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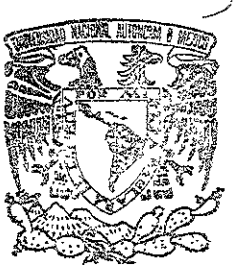
PAPEL DE LA ALCOHOL DESHIDROGENASA (ADH) EN LA FARMACOCINÉTICA DEL ETANOL DURANTE LA REGENERACIÓN HEPÁTICA Y LOS EFECTOS MORFOLÓGICOS Y BIOQUÍMICOS DE LA ADMINISTRACIÓN AGUDA DE ETÁNOL

TESIS

Que para obtener el grado de Doctor en Ciencias (Biología)

Presenta el Médico Cirujano

José Antonio Morales González



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ABREVIATURAS

4-MP	**4-metilpirazola**
ABC	**Areas bajo la curva**
ADH	**Alcohol deshidrogenasa**
ALDH	**Aldehído deshidrogenasa**
ALT	**Alanino aminotransferasa**
AST	**Aspartato aminotransferasa**
EtOH-IG	**Vía oral de administración de etanol**
EtOH-IP	**Vía intraperitoneal de administración de etanol**
GDH	**Glutamato deshidrogenasa**
HP	**Hepatectomía parcial**
LDH	**Lactato deshidrogenasa**
MEC	**Matriz extracelular**
MEOS	**Sistema microsomal de oxidación de etanol**
MMPs	**Metaloproteasas de matriz extracelular**
ODC	**Ornitin descarboxilasa**
OTC	**Ornitin carbamiltransferasa**
Q_{FPM}	**Primer paso metabolico**
RE	**Retículo endoplásmico**
REl	**Retículo endoplásmico liso**
REr	**Retículo endoplásmcio rugoso**
TK	**Timidín cinasa**
TG	**Triacilglicéridos**
TS	**Timidilato sintasa**

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RESUMEN

Cuando existe una pérdida importante de masa hepática funcional, como la producida por remoción quirúrgica del hígado, el tejido hepático remanente sufre un proceso de regeneración (hiperplasia), en el cual el tejido removido es reemplazado en su totalidad. Durante este proceso, la síntesis de DNA se incrementa notablemente y alcanza un pico máximo a las 23-25 horas posteriores a la cirugía (en la rata). Al mismo tiempo, la división celular empieza activamente y por el día 6 al 8 post-cirugía se completa la restauración del hígado. Por lo tanto, la regeneración hepática que se produce como consecuencia de hepatectomía parcial es considerada como un muy buen modelo para el estudio de los mecanismos que regulan la división y diferenciación celulares.

Este proceso es muy sensible a la acción de algunos xenobióticos, en particular la administración aguda o crónica de alcohol (etanol), que ejerce un efecto adverso sobre la regeneración hepática, especialmente cuando éste se administra durante los estadios tempranos del proceso regenerativo. A pesar de que algunos mecanismos de daño ya han sido referidos en la acción del alcohol sobre la regeneración hepática, llama la atención de que prácticamente se desconozca si las acciones de dicho tóxico son por la molécula misma o por las transformaciones metabólicas que experimenta en la célula hepática. En particular, nada está reportado sobre la farmacodinamia y la farmacocinética del alcohol en animales hepatectomizados parcialmente. Por lo tanto se realizó este estudio de la farmacocinética del etanol durante la regeneración hepática en animales sujetos a hepatectomía parcial y los efectos morfológicos y bioquímicos de la administración aguda de etanol en el hígado regenerante.

A ratas machos wistar se les realizó una hepatectomía parcial (HP) del 70%. El modelo se dividió en dos grupos de animales experimentales, dependiendo de la vía de administración del etanol. Al primer grupo se les administró etanol por sonda gástrica (IG), a una dosis de 1.5 g/Kg de peso en una solución al 40%, a diferentes tiempos de regeneración hepática (0-96 h). Al segundo grupo se le administró el etanol por inyección intraperitoneal (IP) a la misma dosis y concentración, así como a los mismos tiempos de regeneración. Se obtuvierón muestras de sangre para determinar las etanolemias hasta por 8 horas. Al final del experimento, las ratas se decapitaron para obtener el hígado y recolección de sangre. Del hígado se determino la actividad de la alcohol deshidrogenasa (ADH) y la actividad específica de la timidín cinasa (TK) como parámetro de regeneración; además las muestras de hígado fueron obtenidas para el estudio histológico por microscopia electrónica y de luz. De la sangre recolectada su obtuvo suero el cual se uso para determinar metabolitos séricos (albúmina, glucosa, triacilglicéridos y bilirrubina), y la actividad de las enzimas marcadoras de daño hepático (ALT, AST, LDH, GDH y OTC).

Los resultados demostraron lo siguiente:

En el primer paso metabólico (FPM) del etanol, el estómago tiene un papel transitorio, pero efectivo en las modificaciones farmacocinéticas del etanol a tiempos tempranos posteriores a la hepatectomía parcial en la rata, constituyendo una barrera protectora contra los efectos adversos del etanol en esos animales. El hígado en regeneración incrementa su capacidad para oxidar el etanol. El aumento del metabolismo del etanol puede potenciar los efectos deletéreos que la droga ocasiona a la regeneración hepática. Los cambios coordinados en el metabolismo del etanol por el estómago y el hígado muestran una interesante interrogante de una comunicación interórganos que ocurre durante la proliferación celular del hígado, posterior a una hepatectomía parcial.

Una dosis aguda de etanol es capaz de inhibir constantemente la regeneración hepática que se induce por la HP, lo cual es dependiente del tiempo y ruta de administración. La administración intragástrica de etanol ocasiona más inhibición al proceso de la regeneración hepática que la ruta intraperitoneal; esto está probablemente relacionado a las diferencias hepáticas del catabolismo del etanol durante la regeneración hepática, la cual varía de acuerdo a la ruta de administración. Se han dado las primeras evidencias de una liberación selectiva de enzimas por parte del hígado durante la regeneración hepática.

La administración de dosis bajas de etanol en ratas sujetas a HP ocasiona diferentes modificaciones en la ultraestructura del hígado regenerante, dependiendo fuertemente de la ruta de administración. La administración intragástrica de etanol disminuye prácticamente todos los cambios adaptativos de los hepatocitos regenerantes. La administración intraperitoneal de etanol promueve modificaciones ultraestructurales, indicativas de proliferación celular.

La administración de dosis bajas de 4-MP (25 y 50 mg/kg) en ratas HP, se asocia con ligeros cambios en la actividad de la ADH hepática y en cambios metabólicos que disminuyen ligeramente la regeneración hepática. La administración de dosis altas de 4-MP (100 y 200 mg/kg)en ratas sujetas a HP, disminuye importantemente la actividad de la ADH hepática, la cual se asocia con cambios en la función y la estructura que precede aparentemente a los hepatocitos para la replicación celular. Estudios en el comportamiento de la ADH hepática se pueden utilizar para identificar la patogenia de la inhibición de la regeneración hepática que se conoce ocurren en la enfermedad hepática por alcohol en el humano.

Una de las interrogantes, de los resultados obtenidos, es saber por qué el etanol ocasiona diferentes efectos, dependientes de la ruta de administración, en la inhibición de la regeneración hepática. Por lo tanto, se deben de continuar realizando estudios encaminados a elucidar los mecanismos por el cual el etanol es capaz de ocasionar este fenómeno.

SUMMARY

Surgical removal of the liver up to 70% produces in the remnant liver the hyperplasia a process named liver regeneration. Liver regeneration (LR) in duces increases DNA synthesis and the maximun increase is present at 23-25 hr (in the rat) after the surgical procedure. At the same time, the cellular proliferation is actived and remains up to 6-8 days after partial hepatectomy (PH) whe the liver mass is restored. Liver regeneration after partial hepatectomy is considered a goodl model for the study of the mechanism that regulate cell divition and cellular differentiation.

Liver regeneration is a very sensitive process and can be damaged by xenobiotic as such as the ethanol intake at acute or chronic. Acute ethanol intake produces damage to LR if is administered at early times of the process of regeneration. The study of the adverse effects of ethanol intake upon liver regeneration has been document, but it is not clear what is the exact mechanism of action of the ethanol. It has been proposed that the ethanol molecule is by *per se* the responsible the of damage, but others are refered that the metabolic products of ethanol oxidation are responsible for the deleterious actions of ethanol up on LR.

None is document above the pharmacological action of ethanol in the aspects of to the pharmacokinetic and pharmacodinamics actions during LR after PH. By hence, we performed the study of the pharmacological parameter of the ethanol in the regenerating liver of the rat. We explored the morphological, biochemical and pharmacological aspects of the ethanol intake in the model of LR produced by PH.

Male wistar rats were subjet to PH (up to 70% of the liver). After PH, the animals were grouped according to the ethanol intake status. At the first group, ethanol was administred by intragastric route (IG) at 1.5 g/Kg bw, 40% v/v. The second group, ethanol was give by intraperitoneal route at the same concentration. Ethanol was administred in both groups during 0-96 hr after PH.

At different times after LR, animals were sacrificed by decapitation and blood was collected for the determination of the ethanol concentration of up to 8 hr. Liver was obtained for the determination of the specific activity of the alcohol dehydrogenase (ADH) and thymidine kinase (TK). Liver samples were `repared for a microscopic study. Light and electronic microscopic studies were dace. In the serum, metabolites and enzymatic activitie were determined. Serum metabolites included: albumin, glucose, tryacylglycerides and bilirubin. Serum enzymes were ALT, AST, LDH, GDH and OTC as marker of liver damage.

Results shows that:

The first pass metabolism (FPM) of the ethanol by the stomach had a transitory, but active role in the pharmacokinetic action of the ethanol in the early times of LR in the rat. These

results show that the stomach constituts a protection barrierfor the advers effects of ethanol on LR.

The regenerating liver increased the capacity for ethanol oxidation. This increase of ethanol oxidation as potentiated the adverse effects of ethanol in the regenerating liver. The interelationships between the stomach and the liver during LR and the changes produced by the ethanol intake are subject several. The latter can be show as an exemple of inter-communication between the stomach and the liver during the process of LR after PH.

An acute dose of ethanol produced inhibition of the LR induced by PH. This inhibition are deppending of both, the time and route of administration. The intragastric ethanol administration produced more inhibition of the LR process in comparasion of the intraperitoneal route. These latter was related with the different ethanol oxidation route by the liver during the process of LR. We described for the first time that selective release of enzymes by the liver during the process of LR.

Low administration of ethanol in the regeneratins liver of rats, produced alterations of the microscopic ultrastructure in the hepatocytes. These were depending of the route of ethanol administration. Intragastric ethanol administration produced a diminution of the normal changes produced in the liver during LR. The intraperitoneal ethanol administration produced a retardation in the appearance of the proliferative profile of hepatocytes during LR.

The 4-MP (25 and 50 mg/Kg) administration to the rat during LR, are associated with low changes in the ADH activity of the liver. Minor metabolic changes were produced by 4-MP. A dosage between 100 to 200 mg/Kg of 4-MP to rats subjected to PH produced a very important diminution in the specific activity of the ADH. These changes were associated with changes in both, the function and the structural changes during the process of the liver regeneration in the hepatocyte. The study of the changes in the specific activity of the ADH can be used in the identification of the mechanism associated with the pathogenesis of inhibition of proliferation of liver in the diseased organ of the human.

It is a question to resolve the mechanisms by involved in the ethanol-induced LR by different routes of its administration. By the same way, it is clear that the investigation of the exact mechanisms in the damage produced by the ethanol has to be advocated.

a) Cantidad. Algunas investigaciones refieren que una importante fracción de una dosis usual de etanol (alcoholismo social) no pasa a la circulación sistémica, sino que es oxidada principalmente en estómago (Fraser y cols. 1992 y 1993).

b) Concentración. Se ha encontrado que a mayor concentración de etanol, se retrasa el vaciamiento gástrico por inhibición de la motilidad intestinal, permaneciendo un mayor tiempo en el estómago, que aquellas con diluciones menores (Roine y cols. 1991; Sharma y cols. 1993).

c) Alimento. La presencia de alimentos en el estómago, al reducir la velocidad de vaciamiento gástrico, demora la absorción de etanol (Roine y cols. 1991) y permite que se dé una absorción gástrica importante de éste, o que se metabolice el etanol por la presencia de alcohol deshidrogenasa en la mucosa del estómago.

d) Ayuno. El ayuno prolongado aumenta la concentración máxima de etanol en sangre, ya sea por una disminución en el tiempo de exposición del etanol a la ADH gástrica por un tránsito acelerado o por un vaciamiento gástrico acelerado y un aumento en la absorción intestinal (Di Padova y cols. 1987).

e) Medicamentos. Con algunos medicamentos, como los antagonistas de los receptores H2 (cimetidina, ranitidina y nizatidina pero no famitidina), se ha visto que se aumenta significativamente la concentración máxima de etanol en sangre después de la ingesta de éste. Esto puede deberse a un aumento en la absorción del etanol, o bien a que se disminuye el metabolismo gástrico de etanol al inhibirse la ADH gástrica (Caballería y cols. 1989b; Hernández Muñoz y cols. 1990a; Palmer y cols. 1991; Di Padova y cols. 1992).

2.- METABOLISMO DEL ETANOL

a) Metabolismo Hepático

(1) Sistema de la alcohol deshidrogenasa (ADH)

El sistema de las alcohol deshidrogenasas se compone de varias enzimas, que en los mamíferos se dividen en seis clases distintas que comprenden 20 isoenzimas diferentes, codificadas por más de 7 genes (Danielsson y cols. 1994; Arnon y cols. 1995). Estas se distribuyen ampliamente en los diferentes órganos y alcanzan su mayor actividad en el hígado (Boleda y cols. 1989; Kennedy y Tipton 1990). Son moléculas diméricas con subunidades de aproximadamente 40 kDa; y cada subunidad se caracteriza por tener 2 átomos de zinc (Das y cols. 1984; Cortot y cols. 1986; Zakim y Boyer 1990) unidos por cisteína, los cuales ayudan a estabilizar la estructura de la enzima (Jelokova y cols. 1994).

Por técnicas inmunohistoquímicas se han localizado estas enzimas alrededor de la vena central (zona 3) del hígado. La ADH hepática se localiza preferentemente en citosol y convierte al etanol en acetaldehído, utilizando como coenzima nicotinamida adenín dinucleótido (NAD^+). El H^+ es transferido del sustrato (etanol) al cofactor (NAD^+), produciendo NADH y acetaldehído (reacción 1)(Lieber 1984c). Posteriormente el acetaldehído es oxidado, predominantemente en las mitocondrias y secundariamente en el citosol (Arnon y cols. 1995), produciendo acetato, por medio de la enzima aldehído deshidrogenasa (ALDH) que utiliza nuevamente como coenzima NAD^+ para producir como productos acetato y NADH (reacción 2).

$$CH_3\text{-}CH_2\text{-}OH + NAD^+ \xrightarrow{ADH} CH_3\text{-}CHO + NADH + H^+ \qquad (1)$$

$$CH_3\text{-}CHO + NAD^+ \xrightarrow{ALDH} CH_3\text{-}COO^- + NADH + H^+ \qquad (2)$$

Cuantitativamente hablando, la mayor parte del etanol es metabolizado por estas dos enzimas (ADH y ALDH) y una fracción pequeña se metaboliza por los otros sistemas. El equilibrio de la reacción de la ADH está desplazada hacia la formación de

etanol; sin embargo, la rápida e irreversible reacción catalizada por la ALDH facilita el metabolismo del etanol (Lieber 1974; Tottamer 1973; Peter 1982).

En los humanos, la alcohol deshidrogenasa y la aldehído deshidrogenasa son las dos enzimas que catalizan la oxidación de etanol hacia acetaldehído y acetato, respectivamente (Boosron y Li, 1986; Yin y Li, 1989). Basados en sus cinética y sus características estructurales, las isoenzimas de las ADH humanas se han clasificado en 5 clases (Vallee y Bazzone, 1983; Yoshida y cols. 1991) (Tabla I). Los rangos de Km para el etanol son los siguientes: ADH clase I (α,β,γ) 4mM; ADH clase II (π) 34 mM; ADH clase III (χ) 3 M; ADH clase IV (μ) 18 mM (Yin y cols. 1990); ADH clase V 28 mM (Chen y Yoshida, 1991). La clase I es altamente sensible a la inhibición por 4-Metilpirazola (4-MP) con una Ki menor a 2 µM; clase II Ki = 2 mM; clase III es virtualmente insensible a la inhibición; clase IV Ki = 320 µM. La homología de las secuencias de las proteínas o de los nucleótidos intraclase es aproximadamente del 90% y de interclase es del 60 % (Yasunami y cols. 1991; Yoshida y cols. 1991; Parés y cols. 1992).

Se han purificado y caracterizado de hígado y estómago de humano 5 isoenzimas de la aldehído deshidrogenasa (Tabla II): ALDH1, ALDH2 (Greenfield y Pietruszko, 1977), ALDH3 (Wang y cols. 1990), ALDH4 (Forte-McRobbie y Petruszko, 1986), y ALDH5 (Kurys y cols. 1989). Basados en las constantes de Michaelis para aceltadehído, en el humano se dividen en alta K_M en el rango mM (ALDH3 83 mM; ALDH4 5mM) (Forte-McRobbie y Pietruszko, 1986; Yin y cols. 1989) y bajo K_M en el rango µM (ALDH1 30µM; ALDH2 3µM; ALDH5 50µM) (Greenfield y Pietruszko, 1977; Kurys y cols. 1989). Aproximadamente el 50% de los orientales tienen deficiencia en la actividad de la ALDH2 mitocondrial de bajo K_M (Harada y cols. 1980; Yin y cols. 1988), y esta alteración es atribuible a la sustitución de un simple aminoácido (ácido glutámico por lisina) en la posición 14 del carboxilo terminal (Yoshida y cols. 1984; Hempel y cols. 1984). Múltiples formas de ALDH3 se encuentran en la mucosa del estómago. Se ha propuesto el modelo de 2 genes para la ALDH3, que codifican para las cadenas polipeptídicas A y B (Yin y cols. 1988).

Las isoenzimas de la ADH y de la ALDH tienen una distribución en tejidos específicos (Smith, 1986; Yin y Li, 1989; Yoshida y cols. 1991), todas las isoenzimas de la ADH clase I y clase II se expresan en el hígado. La ADH-β se expresa en el pulmón y ADH-γ se expresa en el tracto gastrointestinal; la ADH clase III tiene una distribución universal; la ADH clase IV se encuentra presente en el estómago. Las isoformas de la

aldehído deshidrogenasa, ALDH1 y ALDH2 se expresan en hígado, pulmón y tracto gastrointestinal. Múltiples formas de la ALDH3 se han detectado en estómago y pulmón pero no en hígado. La carencia de la actividad de la ALDH2 en los orientales causa reacción de sensibilidad al alcohol (Harada y cols. 1980; Agarwal y Goedde, 1989).

TABLA I Clases de isoenzimas y distribución de la alcohol deshidrogenasa en los seres humanos.

Clase	Isoenzimas	K_M etanol	Sensibilidad 4-MP	Distribución
I	α β γ	4 mM	Ki menos 2μM	Hígado Hígado, pulmón Hígado, TD
II	π	34 mM	Ki = 2mM	Hígado
III	χ	3 M	Insensible	Universal
IV	$\mu(\sigma)$	18 mM	Ki=320 μM	Estómago
V		28 mM		Estómago

TABLA II Isoenzimas y distribución de la aldehído deshidrogenasa en los seres humanos.

Isoenzima	K_M acetaldehído	Distribución
ALDH1	30μM	Hígado, pulmón, TD
ALDH2	3 μM	Hígado, pulmón, TD
ALDH3	83 mM	Estómago y pulmón
ALDH4	5 mM	Hígado, riñón
ALDH5	50μM	Cerebro, estómago

(2) Sistema microsomal de oxidación de etanol.

