Regulation of *N*-Acetylneuraminic Acid Synthesis Following Injury and Partial Hepatectomy (39763)

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Introduction. Many pathological, inflammatory states lead to increased concentrations of a spectrum of circulating plasma glycoproteins, often referred to as the "acute phase reactants". It is now well established that the increased concentrations are the result of increased *de novo* synthesis of these compounds by the liver, and it has been proposed (1–3) that this phenomenon is an "induction" process involving the increased production of specific messenger RNAs.

Because of the central structural and functional roles played by hexosamines and hexosamine derivatives in the carbohydrate moieties of glycoproteins, an increasing interest has been focused on the regulation of synthesis of these compounds in various pathological states. Previous investigations have shown, for example, that the key enzyme involved in glucosamine synthesis, L-glutamine:D-fructose-6-phosphate aminotransferase (aminotransferase), is also increased by injury and partial hepatectomy in a manner indicating that it may be part of the acute phase response (4, 5). These increases observed in enzyme specific activity are also probably the result of an "induction" process since they can be blocked by the appropriate administration of cycloheximide or actinomycin D (4, 6).

The present report extends these studies to a second key enzyme of hexosamine metabolism, UDP-*N*-acetylglucosamine 2'-epimerase. This enzyme catalyzes the key first step to the sialic acids.

Materials and methods. Animals. Male, Wister–King A rats were housed in environmentally controlled animal quarters and were maintained until used on standard laboratory chow.

Laparotomy and partial hepatectomy. Injuries were inflicted by performing laparotomies. Partial hepatectomies were carried out according to the procedure of Higgins and Anderson (7).

Assay of UDP-GlcNAc 2'-epimerase. Enzyme extracts were prepared as previously described (4). The procedure for the enzyme assay was based upon the measurement of *N*-acetylmannosamine (Man*N*Ac) produced in the reaction mixture using a modified Morgan–Elson procedure as described by Spivak and Roseman (8). The incubation mixture contained 2 m*M* UDP-*N*-acetylglucosamine (UDP-Glc*N*Ac); 200 m*M* Tris–HCl buffer, pH 7.5; 80 m*M* $MgSO_4$, and 0.1 ml of enzyme extract in a total volume of 0.25 ml. Incubation was carried out at 37° for 20 min and the reaction was terminated by heating in boiling water for 2 min. The protein precipitate was removed by centrifugation and a 0.1-ml aliquot of the supernatant was placed in a test tube containing approximately 80–100 mg of Dowex-1 acetate (20 mesh). Water (0.3 ml) was added to increase the volume. The tubes were mixed vigorously and the mixture was allowed to react with the resin for 5 min. The resin was removed by centrifugation and 0.2 ml of the supernatant was used for the estimation of Man*N*Ac. The enzyme specific activities were expressed as nanomoles of Man*N*Ac formed per milligram of protein per hour.

Assay of CMP-N-acetylneuraminic acid. The hepatic concentrations of CMP-*N*-acetylneuraminic acid (CMP-*N*ANA) were determined on trichloroacetic acid and heat-treated 105,000*g* liver supernatants by a modification of the method of Jourdian *et al.* (9). One volume of 50% (w/v) TCA was added to nine volumes of 105,000*g* supernatant. After removing the acid-insoluble material by centrifugation, the supernatant was heated 10 min in a boiling-water bath to hydrolyze the CMP-*N*ANA. The solution was centrifuged again to remove heat-coag-

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ulated materials and 0.5 ml of the supernatant was mixed with 0.1 ml of 0.04 *M* periodic acid solution and allowed to stand in ice-cold water for 20 min. After the addition of 1.25 ml of resorcinol reagent, the solution was mixed, placed in an ice bath for 5 min, and then heated at 100° for 15 min. The reaction mixture was cooled in tap water, 1.25 ml of tertiary butyl alcohol was added, and the mixture was agitated vigorously. The tubes were placed in a 37° water bath for 3 min, cooled to room temperature, and the absorbance was measured at 630 nm.

Protein assay. The protein content of the 105,000*g* supernatant was determined by the biuret method of Wolfson *et al.* (10) using crystalline bovine albumin as the standard.

