous studies (33) indicates that the availability of mRNA is the limiting factor in determining the rate of albumin synthesis. Such results are evidence against the existence of a seques-tered, nontranslatable pool of albumin mRNA which has been proposed as a regulatory mechanism for controlling albumin synthesis in fasted rats (48). Although the defect responsible for the rapid fall in albumin mRNA in diabetic rat liver is presently unknown, potential sites of regulation include mRNA transcription, processing, transport from the nucleus, cytosolic sequestration, and degradation. In addition to albumin mRNA, the relative abundance of mRNAs for other plasma proteins also appeared to be altered following cessation of insulin therapy, explaining the reduced concentrations of several plasma proteins which existed in this condition.

This investigation, as well as a previous study (33), provide evidence that insulin can control the synthesis of albumin by modulating the amount of albumin mRNA. This is the apparent mechanism by which the hormone regulates the synthesis of a number of other liver proteins, specifically plasma proteins (Fig. 4). To our knowledge, there are only three identified examples of liver proteins other than albumin whose syntheses are modulated by insulin through altered amounts of mRNA.
These proteins are α2-globulin (43), fatty acid synthetase (41), and phosphoenolpyruvate carboxykinase (1, 5), the first of these being a protein which is secreted from the liver and the latter two being intracellular enzymes. With respect to other tissues, insulin has also been shown to affect the amylase mRNA content of pancreatic acinar cells (18). From the results obtained in the present study, it is clear that insulin may be regulating the synthesis of a number of other proteins through a similar mechanism. An appropriate question to ask is whether insulin-induced changes in specific mRNAs represent a direct action of the hormone since the observations presented here and the examples cited above are based on studies in which insulin was administered to diabetic animals. Recent reports of effects of the hormone on plasma protein production by cultured rat (7), chicken (22), and amphibian (44) hepatocytes, and on phosphoenolpyruvate carboxykinase mRNA in cultured hepatoma cells (1) indicate that insulin acts directly to bring about changes in specific mRNAs.

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