El sistema microsomal de oxidación de etanol (MEOS por sus siglas en ingles: Microsomal Ethanol Oxidizing System) (Lieber y De Carli 1968; Rubin y cols. 1968; Zakim y Boyer 1990), es el responsable principal del metabolismo de los fármacos y otras sustancias xenobióticas (Winter 1993), como el acetominofén, la acetona, el fenol, entre otros (Morgan y cols. 1982 y 1983; Ingelman y Johansson 1984; Koop y Casazza 1985; Yang y cols. 1985; Koop y Tierney 1990), incluyendo a los barbitúricos, lo que explica la resistencia que tienen los alcohólicos a este tipo de drogas. Cabe destacar que cuando se ha ingerido una gran cantidad de etanol, el MEOS metaboliza

preferentemente etanol por lo que los barbitúricos dejan de metabolizarse, ocasionando resistencia a esta droga.

Este sistema se localiza en las cisternas del retículo endoplásmico liso del hepatocito. Se ha descrito la presencia del MEOS en las microsomas de células de diferentes tejidos (Gonzalez 1992) y se conoce que involucra la participación de varias enzimas, las cuales catalizan la oxidación de etanol a acetaldehído.

El MEOS se ha purificado y se encontraron tres componentes diferentes, la citocromo C reductasa (citocromo P-450 reductasa), el citocromo P-450 y un fosfolípido (lecitina). El MEOS es un sistema que se induce por el alcoholismo crónico, en donde participan varias isoenzimas del citocromo P-450 en la oxidación del etanol, siendo el citocromo P-450 2E1 el encargado del metabolismo del etanol (Koop y Casazza 1985; Koop 1989), el cual es capaz de catalizar la oxidación del etanol hacia acetaldehído, utilizando como coenzima NADPH y de oxígeno molecular (reacción 3) (Kennedy y Tipton 1982; Lieber 1991). Posteriormente, el acetaldehído así formado es convertido en acetato por la ALDH utilizando como coenzima NAD^+.

$$CH_3\text{-}CH_2\text{-}OH + NADPH + H^+ + O_2 \xrightarrow{\text{MEOS (P-450 2E1)}} CH_3\text{-}CHO + NADP^+ 2H_2O \quad (3)$$

Normalmente, el etanol se metaboliza preferentemente por la ADH hepática y en menor cantidad por el MEOS, debido a una baja afinidad por el etanol de este último ya que posee una K_M = 8.6 mM o 40 mg/ 100 ml en comparación con la ADH en con una K_M = 2 mM o 9 mg/ 100 ml. Esto se traduce en que el MEOS necesita cantidades mayores de etanol en sangre para saturarse. En un estudio se reportó que el MEOS cataliza el 42% del metabolismo del etanol sí se encuentra a una concentración de 10 mM, y el 62% si ésta es de 50 mM (Takagi y cols. 1986).

La contribución del MEOS y de la ADH en el metabolismo del etanol depende de la concentración sanguínea de alcohol y la duración de su consumo.

(3) Sistema de la catalasa

El hepatocito contiene catalasa en los peroxisomas (Lieber 1984) y utiliza peróxido de hidrógeno para oxidar el etanol. Este sistema está en relación estrecha con el sistema de glutatión oxidado-reducido y al igual que el sistema MEOS, es inducido por el alcoholismo crónico (Thurman y Handler 1989; Lieber 1991). El etanol puede servir como donador de hidrógenos en la reacción.

Esta reacción es dependiente de la disponibilidad de peróxido de hidrógeno por lo que un incremento de este o un decremento en la catalasa puede activar o inhibir esta reacción. Precisamente, la velocidad de producción de H_2O_2 es un factor limitante (Peter 1982), debido a que la producción del peróxido es tan sólo de 3.0 a 3.6 µmol/h/g hígado y este sistema apenas representa el 2% de la oxidación de etanol *in vivo* (Lieber 1994).

El etanol se oxida hacia acetaldehído por el sistema de la catalasa, que utiliza como coenzima H_2O_2 (reacción 4). El acetaldehído sigue el mismo camino para convertirse a acetato por medio de la ALDH.

$$CH_3\text{-}CH_2\text{-}OH + H^2O^2 \xrightarrow{\text{Catalasa}} CH_3\text{-}CHO + 2H_2O \qquad (4)$$

Los tres sistemas principales de oxidación de etanol (ADH, MEOS y la catalasa) producen acetaldehído el cual se convierte en acetato por la enzima ALDH; así mismo, el acetato que es el resultado final del metabolismo del etanol, se libera a la sangre para ser metabolizado hasta CO_2 por los demás tejidos periféricos (Kennedy y Tipton 1990; Lieber 1991)

Asimismo, el aumento en la concentración intracelular de NADH y NADP es el responsable de las alteraciones en el estado redox, provocando las anormalidades metabólicas hepáticas en el alcohólico (Pösö y Forsander 1976; Christensen y Higgins 1979; Lieber 1991), aunado a la alta toxicidad que produce el acetaldehído y los demás metabolitos secundarios que se producen en la oxidación del etanol, como los radicales libres.

b) Metabolismo extrahepático

El metabolismo gástrico del alcohol es una determinante importante en la regulación de los niveles séricos de etanol en donde se ha encontrado a la enzima ADH en la mucosa gástrica (Di Padova y cols. 1987 y 1988; Frezza y cols. 1990).

De hecho, se ha considerado que la capacidad oxidativa de alcohol por el estómago constituye una "barrera protectora" contra los efectos sistémicos del tóxico. Debido a que concentraciones altas de alcohol pueden retrasar el vaciamiento gástrico, se podría esperar que soluciones concentradas de alcohol permanecieran por más tiempo en el estómago que aquellas más diluidas (Lolli y Rubin 1943). Sin embargo, nada se sabe con relación a los factores que están regulando dicha absorción y oxidación gástrica del alcohol en animales sujetos a hepatectomía

La ADH gástrica contribuye a la oxidación del etanol ingerido y por ende disminuye los niveles séricos de etanol, lo que se considera como el primer paso en el metabolismo del etanol (Julkunen y cols. 1985a y 1985b; Hernández Muñoz y cols. 1990a). Este primer paso es un factor determinante importante para las concentraciones sanguíneas de alcohol (etanolemia) y de las áreas bajo la curva (ABC) del alcohol en la circulación sistémica alcanzados por la ingesta de etanol, tanto en humanos (Julkunen y cols. 1985a; Caballería y cols. 1989a) como en animales experimentales (Julkunen y cols. 1985a y 1985b).

La actividad de la ADH gástrica se encuentra afectada por el género (Frezza y cols. 1990; Seitz y cols. 1990), edad (Seitz y cols. 1990), ayuno (Di Padova y cols. 1987), alcoholismo crónico (Di Padova y cols. 1987; Frezza y cols. 1990), algunas drogas (Caballería y cols. 1989b; Roine y cols. 1990a y 1990b) y por microorganismos (Thuluvath y cols. 1994). Esto da como resultado el aumento en los niveles de la etanolemia y de las ABC posteriores al consumo de alcohol (Seitz y cols. 1984; Caballería y cols. 1989b; Roine y cols. 1990a y 1990b) que produce un incremento en la susceptibilidad de daño hepático con relación a los niveles de etanol alcanzados.

Algunos estudios demuestran diferencias en las concentraciones de etanol en sangre después de administrarlo por vía oral en comparación con la vía intravenosa; donde se puede observar que las concentraciones de alcohol en sangre son más bajas después de administrarlo intragástricamente, en comparación con la administración por vía intravenosa con una misma dosis de alcohol tanto en humanos (Di Padova y cols. 1987) como en ratas (Julkunen y cols. 1985a y 1985b). Se concluye que esta diferencia se debe al primer paso metabólico del alcohol, debido a la actividad de la

ADH gástrica, que tiene una isoenzima con una alta K_M para el etanol que no exhibe inhibición con altas concentraciones de sustrato, como ocurre con la ADH hepática (Hernández Muñoz y cols. 1990a).

Más aún, Roine y cols. (1991), al utilizar diferentes vías de administración de etanol (intravenosa, intraduodenal, intraportal), en un análisis comparativo de la farmacocinética de la vía intragástrica por estas diferentes rutas, encontraron que en la vía intragástrica las etanolemias son inferiores que cuando se administra por otras rutas. De aquí que el primer paso metabólico se encuentre asociado con concentraciones inferiores de etanol en la circulación sistémica, así como una menor biodisponibilidad del etanol. Por otra parte, Smith y cols. (1992), sugieren que una velocidad lenta de absorción podría contribuir en el primer paso del metabolismo hepático y concluyeron que el metabolismo del etanol por el estómago es en realidad insignificante.

3.- REGENERACIÓN HEPÁTICA

Se ha reconocido por años que, posterior a un daño químico o retiro quirúrgico parcial de su tejido, el hígado inicia una serie de cambios conocidos en conjunto con el nombre de regeneración, que están encaminados a la recuperación del tejido perdido o afectado en el aspecto anatómico y funcional (Higgins y Anderson, 1931). Los mecanismos exactos por los cuales el hígado se regenera aún no se conocen con precisión y han recibido mucha atención en los últimos tiempos, ya que la regeneración hepática es un excelente modelo experimental para estudiar los procesos que determinan la proliferación celular (Michalopoulos, 1990).

Los mecanismos que regulan la proliferación de los hepatocitos se han estudiado en modelos de hígado fetal, cáncer hepático y regeneración hepática. En todos estos casos, la proliferación celular es el factor común a todos ellos y es este fenómeno el que está sujeto a regulación precisa por parte de la célula, el tejido y el órgano en general (Fausto y Webber, 1993).

De los modelos más utilizados para el estudio de la regeneración hepática, el más común es la hepatectomía parcial (HP, retiro quirúrgico del 70% del tejido hepático) en animales de laboratorio (ratas, conejo, cuyo, perro). Puesto que el hígado está dividido en lóbulos, es posible quitar algunos de ellos que representen el equivalente del 70% del tejido hepático total y dejar un remanente del 30%. El proceso de crecimiento del

hígado implica la proliferación de las células de el (los) lóbulo(s) remanente(s). Esto último no significa la restauración de los lóbulos escindidos; esto es, no debe confundirse el fenómeno de crecimiento y restitución de una parte amputada (como la amputación de la cola en una lagartija), con el fenómeno de la regeneración hepática. En este último caso, el (los) lóbulo(s) remanente(s) entra(n) en proliferación celular hasta restituir al tejido hepático funcional que originalmente tenía. Este crecimiento y proliferación terminan a los 10-14 días en todas las especies examinadas, después de la cirugía (Higgins y Anderson, 1931; Fausto y Webber, 1993). Una pregunta importante que todavía no ha sido contestada, es explicar el funcionamiento óptimo del hígado remanente que mantiene al tejido funcional. Responder a esta pregunta requiere un detallado examen de los mecanismos que inician, mantienen y terminan la regeneración hepática. En este tipo de estudios, es de ayuda el identificar las fases del proceso regenerativo e intentar identificar los eventos que regulan cada una de ellas. En fibroblastos en estado de reposo y en algunos tipos de células epiteliales en cultivo, la activación del crecimiento parece estar dividida en dos fases: competencia y progresión. En el estado de competencia (o competente), la célula pone en marcha los mecanismos para poder entrar al ciclo celular (paso de la célula de la fase G0 a G1). En el estado de progresión, la célula que ha entrado a la fase G1 avanza a la fase S y después a la fase M hasta completar un ciclo celular. La separación entre estas dos fases es hasta cierto punto arbitraria, pero la importancia de ambos conceptos radica en que permite la búsqueda de factores cuya expresión esta restringida a un tipo determinado durante el ciclo celular. Con ello, es posible determinar las diferencias fundamentales entre una célula en estado de reposo (en estado G0) y otra que ha iniciado la proliferación (estado G1) (Fausto y Webber, 1993; Fausto y cols. 1995)

La proliferación hepática se inicia 12 o 14 horas después de la hepatectomía parcial lo que permite una separación entre un estado pre-replicativo (0 a 14 horas) y un estado replicativo (14 a 24 horas) (Steiner y cols. 1966). Por conveniencia, se distingue una fase inicial que es la fase de competencia (o fase de iniciación) que corresponde a las primeras 4 horas después de la HP (paso de la fase G0 a la fase G1), y una segunda fase de progresión que indica el paso de la fase G1 a la fase S. La fase G1 inicia en diversas áreas del parénquima hepático remanente y su duración es variable por lo que la fase de progresión es menos sincrónica que la de iniciación pero termina cuando la célula sintetiza DNA, el cual tiene un pico de síntesis en la rata a las 22-24 horas. La sucesión entre la fase de inicio y progresión depende directamente o

indirectamente de la activación/inhibición de genes, la activación y regulación de circuitos autócrinos y parácrinos y la activación de la maquinaria necesaria para la replicación del DNA (Fausto y Webber 1993).

Es precisamente en estas fases tempranas de la regeneración cuando el proceso es más susceptible de ser interrumpido o alterado, ya que las señales iniciales pueden ser bloqueadas dando como consecuencia la no-regeneración del tejido (Fausto y Webber 1993).

Los eventos bioquímicos que siguen después de la HP, por lo menos en animales en particular en la rata y el ratón, es un incremento temprano de la actividad de la ornitin descarboxilasa (ODC), enzima que se encuentra involucrada en la síntesis de poliaminas, teniendo su pico de actividad entre 4 a 6 horas después a la cirugía y para su retorno a la normalidad en las siguientes horas. El incremento en la actividad de la ODC, es seguido por un incremento en la actividad de la timidín cinasa (TK) con su pico a las 24 horas de HP; esta enzima se encuentra involucrada en la síntesis del DNA (Diehl y Rai, 1996). Estos cambios en los niveles de las enzimas, se han asociado más recientemente a cambios que ocurren en los niveles de las hormonas sexuales durante tiempos tempranos de la regeneración hepática. Específicamente, el hepatocito contiene el receptor para el estrógeno que particularmente aumentan en el núcleo y disminuyen en el citosol durante la regeneración hepática. Simultáneamente, el receptor para los andrógenos disminuye en ambos compartimentos durante la proliferación hepática. Por otra parte, tanto en hombres como en animales, los niveles plasmáticos de estradiol se incrementan mientras que los niveles de testosterona disminuyen (Van Thiel y cols. 1991).

Estudios hechos en animales (perro y primates) y en humanos, han establecido que la regeneración hepática responde en forma proporcional a la cantidad del hígado removido. En resecciones pequeñas (menores del 10%), sigue una respuesta proporcional para la recuperación del hígado. Asimismo, se ha visto que cuando el hígado de perros grandes es transplantado a perros pequeños, aquél disminuye gradualmente de tamaño, siendo al final proporcional al tamaño del nuevo huésped. Por otra parte, cuando se transplanta el hígado de un perro pequeño a un perro grande, el órgano crece rápidamente (semanas) siendo nuevamente proporcional al tamaño del nuevo huésped. Este tipo de estudios ha demostrado que la masa hepática es altamente regulada y que existen señales que controlan en forma positiva así como negativa al hígado para que este tenga su tamaño correcto (Francavilla y cols 1988).

En contraste con otros órganos o tejidos, la regeneración hepática no depende de un grupo pequeño de células progenitoras. La regeneración hepática posterior a la HP es realizada por la proliferación de todas las poblaciones celulares maduras que componen al órgano intacto (Thorgeirsson, 1996). Esto incluye a los hepatocitos (son las principales células funcionales), células epiteliales biliares (forman a los ductos biliares), células endoteliales (son células que dan un máximo contacto entre la sangre y los hepatocitos), células de Kupffer (macrófagos en los sinusoides hepáticos) y las células de Ito (únicas células estelares que se localizan en el hígado, se ubican abajo de los sinusoides, sintetizan tejido conectivo, secretan varios factores de crecimiento y almacenan vitamina A) (Gressner, 1995). Todas estas células se dividen durante la proliferación hepática, siendo los hepatocitos los primeros en hacerlo. La cinética de la proliferación celular difiere ligeramente de una especie a otra. El primer pico de la síntesis del DNA ocurre a las 24 horas, con un segundo pico pequeño entre las 36-48 horas, para completar todo el proceso proliferativo del día 7-10 (Higgins y Anderson, 1931).

En las primeras 24 horas posteriores a la HP, no existen cambios significativos notables de la matriz extracelular (MEC). Existe una actividad mitótica prominente en las zonas periportales, el número de hepatocitos tiene un incremento notable, mientras que la MEC se mantiene sin modificaciones, por lo tanto se presenta un incremento en la relación células/MEC, creado aparentemente por la disminución en la MEC. La actividad mitótica continúa (por el día 3 de la HP), dando como resultado la formación de grupos de hepatocitos periportales de cerca de 10 a 14 células sin la intervención de sinusoides o de la MEC. De esta manera, la relación que guardan los hepatocitos con la vascularización normal es baja. Después a la HP, las primeras células que se encuentran en mitosis son los hepatocitos periportales (zona 1) (16 horas); seguidos por los hepatocitos que se localizan en la región media de los lóbulos (zona 2) (24 horas), y posteriormente se localizan en la región centrolobular (zona 3) (48 horas). Estas "*oleadas*" *de mitosis probablemente* reflejan la diferencia de la fase G1 entre los hepatocitos periportales (G1 corta) y los hepatocitos centrolobulares (G1 larga). Esto tiene como consecuencia directa probables oleadas de replicación, los centros celulares se forman primero en las áreas periportales y posteriormente en las áreas centrolobulares. Esta división de los hepatocitos se alcanza sin degradación *a priori* de la MEC. Estos datos se basan en estudios de inmunohistoquímica para componentes de la MEC, la cual no muestra evidencias de degradación (Martinez Hernandez y cols. 1991). Por el día 4 de la regeneración hepática la actividad mitótica decrece y esto coincide con

el inicio de los depósitos de laminina en pequeñas cantidades que es producida por las células de Ito (Tanaka y cols. 1990; Martinez Hernandez y cols. 1991), segregando estas cadenas de laminina beta-1, beta-2, y gamma-1; sin embargo, la presencia de cadenas alfa-1 no ha sido demostrada (Wewer y cols 1992). A pesar de la síntesis de laminina, no existe todavía membrana basal. La laminina que esta contenida dentro de las células de Ito rodea a los hepatocitos en islotes, coincidiendo con la aparición de un proceso celular que es la invasión de células endoteliales que separan a los islotes de los hepatocitos para formar placas de células, junto con la aparición de los espacios vasculares de la superficie. De esta manera, la estructura vascular lobular normal es restaurada. Por otra parte, en estos momentos es cuando las metaloproteasas de matriz (MMPs) actúan para la remodelación de la MEC y de esta forma reestablecer el parénquima hepático normal. Cerca del día 7-8 de la regeneración hepática las células de Ito que producen laminina son raras y para el día 10 el patrón normal junto con una distribución normal de la MEC está presente. Por supuesto, las colágenas tipo I, III, IV, V, VI han sido sintetizadas y depositadas en los nuevos sinusoides y los espacios de Disse´s están formados. La síntesis de la MEC se ha completado para estos tiempos como se ha demostrado por técnicas de inmunohistoquímica. La coincidencia temporal de la expresión de la laminina por las células de Ito junto con las actividades fisiológicas de estas células dentro de los islotes de los hepatocitos, sugiere que las células de Ito y los isotipos de laminina específicos pueden mediar la vascularización de los islotes de hepatocitos hacia sinusoides (Martinez Hernandez y Amenta, 1995).

4.- REGENERACIÓN HEPÁTICA Y ETANOL

Como ha sido ya mencionado cuando existe una pérdida importante de masa hepática funcional, principalmente la debida a remoción quirúrgica del hígado, el tejido hepático remanente sufre un proceso de regeneración (hiperplasia), en el cual el tejido removido es reemplazado en su totalidad. Durante este proceso, la síntesis de DNA se incrementa notablemente y alcanza un pico máximo a las 23-25 horas posteriores a la cirugía (en la rata). Al mismo tiempo, la división celular empieza activamente y por el día 6 al 8 después de la cirugía se completa la restauración del hígado (Michalopoulus 1990). Por lo tanto, la regeneración hepática que se produce como consecuencia de hepatectomía parcial es considerada como un muy buen modelo para el estudio de los mecanismos que regulan la división y diferenciación celulares.