Cycloheximide and actinomycin D administration. In experiments where cycloheximide and actinomycin D were used, both were administered intraperitoneally. Cycloheximide was dissolved in sterile isotonic saline to form a 0.05% solution and was administered at a dosage level of 1.0 mg/kg. Actinomycin D (100 μg/ml) was dissolved in a 1:1 mixture of saline–propylene glycol and was injected at a dosage level of 0.7 mg/kg. Control animals received equivalent volumes of saline or saline–propylene glycol.

Chemicals. UDP-Glc*N*Ac and *N*ANA were purchased from Sigma. All other chemicals were the highest quality available from commercial sources.

Results. Specific activity of hepatic UDP-GlcNAc 2'-epimerase (2'-epimerase) following injury and partial hepatectomy. The changes in the relative activity of 2'-epimerase as a function of time following injury and partial hepatectomy are shown in Fig. 1.

The average specific activity of the enzyme for control animals was 91.2 ± 8.1 nmol/mg of protein/hr in these experiments. After laparotomy, the specific activity of the enzyme increased for 3 to 5 days to a maximum value approximately 75% greater than the initial control level. This peak in specific activity was followed by a 50% decrease over the next 5 days, but remained significantly elevated ($P < 0.05$) when compared to control values at 10 days.

Partial hepatectomy resulted in a rapid and sharp increase in the enzyme specific activity to about 90% above the control values at 24 hr. This peak value was followed by a gradual decrease over the next 9 days. At 10 days the specific activity was still significantly greater than the control value ($P < 0.02$).

Hepatic CMP-NANA concentrations following injury and partial hepatectomy. Figure 2 shows the effects of injury and partial hepatectomy on hepatic CMP-*N*ANA concentrations as a function of time after surgery. The measurements were performed on the same supernatant fractions used for the enzyme assays. The concentration of CMP-*N*ANA in control livers averaged 153.6 ±

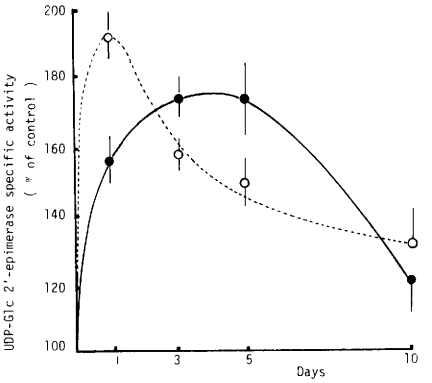

FIG. 1. Changes in UDP-Glc 2'-epimerase specific activity as a function of time following injury and partial hepatectomy. ●——●, injury; ○-----○, partial hepatectomy. Values as presented as mean ± SD. Each point represents the mean of six animals.

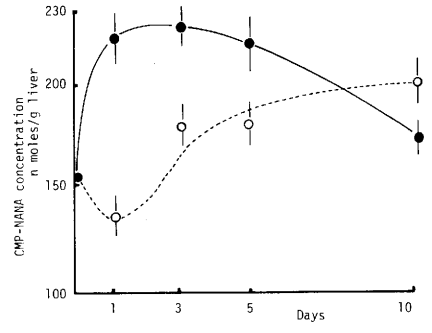

FIG. 2. Hepatic CMP-*N*ANA concentration following injury and partial hepatectomy. ●——●, injury; ○-----○, partial hepatectomy. Values are presented as mean ± SD. Each point represents the mean of six animals.

13.0 nmol/g of liver. CMP-*N*ANA levels showed a 30% increase relative to control levels at 24 hr after injury. These increased levels were maintained until the fifth day, then returned to normal by 10 days.

Partial hepatectomy caused a 20% decrease in hepatic CMP-*N*ANA concentration at 24 hr following surgery ($P < 0.05$). This was followed by a moderate increase at about 3 days and continued to rise more slowly thereafter, showing a 20% increase above normal levels at 10 days.

Effect of actinomycin D and cycloheximide administration on the response to injury. Table I shows the effects of administration of actinomycin D and cycloheximide on the ability of 2′-epimerase to respond to injury. Actinomycin D completely blocked the increase in the enzyme activity usually observed following injury. The expected increase in CMP-*N*ANA concentration was also inhibited by actinomycin D. The administration of cycloheximide also blocked the injury-induced increase in the enzyme specific activity. A significant decrease in hepatic CMP-*N*ANA concentration was observed after this treatment.