Este proceso es muy sensible a la acción de algunos xenobióticos, en particular la administración aguda o crónica de alcohol (etanol), que ejerce un efecto adverso sobre la regeneración hepática, especialmente cuando éste se administró en los estadios tempranos del proceso regenerativo. Por ejemplo, el alcohol es capaz de inhibir la síntesis de DNA (Dugay y cols. 1982), la síntesis de poliaminas, las cuales se encuentran profundamente involucradas en la síntesis de DNA (Diehl y cols. 1990a) y la actividad de la enzima que las sintetiza, que es la *ornitín descarboxilasa (Diehl y cols. 1990b)*. Todo da como resultado una recuperación mucho menor de la masa hepática en ratas hepatectomizadas y sujetas a ingesta de alcohol (Frank y cols. 1979).

La timidín cinasa (TK) es una enzima clave en la generación de sustratos para la síntesis del DNA. Existen reportes del incremento de la actividad específica de esta enzima durante la fase proliferativa de células de eucariontes, incluída la regeneración hepática, posterior a una hepatectomía parcial (Bresnick y cols. 1964; Labow y cols. 1969; *Gutiérrez-Salinas y cols. 1996)*. Para demostrar que el etanol inhibe a la regeneración hepática se han utilizado marcadores que indican la proliferación celular como la determinación de la actividad específica de enzimas relacionadas directamente con la síntesis de DNA (Michalopoulos, 1990).

A pesar de que algunos mecanismos de daño ya han sido referidos en la acción del alcohol sobre la regeneración hepática, llama la atención de que prácticamente se desconozca si las acciones de dicho tóxico son por la molécula misma o por las transformaciones metabólicas que experimenta en la célula hepática. En particular, nada está reportado sobre la farmacodinamia y la farmacocinética del alcohol en animales hepatectomizados parcialmente. Estudios recientes han indicado que una solución concentrada de alcohol (5g/Kg, 60%), a dosis que inhiben el proceso de regeneración hepática, son aparentemente más lentamente absorbidas y más rápidamente oxidadas en animales hepatectomizados (Gutiérrez Salinas y cols. 1996). Sin embargo, otros estudios han encontrado que utilizando dosis menores de etanol (3g/Kg de peso), los niveles sanguíneos de alcohol permanecen elevados durante toda la fase pre-replicativa de hígados en regeneración (Lumpkin y cols. 1992). De ahí que exista una importante discrepancia con relación a la "biodisponibilidad" del alcohol en animales sujetos a hepatectomía parcial.

5.- DAÑO HEPÁTICO POR ALCOHOL

El hígado como principal órgano responsable del metabolismo y eliminación del etanol, también experimenta los efectos más adversos por el tóxico. A continuación se describen algunos mecanismos por los cuales el etanol y sus metabolitos (acetaldehído y acetato), así como el desequilibrio en el estado redox son causantes del daño hepático por alcohol.

El etanol es por sí mismo tóxico para cualquier tejido en general, por ser altamente polar, por lo cual interactúa principalmente con los componentes lípidicos de las membranas celulares, provocando su inestabilidad estructural y afectando su función (Polokoff y cols. 1983).

El metabolismo de la célula es alterado en forma importante cuando el etanol es oxidado por el sistema de la ADH y por la ALDH. Se sabe que estas dos enzimas requieren para su función la coenzima NAD^+, lo cual produce un exceso de NADH tanto en el citoplasma como en el interior de la mitocondria, provocando una alteración en el balance redox general de la célula (Christensen y cols. 1979). A esta desproporción entre $NADH/NAD^+$ resultante de la oxidación del etanol y de su metabolito el acetaldehído se le responsabiliza de una variedad de anomalias en el metabolismo intermedio, especialmente de los carbohidratos y de los lípidos que se observa en los alcohólicos (Lieber 1984b; 1984c). Este desequilibrio redox lleva a una mayor producción de lactato a partir de piruvato y se considera que el incremento de lactato podría ser uno de los principales factores inductores de la fibrogénesis hepática, estimulando algunas enzimas que intervienen en la biosíntesis de la colágena. Por otro lado, la alteración más importante en el metabolismo de las proteínas se expresa mediante un aumento en la síntesis de colágena lo que resulta en la fibrosis del parénquima hepático (Kennedy y Tipton 1990).

El aumento de NADH interfiere en la gluconeogénesis a partir de los aminoácidos y puede explicar la hipoglucemia que presentan algunos alcohólicos. Asimismo, se produce un aumento de glicerofosfato a partir de dihidroxiacetona fosfato (Lieber 1984c) se favorece la síntesis de triacilglicéridos y la disminución de la oxidación de los ácidos grasos, por lo que se induce un estado hipermetabólico en el hígado promoviendo daño hipóxico hepático en la zona 3.

El acetaldehído, derivado del metabolismo del etanol, se le considera de hecho más peligroso que el mismo etanol (Donahue y cols. 1983; Sorrell y Tuma 1985; Shaw y

cols. 1990; Niemela y cols. 1994; Niemela y cols. 1995). La acción tóxica del acetaldehído está dada por su estructura química que le confiere una elevada capacidad de reaccionar con los grupos tiol y amino de las proteínas y de otros constituyentes de las membranas celulares, creando los "aductos" de acetaldehído que alteran la estructura terciaria de las proteínas interfiriendo con la función de éstas. La presencia de "aductos" de acetaldehído no sólo se ha demostrado en las proteínas celulares, sino también en los lípidos y los ácidos nucléicos, lo que pone de manifiesto su toxicidad (Weiner 1979).

El acetaldehído también puede ejercer una acción tóxica al contribuir a la lipoperoxidación de las membranas celulares. El acetaldehído se une a ciertas moléculas como la cisteína y el glutation, que son capaces de aceptar oxígeno y, por lo tanto, son moléculas fundamentales para impedir la lipoperoxidación. De los derivados secundarios producidos por la célula durante el metabolismo del etanol se han descrito como principales a los radicales libres derivados del oxígeno (Lieber 1991). Estos radicales libres son moléculas o átomos que tienen un electrón desapareado por lo que son altamente reactivos y por ende, peligrosos para el organismo; proteínas, lípidos y ácidos nucléicos pueden ser "atacados" por los radicales libres y alterar de manera significativa las funciones celulares.

HIPÓTESIS

Dado que el hígado es el principal órgano que metaboliza al etanol, la inhibición que esta molécula produce sobre la regeneración hepática inducida por hepatectomía parcial estaría dependiendo estrechamente de su metabolismo. Ya que se sabe que la actividad de la ADH es el principal sistema de oxidación de etanol, se esperaría que esta enzima esté profundamente involucrada en el efecto inhibitorio del etanol sobre la regeneración hepática

OBJETIVOS

1.- OBJETIVOS GENERALES

1.- Estudiar la farmacocinética del etanol y la participación del estómago en el primer paso metabólico del etanol, durante la regeneración hepática en animales sujetos a hepatectomía parcial.

2.- Estudiar los efectos del etanol sobre la morfología y la función del hígado en regeneración, después de una hepatectomía parcial.

3.- Estudiar los efectos del etanol sobre la ultraestructura del hígado en regeneración, después de una hepatectomía parcial.

4.- Estudiar sí existe una relación entre el principal sistema de oxidación del etanol en el hígado (ADH) con la regeneración hepática.

2.- OBJETIVOS ESPECÍFICOS

1.- Determinar en animales sujetos a hepatectomía parcial (70% de la masa hepática original), la farmacocinética del etanol durante la regeneración hepática.

2.- Determinar la participación del estómago en el primer paso metabólico del etanol, durante la regeneración hepática.

3.- Determinar en suero de animales sujetos a hepatectomía parcial, la actividad de las enzimas marcadoras de daño hepático [alanino aminotransferasa (ALT), aspartato aminotransferasa (AST), ornitin carbamiltransferasa (OTC), lactato deshidrogenasa (LDH) y glutamato deshidrogenasa (GDH)], a diferentes tiempos de regeneración hepática en presencia o ausencia de etanol.

4.- Cuantificar en suero de animales sujetos a hepatectomía parcial, los metabolitos relacionados con la función del hígado (bilirrubina y albúmina) y los relacionados con la homeostasis general (triacilglicéridos y glucosa).

5.- Estudiar por microscopia de luz la estructura y el índice mitótico (como parámetro de regeneración) de hepatocitos regenerantes.

6.- Determinar en hígados de animales sujetos a hepatectomía parcial, la actividad de la timidín cinasa (TK), como parámetro de regeneración.

7.- Estudiar por microscopia electrónica la ultraestructura del hígado, y la morfología del núcleo de hepatocitos regenerantes.

8.- Estudiar la participación de la ADH en la regeneración hepática al inhibirla con 4-MP, a través de estudios farmacocinético (etanolemias); bioquímico (actividad ADH, ALT, AST y LDH); fisiológico (bilirrubina, albúmina, triacilglicéridos y glucosa); morfológico (microscopia de luz) y parámetros de regeneración (índice mitótico y actividad de la TK).

MATERIAL Y MÉTODOS

1.- MATERIAL

Alcohol deshidrogenasa de levadura (EC 1.1.1.1), 4-Metilpirazola, NAD^+, semicarbazida se obtuvieron de Sigma Chemical Co. (St. Louis, MO). La timidina [^{3}H] (actividad específica 2 mCi/mmol) fue obtenida de Amersham Co. (Arlington Heigts, Illinois). El etanol y los otros compuestos químicos fueron de grado analítico.

2.- ANIMALES

Se utilizaron ratas macho de la cepa Wistar (obtenidas del bioterio del Instituto de Fisiología Celular, UNAM), con un peso de 250 ± 20 gramos. Fueron alimentadas con dieta balanceada para roedores (# 5001, PMI Feeds Inc., St.Louis, MO) y agua *ad libitum*, excepto el día del experimento.

3.- PROCEDIMIENTO QUIRÚRGICO

Se realizó una hepatectomía parcial (HP) del 68 ± 2% acorde a la técnica clásica de Higgins y Andersón (1931). Se utilizó como anestésico éter etílico por el tiempo necesario para realizar la HP (15 minutos aproximadamente). Se realizó una incisión en la parte media del abdomen de aproximadamente 2.5-3 cm, retirando los lóbulos medio y lateral izquierdo del hígado.

4.- ETANOLEMIAS

a) Grupos experimentales

El modelo se dividió en dos grupos de animales experimentales, dependiendo de la vía de administración del etanol. Al primer grupo se les administró etanol por sonda gástrica (IG), a una dosis de 1.5 g/Kg de peso en una solución al 40%, a diferentes tiempos de regeneración hepática (0-96 h). Al segundo grupo se le administró el etanol por inyección intraperitoneal (IP) a la misma dosis y concentración, así como a los mismos tiempos de regeneración.

Como controles se utilizaron ratas falsamente operadas (sham), a las cuales únicamente se les manipula el hígado, regresándolo a la cavidad abdominal. Utilizando en un grupo control solución salina y en otro grupo control la misma dosis de etanol.

b) Procedimiento general

Los animales se colocaron en jaulas de restricción donde se realizó una punción en la punta de la cola, de donde se tomaron muestras por tubos capilares de 50 µl cada 15 minutos las primeras 2 horas, cada 30 minutos las siguientes dos horas y cada 60 minutos hasta que el alcohol no fue detectable. Las muestras de sangre se desproteinizaron con ácido perclórico (6% p/v) y la cantidad de alcohol se determinó por medio de un sistema enzimático, con alcohol deshidrogenasa purificada, tal como lo describieron Bert y Gutmann (1974).

Al final del experimento se anestesió a la rata, utilizando pentobarbital a una dosis de 40 mg/kg por vía intraperitoneal. Se decapitaron las ratas y se recolectó la sangre para obtener suero como se describe más adelante. Se realizó una rápida operación abdominal para extraer el estómago y el intestino delgado, posteriormente el contenido de este último se transfirieron a un tubo con 5 ml de solución fisiológica, agregándole 0.5 ml de ácido perclórico (6% p/v) y posteriormente se centrifugó. La determinación de etanol se realizó de acuerdo a lo descrito por Bert y Gutmann (1974).

Las áreas bajo la curva (ABC) fueron calculadas por el método del trapezoide, desde la administración de etanol hasta el tiempo en donde no fue detectable (Rangno y cols. 1981). La cantidad de alcohol absorbido en función de tiempo (Qt) se calculó desde el tiempo t = 0 hasta t = T (donde el etanol ya no fue detectable en la sangre).

Se utilizó una cinética tipo Michaelis-Menten (Myers 1972). En donde se convierte la cantidad absorbida (Qt) en unidades de la dosis (g/Kg). Se evaluó la Q posterior a la administración de etanol intraperitonealmente (Q_{IP}). Siendo utilizado éste como parámetro en cada grupo para la obtención de Q_{IG}.

La biodisponibilidad del alcohol está representada por la proporción de la dosis intragástrica que se alcanzó en la circulación sistémica y se obtiene dividiendo la cantidad de alcohol alcanzado en la circulación sistémica de la vía intragástrica (Q_{IG}) entre la cantidad alcanzada en la circulación sistémica por la vía IP (Q_{IP}).

El primer paso metabólico (Q_{FPM}), se obtiene de la diferencia que existe entre Q_{IP} y Q_{IG} menos la cantidad de alcohol remanente encontrada en estómago (Q_{GIT}), que se determinó en cada experimento ($Q_{FPM} = Q_{IP} - Q_{IG} - Q_{GIT}$) (Roine y cols. 1991).

5.- MUESTRAS DE SUERO E HÍGADO

Al final del experimento, las ratas se decapitaron recolectando la sangre en un tubo de vacutainer con gel separador de plasma, centrifugándose éste por 10 min en centrífuga clínica. Al final se extrajo el sobrenadante que es el suero.

Las muestras de hígado fueron obtenidas para el estudio histológico y para fraccionamiento subcelular. La fracción citosólica fue obtenida por centrifugación diferencial y la pureza de la fracción fue evaluada con la actividad de la LDH (Aguilar Delfin y cols. 1996). Asimismo, la fracción es congelada a -70°C hasta su uso. Para el análisis histológico, las muestras de hígado fueron tratadas como se describe más adelante.

a) Determinación de metabolitos séricos

Los metabolitos séricos que se determinaron fueron la albúmina, la glucosa, los triacilglicéridos y la bilirrubina. Se utilizó el plasma recolectado y las determinaciones se realizaron utilizando Kits de reactivos marca Sigma (Sigma Chemical St. Louis MO) siguiendo las instrucciones del fabricante.

b) Ensayos enzimáticos en suero

Las actividades de las enzimas en suero se realizaron de acuerdo a técnicas estándares y las actividades se reportan como unidades internacionales por litro. La actividad de la alanino aminotransferasa (ALT, EC 2.6.1.2) fue determinada por la técnica de Horder y Rej (1983); la aspartato aminotransferasa (AST, EC 2.6.1.1), como lo describe Hernández Muñoz y cols. (1990b); la lactato deshidrogenasa (LDH, EC 1.1.1.27) acorde a Vassault (1983); la ornitin carbamiltransferasa (OTC, EC 2.1.3.3.) acorde a Ceriotti (1993); y la glutamato deshidrogenasa (GHD, EC 1.4.1.4) acorde con Ellis y Goldberg (1972).

c) Histología del hígado

Se utilizaron muestras hepáticas de cada grupo experimental para microscopia de luz. Cada muestra se fijó en formaldehído (10% en solución isotónica), se embebieron en parafina y se tiñeron con hematoxilina-eosina. Las muestras fueron analizadas por dos patólogos independientes, sin el conocimiento del protocolo. Los criterios para el análisis de las anormalidades morfológicas son los reportados por

Niemela y cols. (1995). Adicionalmente, el índice mitótico se cuantificó como se describe a continuación.

d) Marcadores de regeneración hepática

La regeneración hepática se evaluó a través de dos parámetros: determinación de la actividad de la timidín cinasa (TK, EC 2.7.1.21) en la fracción citosólica y por medio del índice mitótico. La actividad de la TK en la fracción citosólica se determinó según la técnica de Sauer y Wilmanns (1983) y se expresa en nmolas de [^{3}H] dTMP/min/mg. La proteína se determinó de acuerdo a la técnica de Lowry y cols. (1951), usando albúmina de suero bovino como estándar. El índice mitótico se evaluó por microscopia de luz (Olympus, CH-30) y se reportó según el número de células en mitosis en 10 campos ópticos con un objetivo de 40X. El índice mitótico se indica como sigue: + 1-3 células en mitosis; ++ 4-7 mitosis; +++, más de 7 mitosis.

6.- MICROSCOPIA ELECTRONICA

Se procesaron muestras hepáticas de cada grupo (HP, HP+EtOH-IG y HP+EtOH-IP) para el estudio en microscopia electrónica de transmisión. Las muestras se fijaron en glutaraldehído al 3% en buffer de fosfato (0.1 molas/L, pH 7.4) por 2 horas a 4°C, y se lavaron toda la noche en buffer de fosfato (0.1 M). Posteriormente, se post-fijaron en tetra oxido de osmio (OsO_4)al 1% en buffer de fosfatos por 2 horas a 4°C, se lavaron en buffer de fosfato y se deshidrataron por cambios de etanol que van del 30-100%. Las muestras fueron transferidas a óxido de propileno (2 cambios; 15 minutos cada uno), infiltradas por 24 horas en una dilución 1:1 de resina de Epon 812, como se describió por Luft (1961), e incluídas en cápsulas de BEEM que contienen resina Epon 812 para polimerizar a 60°C por 48 horas. Los cortes se recuperaron en rejillas de cobre, se tiñeron con acetato de uracilo y citrato de plomo. Posteriormente, se examinaron con un microscopio electrónico JEM 1200 EXII operado a 60 kV. Se tomaron microfotografías de áreas representativas de todos los grupos y se analizaron por 2 observadores independientes, sin conocimiento previo del tratamiento.

7.- ENSAYOS CON 4-METILPIRAZOLA

a) Procedimiento general

A ratas macho se les realizó HP como se describió anteriormente y se agruparon de la siguiente forma: (A) ratas sham que recibieron solución salina (0.9% NaCl) por

vía intraperitoneal (control); (B) ratas sham que recibieron una dosis intraperitoneal de 4-metilpirazola (25-200 mg/kg de peso); (C) ratas sujetas a HP que recibieron solución salina por vía intraperitoneal (control HP); (D) ratas HP con una dosis intraperitoneal de 4-metilpirazola (25, 50, 100 y 200 mg/kg de peso).

En todos los casos, la 4-MP fue aplicada inmediatamente. Los animales fueron sacrificados por decapitación a las 24 hr posteriores a la cirugía, utilizando como anestésico una dosis letal de pentobarbital (40 mg/kg de peso) (Morales González y cols. 1998).

b) Ensayos enzimáticos con ADH.

La actividad de la ADH fue determinada espectrofotométricamente, en la fracción citosolica, determinada como un incremento en la absorbancia a 340 nm, en un espectro Milton Roy 1001 plus. La actividad de oxidación de alcohol se midió en buffer que contenía 0.1 M glicina/NaOH, (pH 9.6), con 10 mM de etanol y 2.4 mM NAD^+ (Roine y cols. 1992). La actividad es reportada en nanomolas por minuto por miligramo de proteína.

c) Estudios farmacocinéticos, bioquímicos, fisiológicos, morfológicos y parámetros regenerativos

Los ensayos en las determinaciones farmacocinéticas (etanolemias), bioquímicas (actividad de ALT, AST y LDH); fisiológicas (bilirrubina, albúmina, triacilglicéridos y glucosa); morfológicas (microscopia de luz), y los parámetros de regeneración (índice mitótico y actividad de la TK), se realizaron según los procedimientos descritos anteriormente.

8.- ANÁLISIS ESTADÍSTICO

Los resultados se expresan como promedio ± EE. Las diferencias significativas entre los grupos se analizaron mediante la prueba t de " Student" Siendo significativo con una $p< 0.01$

ARTÍCULO 1. FARMACOCINÉTICA DEL ETANOL

El etanol es capaz de causar inhibición de la regeneración hepática inducida por hepatectomía parcial (HP) ya que disminuye la síntesis de DNA. Frank y cols. (1979), demostraron que el modelo de administración aguda de etanol por vía intragástrica (dosis de 8 gr/kg de peso) durante la regeneración hepática inducida por HP, inhibe la síntesis de DNA y esta inhibición depende del tiempo de administración (0-16 hrs pos-HP). De hecho a las 22 horas poscirugía no se ve esa inhibición. Por otra parte Pösö y Pösö (1981) al utilizar una dosis baja de etanol (3 g/kg peso) encuentran una disminución en la actividad de las enzimas ornitín descarboxilasa y tirosina aminotransferasa, lo que pone de manifiesto el efecto deletéreo del etanol sobre el metabolismo hepático durante la regeneración. Por otra parte, Yoshida y cols. (1997), al administrar etanol (0.5-1.5 gr/kg), por vía intraperitoneal inmediatamente después de la HP, demuestran que se inhiben la transcripción y la actividad de la timidilato sintasa (TS) y de la timidín cinasa (TK), las cuales son enzimas que se relacionan directamente con las síntesis del DNA; además, se encontró que este efecto inhibitorio es independiente del tiempo pos-HP. Estos datos indican que la dosis y el tiempo posquirúrgico de administración del etanol son determinantes de los efectos deletéreos del etanol sobre la regeneración hepática. En este mismo contexto, Duguay y cols. (1982) al utilizar la administración crónica de etanol y ver el efecto del tóxico en la regeneración hepática, encuentran que el efecto inhibitorio del etanol sobre la proliferación hepática está presente hasta por 72 horas después de que el etanol ya no es detectado en sangre. Por otro lado, Wands y cols. (1979) encontraron que el efecto inhibitorio del etanol sobre la regeneración hepática está en relación directa con los niveles de las etanolemias; es decir, a mayor etanolemia mayor es la inhibición de la regeneración.

Es claro que la presencia del etanol pone en serios compromisos el proceso de la regeneración hepatica inducida por la HP. Esto es debido a que al quitar parte de la masa hepática se influye en la biodisponibilidad del etanol en las ratas con HP. En el presente, estudio demostramos que el etanol presenta una biodisponibilidad y farmacocinética de dos tipos, dependiendo del tiempo de administración: la primera forma se presenta cuando el etanol es administrado a tiempos tempranos de regeneración hepática; por la vía intragástrica se observa que a tiempos de 0-3 hrs, disminuye la biodisponibilidad del etanol encontrado en sangre, lo que probablemente

se deba a un aumento en el primer paso metabólico (FPM) (Tabla 1), que explica también la disminución de la concentración máxima de alcohol en sangre, pero manteniendo el área bajo la curva (ABC) de las etanolemias similar a la del control (Figura 1). Mientras que, a las 12-24 horas que es la fase pre-replicativa, se encuentra una disminución del FPM, lo que ocasiona un aumento de la biodisponibilidad y de las concentraciones de etanol en sangre y por ende de las ABC (Tabla 1 y Figura 1). En tiempos tardíos de regeneración (48-96 hrs), se aumenta la oxidación del etanol durante la regeneración hepática, mientras que el FPM tiende a recuperarse conforme avanza el proceso regenerativo (Tabla 1 y Figura 3C). Lo anterior demuestra que el FPM aumenta a tiempo tempranos de la regeneración y, por ello, puede ser muy importante en el metabolismo inicial del etanol ya que es una barrera protectora contra los efectos adversos de éste hacia el hígado; sin embargo, este efecto es transitorio durante la regeneración hepática ya que se pierde a tiempos medios de regeneración. Nuestros datos demuestran la importancia que el estómago juega en la farmacocinética del etanol y probablemente en el efecto de inhibición que tiene éste, ya que de acuerdo a nuestros datos se observa que en el período de 12-24 horas posquirúrgicas, el FPM disminuye y provoca un aumento de la biodisponibilidad que se ve reflejada en el incremento de las etanolemias y esto probablemente ocasione mayor inhibición del proceso regenerativo. Llama la atención que a tiempos tardíos posquirúrgicos, exista un aumento del metabolismo del etanol, como se ve reflejado en el aumento sostenido de la oxidación del etanol por gramo de hígado o por gramo de peso total de la rata (Tabla 2), quedando la duda de que sí esta condición sugiere menos daño al hígado regenerante o que ya no es afectado este proceso por lo tardío de la administración del etanol.

El etanol se absorbe en el tracto gastrointestinal existiendo varios factores que influyen en este proceso, como es el vaciamiento gástrico, la edad, el género, la concentración, el ayuno, etc. Asimismo se ha demostrado por Roine y cols. (1991) y por Lim y cols. (1993), que al comparar diferentes rutas de administración de etanol (intravenosa, intraportal, intraduodenal) se encuentran mayores cantidades de alcohol en sangre en comparación con la vía intragástrica, revelando la participación del estómago en el FPM. Nuestros datos demuestran un aumento del FPM cuando se administra el etanol por la vía intragástrica y una disminución de su biodisponibilidad, para posteriormente incrementarse en relación con el aumento de la masa hepática y volver a valores normales (Tabla 1). Este mecanismo, presumiblemente de origen gástrico, parece intervenir en la farmacocinética del etanol durante la regeneración

hepática. Por lo tanto, para poder valorar más eficazmente el papel del estómago en el FPM y por ende quitar la parte de absorción del etanol y valorar la capacidad del hígado regenerante para metabolizar etanol, se administró éste a través de la vía intraperitoneal y de esta forma, se evitó el contacto del etanol con el estómago, a manera de un "by-pass". En este modelo se encontró que el tejido hepático remanente posee un aumento en su capacidad de oxidar al etanol; sin embargo, es más patente esta situación a tiempos tardíos de la regeneración hepática (48-96 horas) (Tabla 2 y Figura 2). Nuestros datos concuerdan con los reportados por Pösö y Pösö (1979), en donde también encuentran un aumento en la relación del metabolismo del etanol en tiempos tardíos de regeneración, la cual es significativamente alta a las 72 horas posquirúrgicas (Tabla 2). Estos datos sugieren que debe de existir una correlación lineal entre la eliminación del etanol y la actividad de la alcohol deshidrogenasa hepática, lo cual conlleva a un aumento en la producción de NADH. Por otra parte, los datos obtenidos por la vía intraperitoneal muestran una gran diferencia de las etanolemias a las mismas dosis dadas por vía intragástrica (Figuras 3 y 4) lo que concuerda con lo reportado por Roine (1991) y Lim (1993), demostrando de esta forma que durante la regeneración hepática existe una mayor participación del estómago en el proceso oxidativo del etanol.

Asimismo, nuestros datos demuestran que independientemente de la vía de administración del etanol, en el período de las 12-24 horas posquirúrgicas, existe aumento en las etanolemias, lo que da como consecuencia un aumento de las concentraciones máximas de etanol en sangre y de las ABC (Figuras 1 y 2). Sin embargo, en la administración intragástrica son menores estos parámetros lo que demuestra la participación del estómago en el FPM. Ya que por la vía intraperitoneal se obtuvieron las mayores etanolemias (Figura 4), esto debe de correlacionarse con alteraciones en los parámetros de regeneración hepática y de daño al órgano. Por ello, es que se debe explorar sí efectivamente la ruta intraperitoneal inhibe más la regeneración hepática que la vía intragástrica. Por otra parte es necesario realizar estudios en donde se aclare el papel de la alcohol deshidrogenasa en el metabolismo del etanol durante la regeneración hepática ya que esta enzima es el principal sistema de oxidación del etanol en el hígado.

Este trabajo de farmacocinética del etanol demostró la participación del estómago en el FPM del etanol y la gran capacidad del tejido hepático remanente para metabolizar al etanol (Tablas 1 y 2). También demostró la existencia de una comunicación interórganos (estómago-hígado), que se ve reflejada durante la

regeneración hepática. Por otra parte, abrió la expectativa de estudiar sí las concentraciones de etanol en sangre correlacionan linealmente con la inhibición de la proliferación hepática, como lo reportado por otros autores y conocer la influencia de la ruta de administración del etanol en el proceso regenerativo. Otro punto que llama la atención es estudiar el mecanismo responsable del aumento de la oxidación del etanol durante la regeneración hepática. Puesto que durante el proceso regenerativo existe un aumento sostenido de la oxidación del etanol por el hígado remanente, se debe de estudiar en primera instancia a la ADH ya que esta enzima es la encargada principal del metabolismo del etanol en el hígado y, en segundo lugar, dependiendo de los resultados obtenidos, estudiar a los otros sistemas del metabolismo del etanol. Con ello, podría aclararse sí durante el proceso regenerativo los cambios que sufre el hígado por este proceso, son vulnerables a los efectos tóxicos del etanol ya sea por vía directa o por sus metabolitos.

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Pharmacokinetics of the Ethanol Bioavailability in the Regenerating Rat Liver Induced by Partial Hepatectomy

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It is well known that a single ethanol administration is capable of inhibiting the two-thirds partial hepatectomy (PH)-induced liver regeneration (LR); nonetheless, it has not been elucidated how ethanol metabolism by the remnant liver is exerting the deleterious ethanol actions on LR. Indeed, pharmacokinetics analysis of ethanol elimination is lacking in rats subjected to PH, which might extend our understanding in the mechanisms that account for the ethanol-induced inhibition on LR after PH in the rat. Therefore, the present study is a pharmacokinetics analysis comparing intragastric and intraperitoneal administrations of ethanol to rats under PH, at several times after surgery (0 to 96 hr postsurgery). Our results show that PH rats had a much lower blood ethanol peak than sham-operated, when intragastrically administered during the first 4 hr after surgery that was transient and normalized at 6 hr post-PH. The area under the curve for blood ethanol was higher in PH animals, starting after 6 hr postsurgery and extended to the all replicative period, and returned within the control values thereafter. The quantity of ethanol absorbed after its intraperitoneal injection was essentially the same as the administered dose for all of the groups tested. Hence, ethanol bioavailability diminished due to an enhanced rate of the first-pass metabolism for ethanol in PH rats at the very early times post-PH. At later times of PH, ethanol bioavailability was practically normalized, and these effects were accompanied by a drastic increase in the liver capacity to metabolize ethanol, mainly at 48 to 96 hr after surgery, as calculated as ethanol elimination per gram of liver, as well as by total body weight. The very early changes in ethanol bioavailability in PH rats were not accounted for gastric ethanol retention in these animals. In conclusion, first-pass metabolism importantly participates in the modified ethanol bioavailability at very early times after PH, an event presumably attained to gastric catabolism of ethanol. However, the very enhanced metabolism of ethanol showed by the regenerating liver, particularly after the first 24 hr postsurgery, seems to be the main factor affecting ethanol pharmacokinetics in rats subjected to PH. The underlying mechanisms in this liver enhancement of ethanol oxidation by PH rats remains to be elucidated.

Key Words: Alcohol, First-Pass Metabolism, Cell Proliferation, Ethanol Oxidation, Alcohol Dehydrogenase.

IT IS well known that a single oral ethanol administration is capable of inhibiting the partial hepatectomy (PH)-induced liver regeneration (LR). This approach is considered an excellent model to explore factors that may alter hepatic regeneration.[1–3] Despite the advance in the knowledge of the underlying mechanism involved in inhibitory action of ethanol on LR induced by PH, it is surprising that pharmacokinetics analysis of ethanol elimination is lacking in rats subjected to PH. Hence, the elucidation of the latter issue could extend our understanding of the regulation of the process involved in LR, and the mechanisms that account for the growth inhibitory actions of this drug.

Indeed, there exists a single report where the rate of ethanol elimination in vivo was studied in PH rats, using 1.5 to 2 g/kg of ethanol (15% w/v), after ethanol clearance from the blood only during the first 3.5 hr. Herein, it was found that ethanol elimination correlated linearly with alcohol dehydrogenase (ADH) activity, which could be surprising because, in the same study, the PH rats showed higher values for ethanol removal and decreased liver ADH activity than controls.[4]

Pharmacokinetic analysis comparing intragastric and intraperitoneal administrations of ethanol to rats indicated that the more concentrated solution resulted in less alcohol reaching the systemic circulation.[5] These findings have stressed the role of the first-pass metabolism (FPM) of alcohol, assumed to be essentially due to the stomach and viewed as having a protective role against the systemic effects of ethanol by limiting its access to the circulation.[6]

Recently, we have observed that, after a single intragastric (oral) administration of concentrated alcohol solutions (5 g/kg of body weight) to PH rats, an important fraction of ethanol did not reach the systemic circulation,[7] indicating that pharmacokinetics of ethanol is majorly affected by PH. Therefore, the present study was undertaken to determine the ethanol bioavailability in animals subjected to PH, taking into account the participation of FPM, by comparing the quantity of ethanol that reaches the systemic circulation by different routes of administration. We also wished to gain some insight in the capacity of remnant liver to metabolize the absorbed ethanol, compared with the intact liver of the sham-operated rats. Results show that FPM of ethanol, presumably attained to stomach ethanol catabolism, could be an important factor influencing the amount of ethanol reaching the systemic circulation when ethanol is administered at very early times after PH rats (0 to 4 hr after surgery). Moreover, the regenerating liver showed a striking enhanced metabolism of ethanol, mainly after the

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Table 1. Effect of Ethanol Administration on the Quantity of Alcohol (Q) Reaching the Systemic Circulation After Intragastric or Intraperitoneal Injection of 1.5 g/kg of Body Weight in Sham and PH Rats, and the Amount Left in the Gastrointestinal Tract 9 hr After Intragastric Intubation

Measures values	Units	Controls (hr)	Time after PH (hr)			
		0–96	0–3	6–12	24	48–96
Q_{IP} (intragastric)	g/kg	1.47 ± 0.15	1.38 ± 0.10	1.32 ± 0.11	1.40 ± 0.13	1.44 ± 0.17
Q_{IG} (intragastric)	g/kg	0.69 ± 0.07	0.44 ± 0.04*	1.05 ± 0.14*	0.74 ± 0.08	0.93 ± 0.10
Q_{GIT} (gastrointestinal tract)	g/kg	0.41 ± 0.04	0.37 ± 0.04	0.10 ± 0.02*	0.37 ± 0.04	0.09 ± 0.02*
Derived values						
Bioavailability (Q_{IG}/Q_{IP})	ratio	0.46 ± 0.05	0.32 ± 0.03*	0.80 ± 0.09*	0.52 ± 0.05	0.57 ± 0.08
Q_{FPM} ($Q_{IP}-Q_{IG}-Q_{GIT}$)	g/kg	0.37 ± 0.02	0.57 ± 0.05*	0.17 ± 0.02*	0.29 ± 0.04	0.42 ± 0.05

Values obtained from sham-operated animals (n = 10) and PH rats (n = 5 per group) are expressed as the means ± SEM. Because there were no statistically significant differences among the absorption parameters for ethanol in the groups of very early (0–3 hr), early (6–12 hr), and later (48–96 hr) times post-PH, results were pooled and derived values calculated from the average.

* Statistical significance against controls, as described in Fig. 1

first 24 hr postsurgery, which could be considered as the major factor affecting ethanol pharmacokinetics in rats subjected to PH.

MATERIAL AND METHODS

Yeast ADH (EC 1.1.1.1), NAD+, and semicarbazide hydrochloride were purchased from Sigma Chemical Co (St. Louis, MO). Ethanol and other chemicals were of the best quality available.

Animals

Male Wistar rats, weighing 230 to 270 g, were housed under a 12-hr light/dark cycle and allowed an ad libitum consumption of a standard rat pellet chow (PMI Feeds, Inc., St. Louis, MO). Surgical two-thirds PH was performed in the morning (9 to 10 hr), under light diethyl ether anesthesia according to the technique reported by Higgins and Anderson.[8] As controls, sham-operated rats underwent laparotomy without tissue removal.

Experimental Groups

Fed animals were grouped according to their surgical status, and they received ethanol (1.5 g/kg of body weight in 40% w/v solution) either by intragastric intubation (oral administration) or by intraperitoneal injection several times after surgery (0 to 96 hr). The concentrated solution of ethanol (40%) was selected for minimizing the effect of gastric emptying. After ethanol administration, the animals were placed in restriction cages, without further access to water and food, and blood samples were collected from the wound of excised tips of the tail with 50 μl capillary pipettes. Blood sampling was scheduled every 15 min during the first 2 hr, every 30 min for the next two more hours, and every hour thereafter until 9 hr postadministration. When blood alcohol concentrations (BACs) were no longer detectable in the animals, they were anesthetized with sodium pentobarbital (40 mg/kg), their abdomens were rapidly opened, and samples of stomach and small intestine were taken for ethanol determination, as described previously.[7] All procedures were conducted in accordance with our Institutional Guide for Animal Experimentation (National University of Mexico).

Analytical Procedures

Ethanol was quantified by the enzymatic method described by Bernt and Gutman,[9] in neutralized acid extracts of blood samples, and in samples of gastric and intestinal contents.

Computational Procedures

The area under the curve (AUC) for ethanol elimination from the blood was calculated by the trapezoidal method from the beginning of ethanol administration to the time it was no longer detectable.[10] The quantity of ethanol absorbed as a function of time (Q_t) was calculated by means of an equation analogous to the Michaelis-Menten mathematical approach, as described in detail by Roine et al.[5] According to this approach, the final value of Q_t should be equivalent to the dose of ethanol administered, if all the ethanol had appeared in the systemic circulation. then, the expected value for Q after intraperitoneal administration of ethanol (Q_{IP}) should be essentially the same as the ethanol dose administered. On the other hand, Q_{IG} represents the quantity of ethanol that reaches the systemic circulation by the intragastric route. Finally, the derived values calculated in the present study, such as ethanol bioavailability (proportion of the oral dose that reaches the systemic circulation) and the rate of FPM (the difference between the quantity of ethanol absorbed by the intragastric and intraperitoneal routes), were calculated according to Ref. 5 and also indicated in Table 1. The rate of ethanol elimination by the liver was estimated by blood ethanol disappearance, after its intraperitoneal administration, and calculated per gram of liver and per 100 g of the body weight of PH and sham rats.

Statistical Analysis

Results are expressed as the mean ± SEM. The significance of the differences among groups was analyzed by two-way ANOVA and by Student's t test. Statistical significant differences ($p < 0.01$) between sham-operated and PH rats are indicated by an asterisk.

RESULTS

There were different BACs in rats at several stages of LR post-PH after intragastric administration of a single ethanol dose (1.5 g/kg of body weight). The sham-operated animals reached a pooled maximum BAC of 21.2 ± 3.9 mM (0 to 96 hr, after surgery); however, the same ethanol dose elicited a much lower peak during the first 12 hr after PH (Fig. 1A). The PH effect on maximal BAC was very transient (when ethanol was administered within 0 to 3 hr postsurgery), since starting at 6 hr after PH (34.2 ± 3.0 mM); during the replicative stage (12 to 24 hr), a significant increase in BAC was observed, which progressively returned to control values thereafter (Fig. 1A). At very early times of surgery (0 to 3 hr), PH animals showed practically the same AUC (15.8 ± 2.2 mM hr) as that found in controls (11.7 ± 1.1 mM hr; Fig. 1B); however, the loss of hepatic mass largely influenced the AUC registered in PH rats at later postsurgical stages. Animals that received ethanol during the replicative period (12 to 24 hr) showed a 3-fold increase of AUC; nonetheless, after 48 hr of surgery, the PH rats showed a decrease in the AUC elicited by intra-

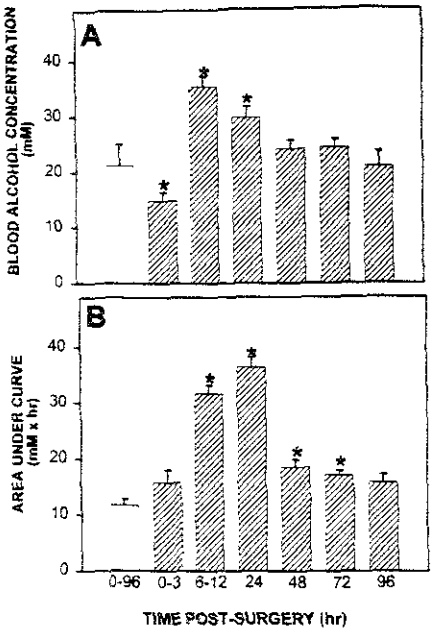

Fig. 1. Maximal blood alcohol levels and AUCs after intragastric administration of ethanol (1.5 g/kg of body weight) in sham and PH rats. (A) Maximal BACs in sham-operated rats (n = 10, white bars) and in animals subjected to PH (n = 5 per time, hatched bars). (B) AUCs obtained from the same animals. Asterisk indicates statistical significance against control. Results are expressed as the means ± SEM.