Effects of actinomycin D and cycloheximide administration on the response to partial hepatectomy. The effects of actinomycin D and cycloheximide on the ability of 2′-epimerase to respond to partial hepatectomy are shown in Table II. The administration of actinomycin D completely blocked the increase in the enzyme activity usually observed after partial hepatectomy. Hepatic CMP-*N*ANA concentration decreased as a result of treatment with actinomycin D.

The administration of cycloheximide also blocked the increase in the enzyme activity normally observed after partial hepatectomy. The inhibitory action of this drug was

TABLE I. EFFECT OF ACTINOMYCIN D AND CYCLOHEXIMIDE ON UDP-Glc*N*Ac 2′-EPIMERASE ACTIVITY AND CMP-*N*ANA CONCENTRATION FOLLOWING INJURY.[a]

Treatment	UDP-Glc*N*Ac 2′-epimerase activity (units)	Percentage of control	Hepatic CMP-*N*ANA concentration (nmole/g of liver)	Percentage of control
Normal	119.1 ± 8.7	100.0	135.6 ± 14.8	100.0
Normal, actinomycin D	94.5 ± 9.9*	79.4	121.1 ± 12.3	89.3
Normal, cycloheximide	84.9 ± 9.3**	71.3	115.0 ± 11.6	84.8
Normal, injury	179.1 ± 13.5	151.0	170.3 ± 9.8*	125.6
Injury, actinomycin D	102.9 ± 10.8***	86.4	130.4 ± 15.3	96.2
Injury, cycloheximide	91.8 ± 7.5**	77.1	120.1 ± 15.4	88.5

[a] The values are expressed as means ± SD of six animals. Animals subjected to injury were injected with actinomycin D and cycloheximide at the time of surgery and were sacrificed 24 hr following surgery. Animals were fasted for 24 hr prior to sacrifice. The enzyme specific activities are expressed as nanomoles of Man*N*Ac formed per hour per milligram of protein.

* $P < 0.02$.

** $P < 0.01$.

*** $P < 0.05$.

TABLE II. EFFECT OF ACTINOMYCIN D AND CYCLOHEXIMIDE ON UDP-Glc*N*Ac 2′-EPIMERASE ACTIVITY AND CMP-*N*ANA CONCENTRATION FOLLOWING PARTIAL HEPATECTOMY.[a]

Treatment	UDP-Glc*N*Ac 2′-epimerase activity (units)	Percentage of control	Hepatic CMP-*N*ANA concentration (nmole/g of liver)	Percentage of control
Normal	100.5 ± 9.0	100.0	140.2 ± 13.6	100.0
Normal, hepatectomy	164.7 ± 15.6	164.0	119.3 ± 10.5*	85.1
Hepatectomy, actinomycin D	82.8 ± 12.3**	82.4	108.4 ± 11.4**	77.3
Hepatectomy, cycloheximide	69.3 ± 9.6**	69.0	93.8 ± 12.1**	66.9

[a] The values are expressed as mean ± SD of six animals. Animals subjected to hepatectomy were injected with actinomycin D and cycloheximide at the time of surgery and were sacrificed 24 hr following surgery. Animals were fasted for 24 hr prior to sacrifice. The enzyme specific activities are expressed as nanomoles of Man*N*Ac formed per hour per milligram of protein.

* $P < 0.001$.

** $P < 0.05$.

more pronounced than that observed with actinomycin D. Hepatic CMP-*N*ANA concentrations were decreased by cycloheximide treatment.

Discussion. Peak values of 2′-epimerase specific activity were reached within 24 hr after partial hepatectomy, whereas it required 3–5 days following injury. The peak values reached after partial hepatectomy were also considerably higher than those observed following injury. Thus it appears that the enzyme response to partial hepatectomy is sharper, more intense, and less long-lived than after injury.

This pattern of response seems to be the mirror image of the response previously observed for L-glutamine:D-fructose-6-phosphate aminotransferase (aminotransferase) (4, 5). In this case aminotransferase specific activity rapidly peaked at 18–24 hr following injury but did not reach its maximum until 2–4 days after partial hepatectomy.