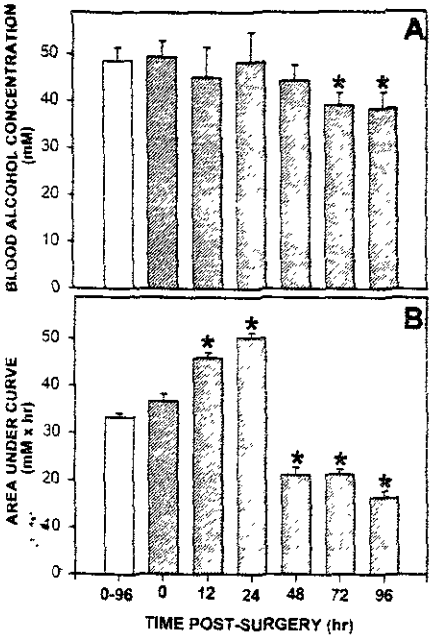

Fig. 2. Maximal blood alcohol levels and AUCs after intraperitoneal administration of ethanol (1.5 g/kg of body weight) in sham and PH rats. (A) Maximal BACs. (B) AUCs for the same number of sham-operated and PH rats indicated in Fig. 1. Symbols and statistical significance are as described in Fig. 1.

gastric ethanol administration, which almost reached the AUC found in the sham-operated rats within 72 to 96 hr after surgery (Fig. 1B).

To gain some insight on the origin and nature of this differential metabolic management of ethanol by hepatectomized animals, we administered the same ethanol dose, but by intraperitoneal route. Interestingly, the maximal BACs obtained after intraperitoneal ethanol administration in PH rats (Fig. 2A) early after surgery (0 to 48 hr; n = 5 per group) ranged from 40.3 ± 6.3 to 49.6 ± 3.4 mM and were not significantly different, compared with sham rats (BAC: 48.7 ± 2.9 mM; n = 10). However, more advanced LR (Table 2) promoted a decreased BAC in PH rats (n = 5 per group) when ethanol was administered intraperitoneally (72 hr: 39.2 ± 2.7 mM; 96 hr: 38.4 ± 3.5 mM; $p < 0.05$ vs. controls; n = 10).

The quantities of ethanol reaching the systemic circulation by the intragastric and intraperitoneal routes in sham and PH rats are presented in Figs. 3 and 4, as well as in Table 1. The quantity of absorbed ethanol after intraperitoneal injection (Q_{IP}) was essentially the same as the administered dose, thus confirming that Q_{IP} was equal to the administered dose and validating the procedure (Table 1). In contrast, the quantity of ethanol that reaches systemic circulation after intragastric administration (Q_{IG}) was clearly less than Q_{IP}. In accordance with the low BAC presented by PH rats at a very early postsurgical stage (0 to 3 hr), the Q_{IG} for ethanol was considerably lower in these animals, without significant modification of the Q_{IP} (Fig. 2A). However, the dramatic effect of early times of PH on

Table 2. Effect of PH on the Liver and Body Weight Rate of Ethanol Elimination and Its Relationship with the Gain of Liver Mass

Groups (time after PH (hr))	Liver weight (%) (g)	Ethanol oxidation (mmol/hr) Per gram of liver	Per 100 g of body weight
Controls	11.1 ± 0.8 (100)	0.57 ± 0.04	2.52 ± 0.17
Hepatectomy (00)	3.1 ± 0.2 (28)*	1.88 ± 0.13*	2.69 ± 0.18
Hepatectomy (12)	3.6 ± 0.2 (32)*	1.80 ± 0.17*	2.22 ± 0.15
Hepatectomy (24)	5.1 ± 0.3 (46)*	0.84 ± 0.07*	1.72 ± 0.12*
Hepatectomy (48)	6.6 ± 0.3 (59)*	1.28 ± 0.11*	3.39 ± 0.24*
Hepatectomy (72)	7.1 ± 0.4 (64)*	1.47 ± 0.12*	4.19 ± 0.30*
Hepatectomy (96)	8.6 ± 0.3 (77)*	1.19 ± 0.09*	4.10 ± 0.31*

Ethanol elimination rate was calculated from the data presented in Fig. 4 after the intraperitoneal administration of 1.5 g/kg of ethanol to control and PH rats. Results are expressed as the means ± SEM for at least five individual observations per group. In parentheses, the percentage of liver mass, compared with controls as a function of postsurgical time.

* Statistical significance against controls, as described in Fig. 1.

the amount of ethanol absorbed after its intragastric administration showed a frank shift to the opposite extreme when ethanol was given 6 hr after surgery and during all the replicative period (12 to 24 hr after PH), maintaining a practically unaltered value for Q_{IP} (Figs. 3B and 3C; Table 1). More advanced PH-induced LR (48 to 96 hr; Table 2) presented Q_{IG} values within the control range, without significant modifications in the intraperitoneal component (Figs. 4A to 4C).

From the data shown in Figs. 3 and 4, it was possible to calculate derived values for the pharmacokinetic analysis of ethanol disposal in sham-operated and PH rats (Table 1). The measured amount of remaining ethanol in the gastrointestinal tract (Q_{GI}), when blood ethanol was no longer detectable, corresponded to 0.41 ± 0.05 g/kg in sham-operated animals that accounted for 27% of the original

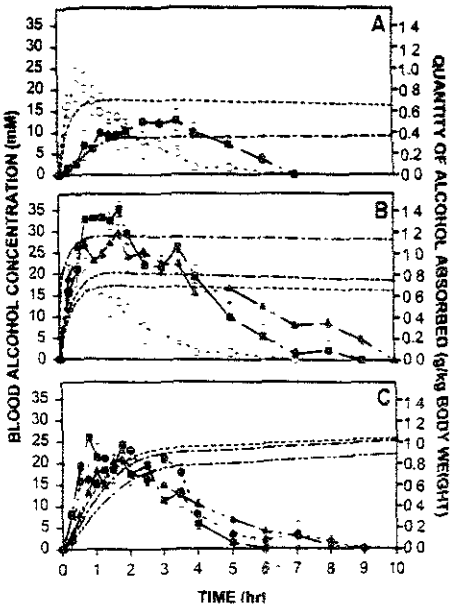

Fig. 3. Effect of ethanol administration on BACs (left scale, curves with mbols) and quantity of ethanol absorbed (right scale, curves without symbols) er intragastric (Q_{IG}) ethanol administration. (A) Sham-operated animals (open cles and dashed line for Q_{IG}) and PH rats after 0 to 3 hr of surgery (filled squares d dash-dotted line for Q_{IG}). (B) Control animals as in (A). PH rats after 6 to 12 (filled squares and upper dash-dotted line for Q_{IG}) and 24 hr after surgery (filled angles, lower dash-dotted line for Q_{IG}). (C) BACs achieved in PH rats after 48 hr ed circles), 72 hr (filled squares), and 96 hr postsurgery (filled triangles). Values Q_{IG} are indicated by the lower and upper dash-dotted lines for 48 and 72 hr er PH, respectively. Dashed line represents that found after 96 hr postsurgery e final values for Q_{IG}) are given in Table 1.

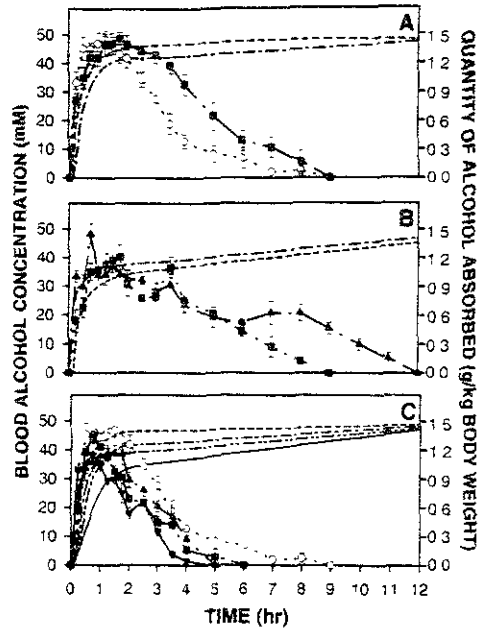

Fig. 4. Effects of intraperitoneal administration of ethanol (1.5 g/kg of body weight) in BACs (left scale) and quantity of absorbed ethanol (Q_{IP}, right scale) in sham and PH rats. (A) Q_{IP} in controls and PH rats (time 0). (B) Results obtained from 12 and 24 hr post-PH. (C) Values from controls and after 48, 72, and 96 hr post-PH. Symbols and lines are the same as in Fig. 3. Because data obtained after intraperitoneal administration of ethanol at 3 and 6 hr after PH did not statistically differ from 0 and 12 hr, respectively, those are not included in the figure. The final values for Q_{IP} are given in Table 1.

ose. This value remained unchanged during the first 3 hr fter hepatectomy, but starting at 6 hr to the replicative eriod, PH rats showed a lower Q_{GIT} that was normalized 24 hr post-PH. However, in the pooled results of PH rats fter 48 to 96 hr, this parameter reached its lowest magnide (Table 1). As expected for the effect of the PH in the osorbed quantity of gastric ethanol, the ethanol bioavailbility (Q_{IG}/Q_{IP}) was significantly diminished in PH rats at ery early times postsurgery (0 to 3 hr). This effect was ccompanied by a significant enhancement in the rate of e FPM for ethanol (Q_{FPM}) (Table 1). However, this effect as transient, because after this time, in the replicative eriod, ethanol bioavailability suddenly increased and a wer FPM was observed, which would correspond to the ght differences in ethanol disposal found between the tragastric and intraperitoneal routes in the PH rats (Fig. A; Table 1). At later times of PH, ethanol bioavailability as practically normalized, and the calculated value for PM for ethanol was progressively enhanced, remaining thin the control range at further post-PH times (48 to 96 ; Table 1).

Finally, the liver rate of ethanol elimination and the nount of removed ethanol by whole body weight was lculated in relation to the recovery of liver mass after PH able 2). Using the maximal BAC achieved and the time urse of ethanol clearance after its intraperitoneal admnration, it was clear that regenerating liver presents a very enhanced capacity for oxidizing ethanol, which allowed rats subjected to PH to maintain practically the same rate of ethanol elimination per 100 g of body weight to that found in sham-operated animals when ethanol was given during the first 12 hr after surgery, despite the obvious lack of liver mass (Table 2). At the onset of increased DNA synthesis (24 hr post-PH), PH rats showed a diminished rate of ethanol elimination due to a reduction in the liver capacity to oxidize ethanol. Thereafter, an increased liver capacity for oxidizing ethanol was again noted in animals subjected to PH, which was associated with the gain of liver mass at later times post-PH, and was reflected in a gradual enhancement of the rate of ethanol elimination in animals subjected to PH, compared with sham-operated controls (Table 2). At the completeness of LR induced by PH (7 to 8 days after surgery), ethanol pharmacokinetics was essentially the same in PH, sham-operated, and intact animals (data not shown).

DISCUSSION

The significance of ethanol-induced reduction in hepatic DNA synthesis during PH remains to be fully clarified. In liver biopsies, ethanol can also suppress mesenchymal cell replication; however, it is assumed that reduction in hepatic DNA synthesis after PH primarily decreases replication in hepatocytes.[11] In the rat, a single ethanol administration is capable of inhibiting the PH-induced LR. In the model of acutely ethanol-treated PH rats, the increase of DNA syn-

thesis was significantly inhibited when a single oral ethanol administration (up to 8 g/kg of body weight) was given immediately after surgery and at 16 hr; but, there were no differences when ethanol was given 22 hr after PH.[1] With a lower ethanol dose (3 g/kg) given 1 hr before operation, a blockade in the stimulated activities of ornithine decarboxylase and tyrosine aminotransferase was found 4 hr after PH.[3] These data indicate that both, ethanol dose and postsurgical time of its administration, are determinants of its toxic effects on LR. Indeed, the inhibitory effect of ethanol depends in part of the time of ethanol intake after PH. Multiple ethanol feedings produced even greater inhibition of DNA synthesis, which persisted for at least 48 hr after PH.[2] Moreover, chronic consumption of ethanol is associated with a significant reduction of LR for up to 72 hr after 70% PH and delayed the peak of regenerative activity by 24 hr, even in the absence of detectable blood ethanol levels.[12]

It is clear that the presence of ethanol seriously compromises the process of LR after PH; then, it is not unlikely to expect that removal of an important fraction of the liver mass is also majorly influencing ethanol bioavailability in PH rats. In fact, the present study shows that, during the first 24 hr after PH, the ethanol reaching the systemic circulation, after its intragastric administration, widely varied in PH rats (Fig. 1). Immediately or up to 3 hr after surgery, time assumed to be critical for the deleterious action of ethanol on the early response for LR, both 1.5 g/kg administered ethanol to PH rats (Fig. 1) or higher doses up to 5 g/kg,[7] elicited lower BACs than sham-operated rats. This initial effect of PH on ethanol availability was mainly due to a decreased amount of ethanol entering into the systemic circulation after its gastric administration (Fig. 3) and indeed could explain why higher ethanol doses, such as 5 g/kg[7] or 8 g/kg,[1] were not lethal for PH animals when administered immediately after surgery, even with the drastic reduction of functional liver mass.

Our results indicate that FPM can account for the lower ethanol concentration reaching the systemic circulation, after its intragastric administration in PH rats at early times postsurgery; this statement is supported by the absence of retained ethanol in the gastrointestinal tract (Table 1). Therefore, it is suggested that FPM plays an important role (transient but very effective) in ethanol pharmacokinetics in PH rats (first 3 hr after surgery). In addition, the liver capacity for oxidizing ethanol of the remnant tissue is largely increased starting 12 hr after PH (Figs. 2 and 3; Table 2), an effect that would became the most relevant factor influencing ethanol pharmacokinetics in rats subjected to PH. Several possibilities could be taken into account for explaining the differential ethanol elimination as a function of the time-course recovery of liver mass in PH rats after surgery (Table 2).

Ethanol is easily absorbed from the gastrointestinal tract, but its absorption varies according to the alcoholic beverage ingested, the rate of gastric emptying, and the amount and kind of food ingested. Hence, it has been assumed that lower levels of blood alcohol after its intragastric administration are due to the slow rate of alcohol absorption from the gastrointestinal tract, compared with that of an intravenous infusion.[13,14] In addition, more concentrated ethanol (40%) has been shown to inhibit gastric emptying in rats even in the absence of food[15]; therefore, a concentrated solution of alcohol will remain in the stomach much longer than a dilute solution exposed to undergo oxidation by the gastric mucosal ADH activity.[16] These findings (lower BACs after intragastric versus intraperitoneal ethanol administrations) have revealed the participation of gastric FPM, rather than the rate of ethanol absorption, as another important factor in controlling gastrointestinal absorption for ethanol.[17,18] Therefore, increased gastric retention of ethanol and a large increase in FPM would explain the lower BACs obtained with concentrated rather than diluted ethanol administrations. Our results agree with these statements, because PH rats showed initially decreased ethanol bioavailability, diminished Q_{IG}, and an enhanced rate of FPM (Q_{FPM}). However, after this initial period (0 to 3 hr after PH), ethanol bioavailability suddenly increased in PH rats due to a drop in the FPM, presumably of gastric origin. Moreover, AUC was greatly increased in relation to the hepatic mass lost (Tables 1 and 2). At later postsurgical times (48 to 96 hr), ethanol bioavailability returned to the control range in PH animals. This seemed to be related to a dramatic increase in liver oxidation of ethanol, as pointed out by the calculated rate of ethanol elimination after its intraperitoneal administration to hepatectomized rats, and associated with normalization of the rate of FPM in PH rats when compared with sham-operated animals (Figs. 2 and 4).

The underlying mechanism in the early adaptation of the rat to increase the FPM for ethanol in response to a loss in functional liver mass is not known. Even though gastric and hepatic participation are important for the event of the FPM of ethanol, it is our belief that the stomach is playing a major role in the rate of FPM at a very early time after PH, when hepatic ethanol elimination would still be expected to be minimal (during the first 12 hr after PH). This is shown in Fig. 3A (intragastric route) when compared with the intraperitoneal administration of ethanol (Fig. 4A).

Nonetheless, this could relate to the fact that very high gastric concentrations of ingested ethanol promote a significant gastric metabolism of ethanol, despite the K_m reported by gastric ADH in rats.[19] Indeed, the rate of gastric FPM is susceptible to be largely influenced by factors capable of decreasing the activity of gastric ADH, such as the inhibition of this enzyme by some commonly used drugs (such as aspirin) or H_2-blockers (such as cimetidine and ranitidine).[20] This would support the fact that early enhanced FPM shown by animals subjected to PH has a gastric origin, rather an important liver participation, and that loss of functional hepatic mass exerts some kind of

control on gastric management of ingested ethanol (namely Q_{FPM}). The latter could be supported by the absence of changes in ethanol pharmacokinetics when comparing sham-operated rats against intact rats receiving the same ethanol doses and administration routes (data not shown).

As for the capacity of remnant liver to oxidize ethanol in PH rats, our results clearly show that rats subjected to PH had increased liver metabolism of the administered ethanol at later times postsurgery. Our data partially agree with those reported by Pösö and Pösö,[4] where they concluded that a great variation in the rate of ethanol elimination was found at later times of LR, with ethanol disposal significantly elevated at 72 hr postsurgery (this calculation was based on grams of liver weight). Indeed, it is suggested that ethanol elimination correlates linearly with the activity of liver ADH in PH rats, which could be related to increased NADH reoxidation. The rate of ethanol elimination was limited by the activity of ADH, which is significantly inhibited in these animals.[4] In this context—despite the fact that ADH is not considered a rate-limiting factor in alcohol metabolism, unless it is dramatically diminished by chronic administration of a low protein diet—rats oxidize ethanol more slowly when it is given in a dose of 5 g/kg than a dose of 1.5 g/kg.[21] Indeed, Pösö and Pösö also found that PH groups showed a significantly higher ethanol elimination rate, compared with the controls at early hours after surgery and slightly higher at 24 hr after PH; the latter seemed to inversely correlate with the liver phosphorylation potential.[22] Our results also show that the increased liver capacity for oxidizing ethanol is still present in the proliferating liver up to 96 hr postoperatively, which is strongly reflected in the rate of ethanol elimination presented by rats subjected to PH.

Despite this, it is difficult to explain the increased liver capacity in oxidizing ethanol by PH rats, when low ADH activity in the remnant liver of these animals has been reported.[4] This opens at least two possibilities: one concerns the real activity of liver ADH in PH rats receiving ethanol, and the other is the participation, in sham and PH rats, of non-ADH pathways for ethanol oxidation. The latter could be very important, because it has been reported that acetaldehyde produced by the non-ADH pathways is degraded more slowly than that produced by the ADH pathway. This acetaldehyde could be an important factor in the development of alcoholic liver disease.[23] Initial data from our laboratory indicate that ethanol treatment to PH rats blocks the LR-induced inhibition of liver ADH activity, which could explain the increased ethanol oxidation found in PH rats previously exposed to ethanol (data not shown).

Whatever the underlying mechanism, the later increase in liver capacity to oxidize ethanol in PH rats could become a major factor influencing the ethanol pharmacokinetics in animals subjected to PH and, perhaps, also influencing the deleterious effect of ethanol on LR. Moreover, our data could suggest a coordinated adaptation of PH rats to handling administered ethanol, because when liver participation is minimal (first hour after surgery), gastric FPM emerges as a protective mechanism against a high concentration of ethanol that can reach systemic circulation despite the fact that the nature of this coordinated mechanism remains obscure. Thereafter, as soon as the remnant liver acquires a greater capacity to eliminate ethanol, the FPM for ethanol elimination would depend entirely on the liver; gastric participation is minimal, thus returning to the level found in control animals.

In conclusion, gastric FPM plays a transient, but effective, role in modifying ethanol pharmacokinetics at very early times after PH in rats, therefore constituting a realiable protective barrier against systemic effects of ethanol in these animals. However, the increased liver capacity for oxidizing ethanol present in the remnant tissue at later times post-PH emerges as the main factor influencing ethanol disposal in PH rats. It might potentiate the deleterious effect that ethanol exerts on LR. Finally, the coordinate changes in the ethanol metabolism of both the liver and stomach seem to be an interesting instance showing the interorgan communication that occurs at the onset of the harmonious liver cell proliferation triggered after PH.

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ARTÍCULO 2 EFECTOS MORFOLÓGICOS, BIOQUÍMICOS Y FISIOLÓGICOS DEL ETANOL SOBRE EL HÍGADO EN REGENERACIÓN

Es bien conocido que la administración aguda de etanol a animales sujetos a HP, reduce la síntesis de DNA del hígado, lo cual es característico de la regeneración hepática. La administración intragástrica de una dosis alta de etanol (5-8 g/kg) inmediatamente después de la cirugía prácticamente inhibe la síntesis de DNA, pero el etanol no produce inhibición de la regeneración hepática cuando se administra a las 22 horas posteriores a la cirugía (Frank y cols. 1979). Adicionalmente, evidencias recientes indican que dosis bajas de etanol (1-1.5 g/kg) administradas intraperitonealmente inhiben fuertemente la actividad de la timidín cinasa y de la timidilato sintasa en hígados remanentes de ratas sujetas a HP (Yoshida y cols. 1997). Estas enzimas están estrechamente relacionadas con la síntesis del DNA. En un reporte reciente, donde se estudió la farmacocinética de la biodisponibilidad del etanol durante la regeneración hepática inducida por HP, se encontró que el manejo del etanol por el tejido remanente depende del tiempo y de la ruta de administración (Morales-González y cols. 1998), evidencias que involucran a la ruta de administración en los efectos deletéreos del etanol sobre la regeneración hepática inducida por HP.

Cuando se remueve una porción importante de la masa hepática, esto influye significativamente en la biodisponibilidad del etanol lo cual es dependiente del tiempo y de la ruta de administración de una dosis baja de etanol durante la regeneración hepática inducida por HP, lo cual debe influir en los efectos adversos de la droga en la regeneración hepática. El presente estudio fue para explorar los efectos del tiempo y de la ruta de administración de etanol en ratas con HP, a través de parámetros morfológicos y bioquímicos.

Morfológicamente, durante la regeneración hepática se observa una acumulación transitoria y moderada de gotas de grasa, inflamación mínima y abundantes imágenes de mitosis con un pico a las 36-56 horas de regeneración hepática (Figura 2B y Tabla 1). Por otra parte, las características histológicas muestran una desorganización hepatocelular la cual puede tener dos probables interpretaciones. Algunos autores (Jordan 1964, Trotter 1964, Lane y Becker 1966) consideran estos cambios morfológicos como evidencias de actividad celular, la cual esta relacionada con la restauración de la masa hepática; por otra parte, otros autores (Aterman 1961, Fisher y Fisher 1963) describen que estos cambios morfológicos reflejan un daño al hígado como consecuencia

de la cirugía. Nosotros interpretamos estos cambios histopatológicos en razón de su asociación con el índice mitótico, ya que los cambios morfológicos que presenta el hígado están en correlación directa con los cambios en la actividad nuclear como consecuencia del proceso regenerativo (Figura 2 y Tabla 1), lo cual concuerda con los datos obtenidos por otros investigadores (Jordan 1964, Trotter 1964, Lane y Becker 1966). En cambio, cuando se administra etanol por cualesquiera de las dos rutas, el patrón de la desorganización celular se modifica al igual que el índice mitótico (Tabla 1).

La administración de etanol por vía intragástrica produce la presencia de gotas de grasa e inflamación en grado mínimo junto con una actividad mitótica discreta por parte del hígado (Figura 2C). Por otra parte, cuando se administra el etanol por vía intraperitoneal, en donde se encontraron las más altas etanolemias (Morales-González y cols. 1998), la acumulación de gotas de grasa y la inflamación fueron similares al grupo que únicamente fue HP (Figura 2D). Además, los cambios estructurales en la desorganización hepatocelular fueron más evidentes y, asimismo, un incremento en el índice mitótico en comparación con el grupo que recibió el etanol intragástricamente (Tabla 1). Estas diferencias en la magnitud de inhibición de la regeneración hepática, la cual depende de la ruta de administración, se han visto por el mantenimiento de la función hepática, que se relaciona con los niveles de etanol en sangre y no con los relacionados con daño al hígado.

El índice mitótico correlaciona con la actividad de la timidín cinasa (TK), los cuales son parámetros de proliferación celular (Figura 1 y Tabla 1). Esta enzima participa significativamente en la formación de pirimidina, catalizando la fosforilación de timidina a timidilato. Esta actividad se ha reportado que se incrementa en la fase de proliferación en las células eucariontes, incluída la regeneración hepática inducida por HP (Tsukamoto y cols. 1991). Los resultados demuestran que en los animales con solo hepatectomía parcial, se encuentra un pico de incremento de actividad de la TK a las 24 hrs de hasta 25 veces sobre el control (Figura 1). Este pico de actividad decrece conforme avanza el proceso regenerativo, manteniéndose siempre por arriba del control. Por otra parte, las ratas HP tratadas con etanol por vía intragástrica presenta una disminución del pico de actividad de la TK, que correlaciona con una disminución en la actividad mitótica (Figura 1 y Tabla 1). Cuando se administró el etanol por vía intraperitoneal, se presenta el pico de actividad disminuído y desplazado hacia las 36 horas de regeneración en lugar de las 24 hrs que son lo normal; lo cual correlaciona con

la disminución y desplazamiento temporal en la actividad mitótica de los hígados, en comparación con el grupo HP (Figura 1 y Tabla 1). Sin embargo, la actividad de TK y el índice mitótico en este grupo es mayor que el que presentó el grupo que recibió el etanol por vía intragástrica. Nuestros datos demuestran que, dependiendo del tiempo y la ruta de administración del etanol, se ven afectados diferencialmente los parámetros de regeneración, lo cual no había sido reportado previamente.

En lo que respecta a la función del hígado en estado proliferativo, los metabolitos hepáticos son buenos indicadores de los efectos deletéreos del etanol, sobre todo si éste es administrado por diferentes rutas (intragástrica e intraperitoneal). Los resultados indican que se podría diferenciar el tipo de daño al hígado dependiendo de la ruta de administración ya que se supondría que el etanol administrado intraperitonealmente debe producir mayor daño a las funciones hepáticas que aquel administrado por vía intragástrica.

Se ha reportado una relación entre la disminución de los niveles séricos de albúmina con disminución de los niveles de ATP en el hígado dañado (Jikko y cols. 1984). Los resultados señalan que el grupo HP no manifiesta un decremento significativo en los niveles séricos de albúmina, mientras que esta proteína disminuye por la presencia del etanol. Sin embargo, esta disminución en la concentración de albúmina sérica cuando el etanol se administró por vía intraperitoneal, retorna a niveles normales al final de la regeneración. En contraste, cuando el etanol es administrado por vía intragástrica, los niveles de albúmina no se recuperan durante todo el proceso regenerativo, manteniéndose siempre por debajo del grupo HP y control (Tabla 2).

Los cambios en los niveles séricos de bilirrubina en todos los grupos estudiados fueron más variables y por ello su análisis e interpretación se dificultan, ya que el patrón de concentración temporal es muy variable entre grupos. Sin embargo, las concentraciones de bilirrubina en el grupo HP con o sin etanol (independientemente de la vía de administración), mostraron un patrón de comportamiento de concentración de bilirrubina distinto del grupo control, lo cual indica un compromiso metabólico importante del hígado por el proceso regenerativo (Tabla 2).

Se ha demostrado que la administración aguda de etanol inmediatamente después a la HP, aumenta los niveles séricos de glucosa y triacilglicéridos (Gutiérrez Salinas y cols. 1996). Los resultados demuestran que la dosis administrada de etanol, independientemente de la ruta y a diversos tiempos posteriores de HP, produce un efecto semejante en relación con los niveles de glucosa (Figura 3); esto es, los niveles de

este metabolito sérico se mantienen por debajo del grupo control en todos los grupos HP con etanol (independientemente de la vía de administración) o sin él.

Los niveles séricos de triacilglicéridos son un reflejo de su utilización y/o producción en diversos tejidos, no solamente en el hígado. Es por ello que pueden tomarse como un índice general de la homeostasis del sujeto y no únicamente como reflejo de daño al hígado; si bien, este órgano es uno de los principales que los regulan (Hernández Muñoz y cols. 1978).

Se ha reportado que el incremento en los niveles de triacilglicéridos en suero es una característica constante del avance de la proliferación hepática, que recientemente se ha atribuído al incremento de la permeabilidad del hígado para los lípidos (Morsiani y cols. 1995). Nuestros datos muestran que en el grupo HP se observan gotas de grasa en el hígado (esteatosis) y movilización de grasa neutra (triacilglicéridos; hipertriacilgliceridemia) en el suero, la cual fue reducida fuertemente por la administración intragástrica de etanol. Por otra parte, la administración intraperitoneal de etanol no modifica significativamente la esteatosis hepática o la hipertriacilgliceridemia (Tablas 1 y 3).

Los efectos deletéreos del etanol sobre la regeneración hepática dependen de la ruta de administración. En este contexto, se ve que la administración del etanol por vía intragástrica da una mayor inhibición de la regeneración hepática inducida por HP. Todos estos cambios en los metabolitos séricos pueden ser un reflejo de los efectos adversos que el etanol produce en la función del hígado y consecuentemente, en los ajustes metabólicos que están involucrados en la síntesis de DNA.

La liberación de enzimas mitocondriales por el hígado se considera una evidencia fuerte de necrosis hepática y se ha asociado con formas específicas de enfermedad hepática (Frederiks y cols. 1984). Los niveles de OTC y GDH en plasma se han relacionado con varias patologías hepáticas y se usan como marcadores de daño mitocondrial en el hígado (Van Waes y Lieber 1977). En el caso de daño agudo al hígado, varias enzimas (ejem. LDH, ALT) se han encontrado que presentan un pico de actividad en forma simultánea, que no correlaciona con el pico en suero de las enzimas mitocondriales específicas (OTC y GDH) (Menache y cols 1980).

El incremento en el patrón en la actividad en suero de la LDH, ALT y OTC después de una HP en la rata ha sido reportado previamente (Sekas y cols. 1979). El incremento en suero de la actividad de estas enzimas durante el comienzo de la proliferación se ha relacionado a necrosis celular o aumento en la permeabilidad de la

membrana. Por lo tanto, durante la regeneración hepática inducida por HP, se ha concluido que la liberación de estas enzimas se relaciona con daño celular o alteración en la permeabilidad, que probablemente involucra un aumento en la síntesis y liberación de las enzimas (Menache y cols 1980). Por otra parte, al utilizar otro modelo de regeneración hepática, en el que se administra tetracloruro de carbono, existe un incremento de los niveles séricos de estas enzimas lo que sugiere que la regeneración hepática se asocia con daño celular (Dinman y Bernstein 1968).

Los resultados apoyan el hecho de que la HP induce una elevación de enzimas en suero que no se relacionan con necrosis hepatocelular y nuestros datos demuestran las primeras evidencias de que el proceso de regeneración hepática se acompaña de una liberación selectiva de enzimas, sobre todo, de predominio mitocondrial (estas tienen una elevación de 30 veces sobre el control, tal como la OTC, mientras que las de localización citosolica apenas se incrementan el doble sobre el control, tal como la LDH; esto ocurre a las 24 horas de regeneración) (Figuras 3 y 4). También se encontró una disminución en la actividad sérica de las enzimas LDH, ALT, y AST (citosolicas) (Figura 3), así como la GDH (mitocondrial)(Figura 4), las cuales se ven en relación con el progreso de la regeneración. Por otra parte, la OTC en suero declina un poco más lentamente durante el periodo de regeneración (Figura 4).

Es evidente que el etanol por sí solo no es un inductor constante de destrucción del hepatocito, sino que aparentemente incrementa la vulnerabilidad del hígado (Dugay y cols. 1982, Lieber y Rubin 1968). Nuestros datos señalan que el etanol, independientemente de la ruta de administración, no produce la esperada elevación de las enzimas séricas provenientes del hígado, como productos del daño hepático que ya habia sido reportada con la administración de tetracloruro de carbono (Dinman y Bernstein 1968). Sino al contrario, tienden a presentar una disminución brusca en la actividad sérica de estas mismas enzimas que se han considerado marcadoras de daño hepático. Todo lo anterior es aplicable a todas las enzimas determinadas, con excepción de la AST, que no es afectada por la presencia del etanol. Estos datos sugieren que existe una liberación selectiva de enzimas al principio de la regeneración hepática, que probablemente influya en el progreso de la proliferación celular. Esto es apoyado por el hecho de que el etanol es un potente inhibidor de la regeneración hepática. En nuestro estudio, existe una disminución de forma abrupta en la actividad específica en suero en animales sujetos a hepatectomía parcial, indicando que la necrosis hepatocelular no está involucrada en este proceso (Figuras 3 y 4, y Tabla 1).

En conclusión, las ratas que se les realizó HP y recibieron una dosis baja de etanol, mostraron una inhibición constante de la respuesta normal de la regeneración hepática inducida por la remoción quirúrgica de la masa hepática, lo cual es dependiente del tiempo y de la ruta de administración. La administración intragástrica de etanol produce más efectos deletéreos en la regeneración hepática que la ruta intraperitoneal y probablemente este efecto este relacionado a las diferencias en el catabolismo hepático del etanol durante la regeneración hepática, el cual varía acorde a la ruta de administración (Morales-González y cols. 1998). Por último, el presente estudio da las primeras evidencias de una liberación selectiva de enzimas por el hígado que probablemente estén involucradas en el proceso de regeneración hepática y que son posibles "blancos" de los efectos adversos del etanol, ocasionando inhibición del proceso proliferativo.

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Morphological and Biochemical Effects of a Low Ethanol Dose on Rat Liver Regeneration

Role of Route and Timing of Administration

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We have demonstrated that in rats subjected to partial hepatectomy (PH), the regenerating liver had an enhanced metabolism of ethanol, which largely depended on the route and timing of ethanol administration. Therefore, the influence of the administration route and timing for ethanol-induced deleterious effects on the regenerating rat liver was evaluated in animals subjected to 70% PH. Remnant liver showed moderate fatty infiltration, extended distortion of hepatocellular structure, and high mitotic index. Intragastric ethanol administration (1.5 g/kg body weight) considerably reduced the PH-induced changes in liver structures. Ethanol treatment also decreased liver thymidine kinase activity, serum albumin, and glucose levels. Intraperitoneal administration of the same ethanol dose to PH rats promoted lesser alterations on liver regeneration. Independently of its administration route, ethanol abruptly shortened a PH-induced selective increase in serum enzyme activities. These data suggest that the inhibitory effect of a low dose of ethanol on PH-induced liver regeneration is dependent on the timing and route of administration.

KEY WORDS: alcohol; hepatectomy; liver function; liver enzyme release, thymidine kinase activity; mitotic index.

Liver regeneration after partial hepatectomy (PH) in the rat has been widely employed as an experimental model to study mammalian cell proliferation (1). Normal liver regeneration after PH is a process dependent on protein (2, 3) and RNA synthesis (3), enhanced mitochondrial respiration (4), and the rate of ATP formation (5). These processes for metabolic adjustment to drive liver regeneration are readily altered by acute or chronic ethanol administration in the rat (6). It is well known that a single intragastric administration of ethanol (ranging from 3 to 8 g/kg of body weight) is capable of inhibiting PH-induced liver regeneration (7).

Indeed, a high ethanol dose (8 g/kg body wt) administered intragastrically to rats subjected to PH promotes a reliable inhibition of DNA synthesis (as assessed by *in vivo* incorporation of radiolabeled thymidine into nuclear DNA) and recovery of the liver mass (7). Furthermore, acute ethanol administration by gastric intubation, 4 hr after PH, significantly inhibited the activity of ornithine decarboxylase, which

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seems to be deeply involved in DNA synthesis (8). Lower ethanol dosage (3 g/kg body wt), administered 1 hr before PH also inhibited RNA and protein synthesis. These data suggest that the inhibitory effect of ethanol depends, at least in part, on the time of ethanol treatment after PH (9).

Recently, it has been shown that ethanol given to rats undergoing PH strongly decreases liver thymidylate synthase and thymidine kinase activities by repressing their expression at mRNA level. These ethanol-induced effects, which can be deeply involved in the inhibitory effect of ethanol on PH-induced liver regeneration, were achieved with low ethanol doses administered intraperitoneally (10). Therefore, the magnitude of the ethanol-induced inhibition on liver regeneration seems to be closely related to its presence in the bloodstream (11).

In this context, we have recently reported a pharmacokinetic analysis, comparing intragastric and intraperitoneal administration of a low ethanol dose (1.5 g/kg body wt) to rats subjected to PH. The data indicate that the regenerating liver has enhanced ethanol metabolism, particularly in the first 24 hr after surgery (12). These data also stressed the importance of the route and timing of ethanol administration to PH animals, for catabolizing ethanol by the proliferating liver. Hence, it can be assumed that the route and timing of ethanol administration, could have a major influence on the appearance of the deleterious effects of this hepatotoxin in the regenerative process after PH in the rat.

Therefore, the present study was aimed at comparing the liver morphological and metabolic effects of a single low dose of ethanol when administered to rats subjected to PH by two different routes: intragastrically and intraperitoneally. The data suggest that intragastric administration of ethanol results in a more pronounced inhibition of the PH-induced liver proliferation. This effect of intragastric ethanol on liver regeneration does not seem to be related to hepatocellular necrosis, but rather to a deficient metabolic adjustment of the regenerating liver in the presence of ethanol.

MATERIALS AND METHODS

Materials. [^{3}H]Thymidine (specific activity 2 Ci/mmol) was purchased from Amersham Corp. (Arlington Heights, Illinois), and enzymes and coenzymes were from Sigma Chemical Co. (St. Louis, Missouri). Other chemicals were obtained from Merck (Merck de Mexico, S.A.).

Animal Treatments. Male Wistar rats (230–270 g) were housed under a 12-hr light–dark cycle and allowed consumption of a standard rat pellet chow and water *ad libitum* before treatment. All manipulations were made according to our Institutional Guide for Animal Experimentation (National University of Mexico).

Partial hepatectomy (PH: removal of 70% of liver mass) was performed according to the technique reported by Higgins and Anderson (13), under light diethyl ether anesthesia. As controls, sham-operated rats were subjected to laparotomy without tissue removal.

After surgery, animals were grouped as follows: (A) sham-operated rats receiving intragastric or intraperitoneal administration of saline solution (0.9% NaCl); (B) sham-operated animals receiving either, intragastric or intraperitoneal ethanol administration (1.5 g/kg of body weight, as a 40% v/v, solution in water); (C) rats subjected to PH receiving saline solution by either administration route (control of PH); (D) PH animals with a single intragastric ethanol administration (1.5 g/kg of body weight); and (E) rats subjected to PH and treated with the same dose of ethanol, but through the intraperitoneal route.