It appears, therefore, that aminotransferase, and consequently glucosamine synthesis, is most responsive to injury, whereas 2′-epimerase, and consequently sialic acid synthesis, is most responsive to partial hepatectomy. The relative responses may reflect the priorities of demand for the two sugar nucleotides following injury and partial hepatectomy, and it is suggested that after injury the stimulated plasma glycoprotein synthesis will create a rapid demand for a much greater supply of hexosamines; whereas, in the regenerating liver the stimulation of membrane glycoprotein and glycolipid synthesis may shift the demand to sialic acid supply.

Although the method employed for measurement of CMP-NANA concentration actually measured free *N*ANA, previous work has shown that almost all of the *N*ANA found in 105,000*g* supernatant of liver tissue exists as CMP-*N*ANA. Liver supernatants not subjected to hydrolysis show only traces of free *N*ANA.

The hepatic concentration of CMP-*N*ANA, the major feedback inhibitor of 2′-epimerase, following injury generally paralleled the specific activity of 2′-epimerase, being elevated above control levels between 1 and 5 days and back to normal concentrations by 10 days. Partial hepatectomy, however, resulted in a marked, transient decrease in hepatic CMP-*N*ANA concentration, decreased to 20% below normal levels at one day in spite of an 80% increase in 2′-epimerase specific activity at this time. The hepatic CMP-*N*ANA concentration then rose gradually from the 1-day level over the next 9 days to levels significantly higher than the control. The transient decrease in CMP-*N*ANA concentration following partial hepatectomy resembles that observed for hepatic UDP-Glc*N*Ac in the same circumstances (4, 5). The instantaneous concentrations of UDP-Glc*N*Ac and CMP-*N*ANA are, of course, functions of the rates at which they are being synthesized and the rates at which they are being utilized. Previous studies have shown that hepatic plasma glycoprotein production is stimulated five- to seven-fold after injury and after partial hepatectomy (11). In addition, cell membrane renewal will cause increased membrane glycoprotein and glycolipid synthesis in the regenerating liver. The increased demand for Glc*N*Ac and *N*ANA should then cause a depletion of the UDP-Glc*N*Ac and CMP-*N*ANA pools unless compensated for by an increased synthesis. The intact liver apparently is able to respond to the increased demand by inducing the rate-limiting enzymes. Despite the marked induction of 2′-epimerase 24 hr after partial hepatectomy, the rate of utilization of CMP-*N*ANA is still greater than its replacement rate and one observes a transient decline in pool size. The rate of utilization slows after one day and the pool size gradually recovers to control levels and beyond.

Actinomycin D and cycloheximide blocked the increases in 2′-epimerase specific activity usually observed after injury and partial hepatectomy indicating that the increases observed were the result of an "induction" phenomenon involving the *de novo* synthesis of mRNA and enzyme protein. A similar induction mechanism has been proposed by Bley *et al.* (4) and Akamatsu and Maeda (6) for aminotransferase. Thus it is interesting to note that two of the key enzymes involved in hexosamine metabolism are subject to induction in response to injury and partial hepatectomy.

Summary. Changes in hepatic UDP-Glc*N*Ac 2′-epimerase specific activity and concentration of CMP-*N*ANA were measured as a function of time following injury and partial hepatectomy. Injured rats exhibited an increase in the enzyme specific activity at 24 hr which reached a maximum between 3 to 5 days at 70–75% above control levels. The specific activity then declined, returning to normal values by 10 days. Partial hepatectomy resulted in a sharp increase in the enzyme specific activity to a peak of 90% above control values at 24 hr. The specific activity then declined steadily over the next 9 days, remaining significantly elevated over the normal at 10 days.

The hepatic concentration of CMP-*N*ANA increased rapidly, showing a 40% increase at 5 days following injury, after which it slowly declined. The values were still significantly elevated above the normal at 10 days. Partial hepatectomy, on the other hand, caused a transient decrease of about 20% below the normal at 1 day. This was followed by a rapid recovery to normal levels and beyond. CMP-*N*ANA levels were significantly elevated above normal at 10 days.

Both actinomycin D and cycloheximide administration completely blocked the increase in the enzyme activity after injury and partial hepatectomy. The expected increase in hepatic CMP-*N*ANA concentrations was also blocked by these inhibitors.

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