Ethanol was administered at 0, 12, 24, 36, 48, 72, and 96 hr after PH, and animals were killed by decapitation 8 hr thereafter (when blood ethanol level was no longer detectable), under general anesthesia with sodium pentobarbital (40 mg/kg body wt) (12).

Serum and Liver Samples: Whole blood was collected and centrifuged at 850g for 15 min. The serum was then separated and kept frozen at −70°C until use. Liver samples were removed for histological assessment and subcellular fractionation. The cytosolic fraction was obtained by differential centrifugation, and the purity of the fraction was evaluated with marker enzyme activities (14). The cytosolic fraction was frozen at −70°C until use. For histopathological analysis, liver samples were treated as described below.

Liver Histology. Hepatic samples from each group were used for light microscopy. Samples were fixed with formaldehyde (10% in isotonic solution), embedded in wax, and stained with hematoxylin–eosin. Biopsy specimens were coded and read blindly without knowledge of the other data by independent observers at two different laboratories (C.V.R. and J.B.R.). Criteria for the analysis of morphological abnormalities were the same as those reported by Niemela et al (15): fatty infiltration (+, mild; ++, moderate, +++, severe; and ++++, very severe); inflammation (+, zonal localization, focal inflammatory cells; ++, moderate, not restricted to one zone of the acinus; +++, diffuse); and hepatocellular disorganization (+, isolated foci in zone 3 of the liver acinus; ++, more widespread, and +++, definitively diffused in the hepatic acini). In addition, the mitotic index of hepatocytes was counted as described below.

Serum Enzyme Assay. The following serum enzymes were assayed according to standard techniques and activities are reported in international units per liter. Alanine aminotransferase (ALT, EC 2.6.1.2) activity was determined according to Horder and Rej (16), and that of aspartate aminotransferase (AST, EC 2.6.1.1) as described by Hernández-Muñoz et al (17), lactate dehydrogenase (LDH, EC 1.1.1.27) according to Vassault (18), ornithine carbamyltransferase (OCT, EC 2.1.3.3) according to Ceriotti (19), and glutamate dehydrogenase (GDH, EC 1.4.1.4) according to Ellis and Goldberg (20).

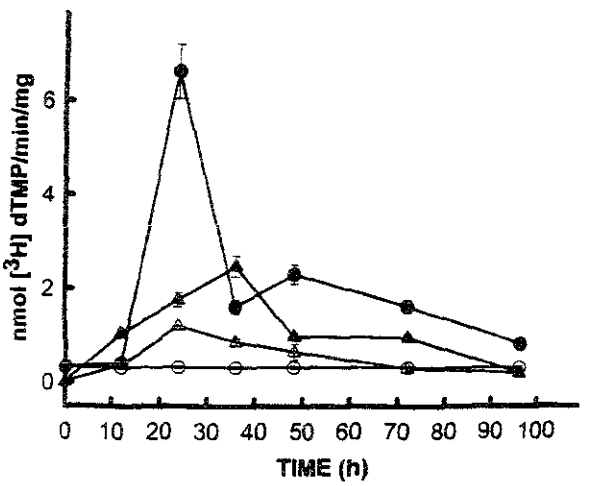

Fig 1. Effect of ethanol upon thymidine kinase (TK) activity during hepatic regeneration. TK was determined in the cytosolic fraction by a radiometric method. Each point is the mean ± SEM for 4–6 independent experiments. Sham: open circles; PH closed circles; IG, open triangles; IP: closed triangles.

Serum Metabolites Assays. Serum concentrations of glucose, triacylglycerols, bilirubin, and albumin were measured in total serum using kit assays from Sigma Diagnostics Co., (St. Louis, Missouri).

Parameters of Liver Regeneration. The rate of liver regeneration was estimated through two approaches: determination of thymidine kinase (TK; EC 2.7.1.21) activity in the cytosolic fraction and by the mitotic index. Cytosolic TK activity was determined according to Sauer and Wilmanns (21), and expressed as nanomoles of formed [^{3}H]dTMP per minute per milligram of protein. Protein was determined according to Lowry et al (22), using bovine serum albumin as standard. Mitotic index was assessed with an optical microscope (Olympus, CH-30) taking into account the number of mitotic cells in 10 microscopic fields with a 40× objective. Mitotic index is indicated as follows: −, 1–3 mitotic cells, −−, 4–7 mitotic cells; and +++, >7 mitotic cells

Statistical Analysis. Statistically significant differences among groups were assessed by variance analysis (ANOVA) and Student's t test, where $P < 0.05$ indicated significance.

RESULTS

The increase in DNA synthesis started 12 hr after PH surgery, as assessed by TK activity, and reached its peak at 24 hr after PH, followed by a lower TK activity peak at 48 hr after PH, declining significantly thereafter (Figure 1). TK activity was significantly increased in the PH group as compared to sham-operated animals at 24 hr (6.59 vs 0.32 nmol [^{3}H]dTMP min/mg; $P < 0.001$) and after 48 hr (2.29 vs 0.32; $P < 0.001$). The effects a single intragastric or intraperitoneal ethanol dose on the activity of cytosolic TK during liver regeneration are shown in Figure 1. Intragastric ethanol administration almost suppressed the PH-induced peak of TK activity at 24 hr after surgery (more than 80% inhibition). The striking enhancement of TK, induced by PH, was not found in this experimental group at any of the times tested after surgery (Figure 1). The intraperitoneal ethanol administration to PH rats reduced TK activity up to 27% of the value found in the PH group not receiving the hepatotoxin (24 hr after surgery); a significant later peak (more than seven fold over the sham-operated group) was noted in PH animals given ethanol by the intraperitoneal route (36–44 hr after PH; Figure 1). Hence, results with intragastric versus intraperitoneal ethanol administration to rats subjected to PH showed significant differences regarding cytosolic TK activity at 12, 24, 36, and 72 hr. Acute ethanol administration resulted in a significant and persistent reduction of TK activity, regardless of the administration route (Figure 1).

The results provided by the mitotic index were similar. Figure 2 shows liver micrographs of control rats (Figure 2A) and PH animals 48–54 hr after surgery with slight fatty infiltration and abundant mitotic images (Figure 2B). Ethanol administered by intragastric route (Figure 2C), did not induce fatty changes, but the rate of cell mitosis was readily depressed by the hepatotoxin. The same pattern was achieved when ethanol was administered by the intraperitoneal route (Figure 2D).

Table 1 shows the consensus of histological interpretations of the coded sequential liver biopsy specimens by two independent observers of the liver structural changes induced by the PH and ethanol administration. As suggested by the micrographs, liver cell mitosis started at 24 hr, peaked 48 hr after PH, and returned thereafter to the range found in sham-operated rats. When intragastric ethanol was administered, mitotic images were practically abolished during the first 48 hr after PH; however, at later times of liver regeneration (72–96 hr), ethanol was unable to modify the late mitotic rates (Table 1). Interestingly, when ethanol treatment was applied through the intraperitoneal route, its adverse effect on the mitotic index was lower as compared to the intragastric route. An unexpected appearance of late mitotic images (at 96 hr after surgery) was found in this group, when compared to both PH alone and PH plus intragastric ethanol administration (Table 1).

The PH-induced fatty liver began at 12–24 hr after surgery and reached its maximal amount around 72 hr after PH (Table 1). The intragastric ethanol administration at different times after surgery induced an

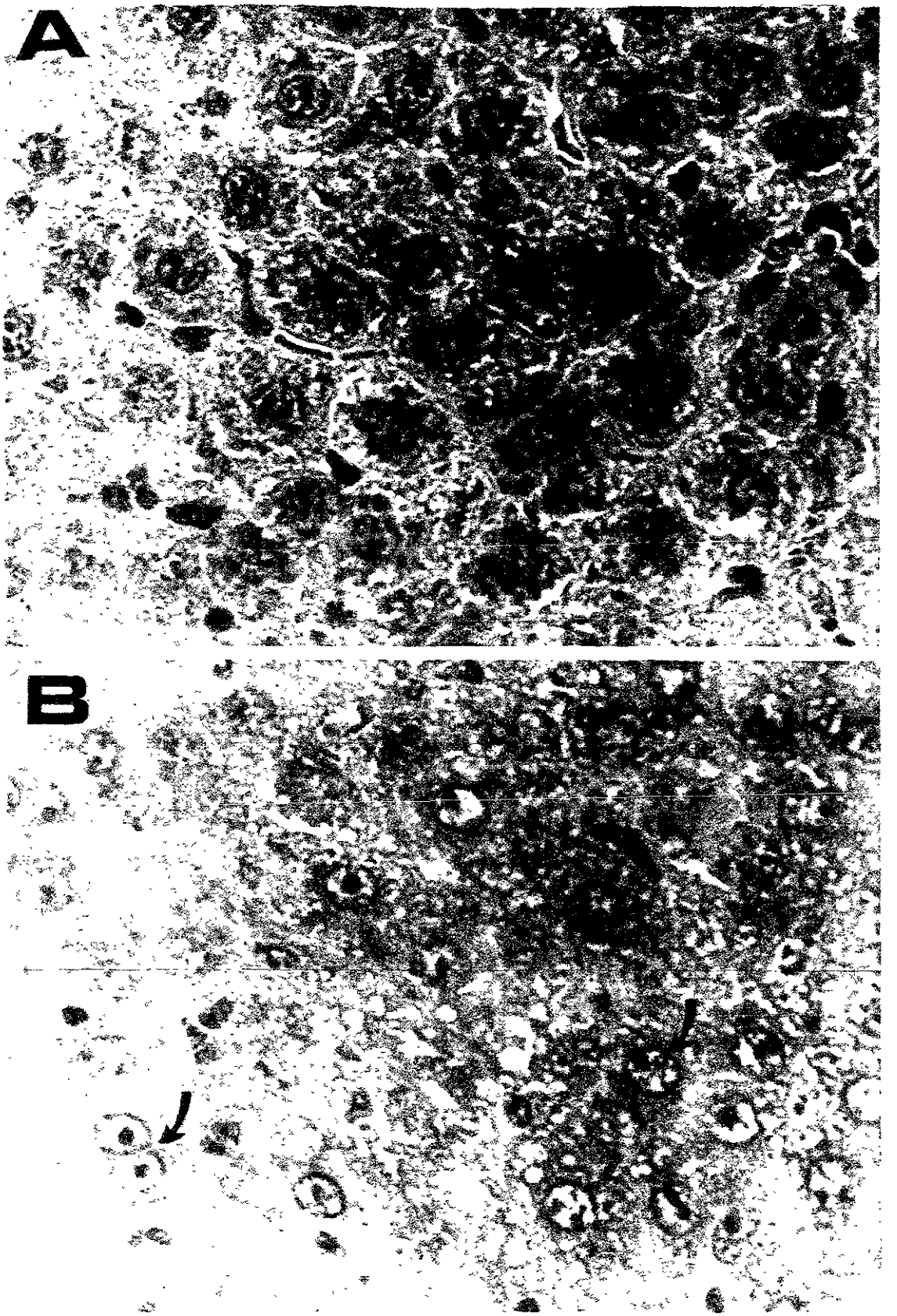

Fig 2. Histological assessment of partial hepatectomized rats treated ethanol. Liver micrographs from control rats (A); partial hepatectomized rats (B), and those from PH animals receiving intragastric (C); or intraperitoneal (D) ethanol administration. The arrows indicate the mitotic images and arrowheads denote fatty infiltration.

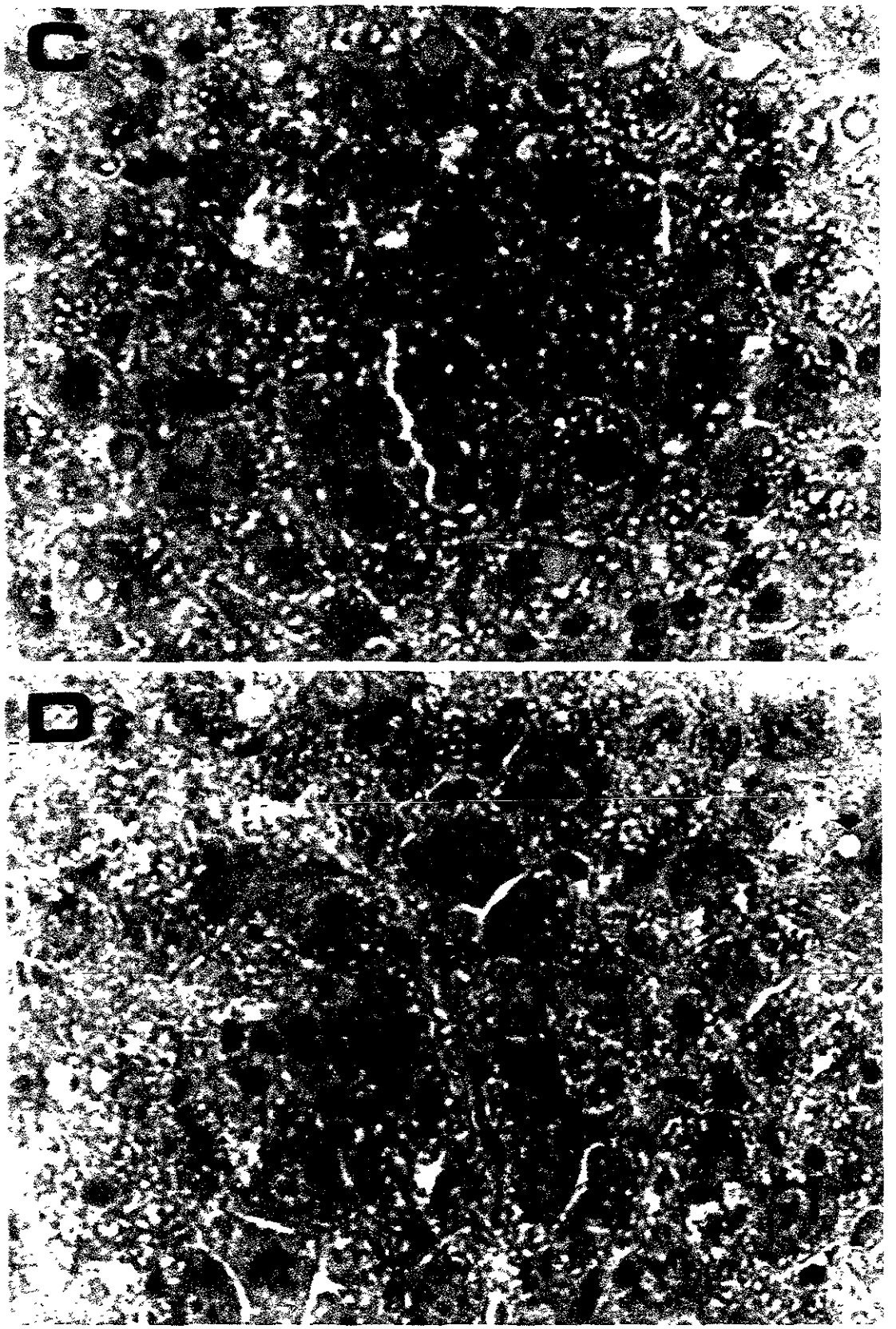

Fig 2. Continued.

TABLE 1. HISTOPATHOLOGICAL CHANGES INDUCED BY PARTIAL HEPATECTOMY AND ETHANOL TREATMENT IN LIVER CELLS*

Group	*Time (hr)*	*Fatty change*	*Inflammation*	*Hepatocellular disorganization*	*Mitotic index*
PH	0	0	0	0	0
	12	+/++	0	++/+++	0
	24	+	0	+	+/++
	36	0	0	+/++	+/++
	48	+	+	+/++	++/+++
	72	++ +++	0	+/++	+/++
	96	+/++	0	0	+
PH + EtOH-IG	0	+	+/++	+	0
	12	+/++	+/++	+	0
	24	+/++	0	+/++	0
	36	++	0	0	+
	48	+	0	+/++	+
	72	+	0	+	+/++
	96	+	0	+	++
PH + EtOH-IP	0	+ ++	+	+/++	0
	12	+ +++	+/++	++/+++	0
	24	++ +++	0	+/++	+
	36	+/++	0	+	+++
	48	+	0	+	+
	72	++	0	+	+/++
	96	+	0	+	++ +++

* Histopathological parameters were evaluated as described in Materials and Methods. For fatty change, hepatocellular disorganization and inflammation: +++, strong; ++, moderate, +, mild; 0, absent. Assessment of the mitotic index is described in Materials and Methods.

earlier accumulation, but of smaller magnitude, of neutral fat in the liver of these animals; ethanol administered by the intraperitoneal route produced the same earlier fatty liver, but at a similar magnitude to that shown by the group of rats undergoing PH without ethanol treatment (Table 1). Inflammation was practically absent in livers from animals subjected to PH; however, ethanol administration by either route elicited an early moderate inflammation, which was confined to the first 12 hr after surgery (Table 1). Finally, the hepatocellular disorganization (necrosis-like images?) was very prominent in animals subjected to PH during the first 72 hr of the regenerative process and disappeared abruptly thereafter (Table 1). Ethanol administration by both routes deeply modified this pattern, since a later times of liver regeneration, ethanol treatment readily blocked the PH-induced effects of hepatocellular organization (Table 1).

Table 2 shows the time course of changes in serum levels of albumin and bilirubin, which reflect liver function integrity, found in the experimental groups. Animals subjected to PH, without ethanol treatment, presented an initial decrease in the level of serum albumin, which was rapidly normalized along with the proliferative process. However, when ethanol was intragastrically administered to these PH rats, the serum albumin content was hardly normalized at the postsurgery times tested (Table 2). A similar situation was found in the group treated with ethanol by the intraperitoneal route, but the serum albumin level was not affected by ethanol at later times after PH (72–96 hr; Table 2).

Regarding bilirubin serum levels in PH animals, an early increase of this metabolite (12–24 hr after surgery) occurred but was rapidly normalized; this was followed by an unexpected increase at the latest post-PH time tested (96 hr; Table 2). The intragastric treatment with ethanol did not significantly modify the PH-induced changes in bilirubin serum levels, but intraperitoneal administration of ethanol actually enhanced the second serum bilirubin peak, found in PH rats (Table 2).

The time course of modifications of other serum metabolites, which depend on liver metabolism, namely glucose and triacylglycerols, were also measured in the experimental groups (Table 3). Partial liver removal induced a sustained diminution of serum glucose, which lasted up to 48 hr after surgery followed by a normal glycemia at more advanced stages of liver regeneration. When ethanol was administered by either intragastric or intraperitoneal routes, the lower glucose levels persisted at later times after surgery (Table 3). The time course of triacylglycerol serum levels in PH rats resulted in a mirror image of the fatty infiltration found in the liver

TABLE 2. EFFECTS OF HP AND ROUTES OF ETHANOL ADMINISTRATION ON SERUM ALBUMIN AND BILIRUBIN*

Metabolite	*Time (hr)*	*PH*	*PH + EtOH-IG*	*PH + EtOH-IP*
Albumin (4.76 ± 0.19 g/dl)	0	4.50 ± 0.27	4.01 ± 0.19	4.12 ± 0.35
	12	3.98 ± 0.15[a]	3.20 ± 0.34[ab]	3.25 ± 0.25[ab]
	24	4.11 ± 0.34	3.89 ± 0.38[a]	3.68 ± 0.35[a]
	36	4.17 ± 0.27	3.72 ± 0.24[a]	3.51 ± 0.25[a]
	48	4.20 ± 0.25	3.81 ± 0.15[a]	3.75 ± 0.32[a]
	72	4.14 ± 0.12	3.04 ± 0.35[ab]	3.98 ± 0.29[a]
	96	4.33 ± 0.18	3.69 ± 0.25[ab]	4.43 ± 0.14[c]
Bilirubin (0.42 ± 0.005 mg/dl)	0	0.45 ± 0.02	0.46 ± 0.06[b]	0.36 ± 0.01[ab]
	12	0.72 ± 0.04[a]	0.91 ± 0.08[a]	0.65 ± 0.07[ac]
	24	0.72 ± 0.05[a]	0.65 ± 0.08[a]	0.47 ± 0.03[bc]
	36	0.44 ± 0.08	0.58 ± 0.04[a]	0.48 ± 0.01[ac]
	48	0.43 ± 0.02	0.34 ± 0.03[ab]	0.43 ± 0.04
	72	0.29 ± 0.04[a]	0.24 ± 0.04[a]	0.75 ± 0.01[abc]
	96	0.84 ± 0.08[a]	0.64 ± 0.01[ab]	0.44 ± 0.02[bc]

* Metabolites were determined in serum according to Materials and Methods. Results are expressed as the mean ± SEM for 4–6 independent experiments. In parentheses are denoted sham group (0–96 hr) values for each metabolite. PH: partial hepatectomy; EtOH: ethanol; IG: intragastric administration; IP: intraperitoneal administration. The statistical significance was as follows: [a], $P < 0.05$ vs the sham group; [b], $P < 0.05$ vs the hepatectomized group, and [c], $P < 0.05$ vs hepatectomized + IG ethanol group.

of the same animals (Table 1): fatty liver was accompanied by lower triacylglycerol serum levels and *vice versa* (Table 3). The same pattern was found in PH rats receiving ethanol by the intragastric route, but there was an initial drop in the serum amount of triacylglycerols (first 8 hr after PH), followed by a sustained increase in the mobilization of neutral fat. When intraperitoneal ethanol was administered, drastic elevations of triacylglycerols were observed in both 36- and 72-hr post-PH groups. Independently of its administration route, ethanol promoted a more pronounced decrease of serum glucose level and a more marked enhancement of circulating triacylglycerols in PH animals (Table 3).

To correlate the PH-induced changes in liver structure with possible liver damage after surgery, besides the assessment of liver function, we also determined the serum activities of marker cytosolic enzymes, such as LDH, ALT, and AST, and of those marker liver enzymes located in mitochondria (OCT and GDH) in the experimental groups. Serum activities of ALT, AST, LDH, OCT, and GDH, as a function of time after surgery and of ethanol treatment, are depicted in Figures 3 and 4. Shortly after liver resection, an elevation of enzyme activities was noted, but the magnitude of this PH-induced effect differed according to the enzyme tested. The activities of cytosolic enzymes, namely ALT and LDH, significantly in-

TABLE 3. EFFECTS OF HP AND ROUTES OF ETHANOL ADMINISTRATION ON SERUM GLUCOSE AND TRIACYLGLYCEROLS*

Metabolite	*Time (hr)*	*PH*	*PH + EtOH-IG*	*PH + EtOH-IP*
Glucose (102.23 ± 3.4 mg/dl)	0	110.0 ± 4.5	69.73 ± 3.2[ab]	63.44 ± 4.3[ab]
	12	56.89 ± 3.8[a]	64.99 ± 0.6[a]	62.75 ± 5.2[a]
	24	82.93 ± 2.7[a]	74.48 ± 6.2[a]	78.10 ± 3.4[a]
	36	72.93 ± 4.2[a]	72.75 ± 2.9[a]	73.96 ± 5.4[a]
	48	66.89 ± 5.2	49.31 ± 4.6[ab]	64.48 ± 3.9[ac]
	72	92.06 ± 3.1	69.31 ± 3.1[ab]	74.13 ± 5.0[ab]
	96	100.5 ± 4.6	49.65 ± 0.9[ab]	46.55 ± 1.4[ab]
Triacylglycerols (56.90 ± 3.7 mg/dl)	0	59.33 ± 5.4	36.39 ± 3.7[a]	58.5 ± 2.5[bc]
	12	79.49 ± 2.7[a]	61.50 ± 4.6[b]	67.3 ± 4.3
	24	92.05 ± 3.5[a]	46.86 ± 2.8[b]	70.2 ± 2.7[abc]
	36	36.82 ± 4.7[a]	67.78 ± 5.2[b]	113.8 ± 5.3[abc]
	48	93.72 ± 3.9[a]	58.57 ± 4.5[b]	82.8 ± 4.9[ac]
	72	46.86 ± 5.3[a]	79.07 ± 2.7[ab]	157.3 ± 2.8[abc]
	96	37.65 ± 2.5[a]	62.76 ± 1.5[b]	62.7 ± 2.6[b]

* Metabolites are expressed as the mean ± SEM for at least four independent observations. Control values from the pooled sham groups (0–96 hr) are given in parentheses. Abbreviations and statistical significance as indicated in Table 2.

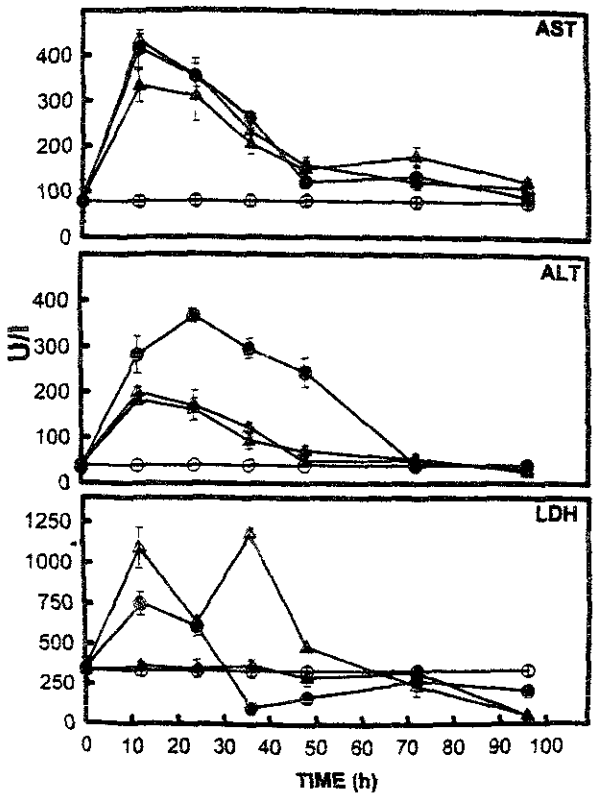

Fig 3. Specific activities of serum alanine and aspartate aminotransferases (ALT and AST) and lactate dehydrogenase (LDH) during liver regeneration and ethanol intoxication. Each point represents the mean ± SEM of 4–6 independent observations. aminotransferase; ALT: alanine aminotransferase; LDH: Lactate Dehydrogenase. Symbols as in Figure 1.

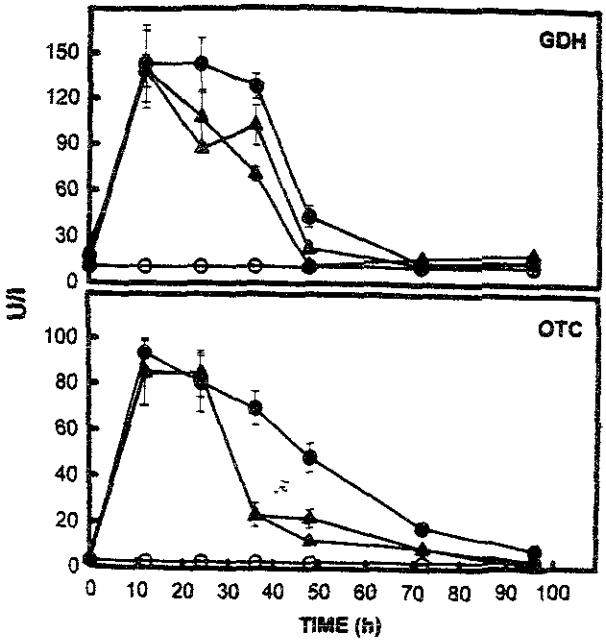

Fig 4. Specific activity of serum "mitochondrial" enzymes during liver regeneration and ethanol intoxication. Each point is the mean ± SEM of 4–6 independent experiments. GDH: glutamic dehydrogenase; OTC: ornithine transcarbamoylase. Symbols are as described in Figure 1 legend.

creased after surgery, peaking at 12–24 hr (three- to sixfold over controls) and returned to normal values thereafter (Figure 3, middle and lower panels), whereas AST, an enzyme located in both the cytosolic and mitochondrial subcellular liver compartments, also showed a maximal serum activity peak at 12–24 hr after surgery (sixfold over controls: Figure 3, upper panel). Animals subjected to PH and receiving ethanol by either intragastric or intraperitoneal routes did not show a modified PH-induced serum pattern of AST activity, but ethanol elicited an unexpected lowering of ALT activity (43–46%, as compared to the PH group), independently of its administration route during the whole proliferative period (Figure 3). The effects of the intragastric versus intraperitoneal routes of ethanol administration on serum LDH activity during liver regeneration are also shown in Figure 3 (lower panel). In the case of intragastric ethanol treatment, two peaks of increased LDH activity were noted at 12 and 36 hr followed by an abrupt drop in the serum LDH activity. On the other hand, intraperitoneal administration of ethanol to PH rats promoted a striking suppression of the PH-induced profile of serum LDH activity during the whole replicative period (Figure 3, lower panel). The effect of ethanol on PH-induced elevation of serum LDH was drastically modified depending on its route of administration.

Figure 4 shows the serum GDH and OCT activities in the treated groups. The profile of activities of these mitochondrial enzymes in serum from rats after PH was the same as that recorded for ALT, AST, and LDH (Figure 3), but the increase of serum activities for GDH and OCT was much higher (10–70 times over controls; Figure 4). This dramatic PH-induced elevation of the latter enzyme activities was greatly modified by ethanol administration: through the intragastric route, ethanol administration did significantly decrease serum GDH activity, when administered to PH rats 24 hr after surgery (Figure 4). In addition, intraperitoneal administration of the same ethanol dose, readily returned GDH activity to basal values, when administered 48 hr after PH. Serum OCT activity was also diminished earlier by intragastric ethanol administration to PH rats, while ethanol administered by the intraperitoneal route produced

minor changes in the PH-induced elevation of serum OCT activity (Figure 4).

DISCUSSION

It is well known that acute ethanol treatment of animals subjected to PH readily reduces hepatic DNA synthesis, which characterizes liver regeneration. Indeed, a single intragastric administration of a high dose of ethanol (5–8 g/kg/day) given immediately after surgery practically abolishes PH-induced DNA synthesis, but ethanol fails to produce inhibition of liver regeneration when administered 22 hr after PH (7). Lower dosage of ethanol (3 g/kg body wt, intragastrically) given to PH animals severely compromises RNA and protein syntheses, and elicits inhibition of some enzymes, such as ornithine decarboxylase and tyrosine aminotransferase (8, 9). These data indicate that both ethanol dosage and postsurgical time of administration are determinants of its toxic effect on liver regeneration.

In addition, recent evidence indicates that even lower doses of ethanol (1–1.5 g/kg body wt) through intraperitoneal administration strongly inhibit thymidylate synthase and thymidine kinase activities in the remnant liver of rats subjected to 70% PH (10). These enzyme activities, which are rate-limiting steps in the DNA synthesis, decrease due to lower protein levels by repressing the expression at the mRNA level. In a recently reported study on the pharmacokinetics of ethanol bioavailability in the PH-induced regenerating liver, we showed that ethanol management by the regenerating liver largely depends on the timing and administration route of ethanol in animals subjected to PH (12), evidencing a significant involvement of the route of administration in the deleterious effects of ethanol on the PH-induced liver regeneration.

Since removal of an important portion of liver mass significantly influences ethanol bioavailability, it could be expected that modifying the timing and route of administration of a low dose of ethanol (1.5 kg body wt) to PH animals will influence the adverse effects of this drug on liver regeneration. The present study was undertaken to explore whether a single low dose of ethanol, administered by two different routes to PH rats, has different effects on liver regeneration as assessed by morphological and metabolic parameters.

Morphologically, the regenerating liver presented transient moderate fatty accumulation, minimal inflammation, and abundant mitotic images, peaking at 36–56 hr after surgery (Figure 2 and Table 1). Furthermore, histological features showing hepatocellular disorganization, which could resemble necrosislike images were found. These early structural alterations are the subject of conflicting interpretations. Many authors have considered these morphological changes as evidence of cellular activity, linked to the restoration of the liver mass (23–25); however, early hepatic alterations have also been regarded as the result of liver damage, mainly attributed to the surgical procedure or the food restriction of the early postoperative period (26, 27). The present data agree with the former interpretation, especially regarding the high mitotic index shown only in the PH group (Figure 2 and Table 1). Indeed, ethanol administration by both routes deeply modified this pattern, since at later times of liver regeneration, ethanol treatment readily blocked the PH-induced effects of hepatocellular organization (Table 1). When ethanol was administered by the intragastric route, fatty infiltration was minimal at later times after PH, whereas inflammation was evidenced earlier; in addition, hepatic mitotic activity was readily decreased. This ethanol-induced effect was accompanied by slight modifications in hepatocellular organization, as compared to the groups of PH animals receiving only saline solution. Interestingly, in the PH group treated with intraperitoneal ethanol, where higher blood ethanol concentrations are reached (12), fatty infiltration was similar to rats with PH only, and inflammation was less prominent as compared to those animals receiving ethanol, but through the intragastric route. Changes in hepatocellular structure were earlier and more evident. An unexpected late increase of the mitotic index was found in this group, as compared with animals subjected to PH and intragastric ethanol administration (Figure 2 and Table 1). This striking difference in the magnitude of the ethanol-induced inhibition on liver regeneration, which was dependent on its route of administration, seems to be related to the maintenance of liver function, rather than the blood ethanol level, and unrelated to a possible liver damage, as discussed below.

The rate of the mitotic index correlated with the TK activity, a reliable parameter of cellular proliferation (Figure 1). This enzyme participates significantly in the pyrimidine salvage pathway, catalyzing the phosphorylation of thymidine to thymidylate. Its activity has been reported to increase in the proliferative phase of eukaryotic cells, including regenerating liver after PH (28). In the present study, animals subjected to PH only increased their liver TK activity

up to 25 times over control (at 24 hr after PH). This enzyme activity remained significantly higher compared to sham-operated rats, during all postsurgical times tested. Treatment with intragastric ethanol in these rats almost flattened the PH-induced TK peaks, which closely correlates with the slight presence of mitotic images in the liver from these animals (Figures 1 and 2). When the same ethanol dose was administered by the intraperitoneal route to PH animals, the decrease of TK activity was smaller, which could explain the late liver mitosis found in this experimental group as compared to animals subjected to PH only and those additionally receiving intragastric ethanol administration. Depending on the timing and administration route, ethanol could promote liver regeneration to some extent, instead of exerting its known inhibitory effect on this process (Figures 1 and 2, Table 1). This latter effect of ethanol has not been reported previously.

Liver function, as assessed by serum levels of metabolites deeply involved with hepatic metabolites, was also a target for the differential effect of ethanol in relationship to its administration route. A decrease in serum albumin level could be related to diminished liver ATP levels, as occurs in the injured liver (29). Although PH by itself did not significantly decrease the serum levels of albumin, this serum protein readily diminished in the presence of ethanol. However, when intraperitoneal ethanol was administered to PH rats, the decrease of serum albumin was less evident and was unaffected by intraperitoneal ethanol administration at later post-PH times, remaining essentially similar to those of sham-operated and only PH-animals (Table 2). Changes in serum bilirubin were more variable, making it difficult to analyze the ethanol effect on this parameter.

Acute ethanol administration to rats, immediately after PH, effectively enhances the magnitude and duration of the PH-induced drop of serum glucose (30). In the present study, lower ethanol doses administered at several times after PH produced the same effects, independently of its administration route (Table 3).

Acute ethanol treatment to intact rats induces fatty liver accompanied by an increased output of neutral fats (triacylglycerols), which is not a process that follows first-order kinetics. Therefore, serum levels of triacylglycerols might not only reflect liver output, but a balance between production and utilization of neutral fat (31). Fatty accumulation seems to be a constant feature linked to the progression of liver proliferation, recently attributed to an increased liver wall permeability to lipids, resulting from a 10-fold increase in the sinusoidal wall porosity of the regenerating liver (32). Our data show that PH-induced fatty liver and mobilization of neutral fat, as assessed by serum triacylglycerols, was strongly reduced by intragastric administration of ethanol, even though liver ethanol oxidation would promote the opposite effect (31). On the other hand, the intraperitoneal administration of the same dose of ethanol did not significantly modify either hepatic steatosis or hypertriglyceridemia (Tables 1 and 3).

The aforementioned agrees with the suggestion that the deleterious effect of low ethanol administration primarily depends on its administration route. The intragastric ethanol treatment would be a more reliable inhibitor of PH-induced liver regeneration, probably associated with its adverse effects on liver function and, consequently, on metabolic adjustment, which enhances DNA synthesis by the regenerating liver.

Release of mitochondrial enzymes from the liver is considered to provide strong evidence for hepatic necrosis and has also been associated with specific forms of liver disease (33). Plasma OCT and GDH may rise markedly in various forms of liver pathology, and their use as markers of mitochondrial liver damage has been advocated (34, 35). In the case of acute liver injury, several enzymes (ie, LDH and ALT) reach peak activity simultaneously, but the disappearance rate of specific mitochondrial enzymes (OCT and GDH) from plasma was, by comparison, slower than for other enzyme tested (36, 37).

Increased serum patterns of LDH, ALT, and OCT activities after 70% PH in rats have been reported previously (38, 39). The increase of these serum enzyme activities during the onset of liver proliferation could be related to cell necrosis or enhanced cell membrane permeability. Indeed, it has been concluded that increased serum activities of some enzymes after PH could be due to early release either from damaged cells or from cells with altered permeability, probably involving enhanced synthesis and release of enzymes (39, 40). In addition, in acute CCl_4-induced hepatotoxicity, another model for inducing liver proliferation, the increase of serum enzymes was distinctly separable from prior elevations associated with acute tissue damage, leading to the suggestion that liver regeneration might be somehow associated with increased serum enzyme activity (41).

Our results strongly support the statement that PH-induced elevation of serum enzymes is not related

to hepatocellular necrosis and, to our knowledge, provide the first evidence that liver regeneration is accompanied by a selective release of enzymes, predominantly of mitochondrial location (for instance, OCT activity was elevated almost 30-fold over control, as compared to LDH, which barely doubled over control activity at 24 hr after PH; Figures 3 and 4). While decay of serum activities of LDH, AST, and ALT (primarily cytoplasmic enzymes), as well as GDH, was clearly in function of the progress of regeneration, serum OCT activity declined consistently slower during the proliferative period (Figures 3 and 4).

Even if ethanol by itself does not constantly produce destruction of hepatocytes, it appears to increase the vulnerability of liver cells to additional trauma, which can include the surgical removal of an important fraction of the liver mass (11, 42). However, under our experimental conditions, ethanol administration by both routes to rats subjected to PH did not produce the expected extra elevation of liver enzymes, but rather promoted an earlier blood enzyme clearance, when administered at variable times during the proliferative period. These findings suggest that selective liver enzyme release, due to PH in the rat is an event associated to the onset of liver regeneration, probably influencing the progress of the proliferative process. This statement is supported by the fact that ethanol, proven to be a potent inhibitor of PH-induced liver regeneration in the present study, abruptly lowers liver-specific enzyme activities in serum from animals subjected to PH (Figures 3 and 4), indicating also that hepatocellular necrosis is not readily involved in this phenomenon.

In conclusion, rats undergoing PH and receiving a single low dose of ethanol showed a reliable and constant inhibition of the normal hepatic regenerative response induced by surgical removal of liver mass, which was strongly dependent on timing and route of administration. The intragastric administration of ethanol was more deleterious than the intraperitoneal route in inhibiting the regenerative process, an effect probably related to the differential hepatic ethanol catabolism of the regenerating liver, which varies according to the route of administration (12). Furthermore, the present data provide the first evidence that a selective liver enzyme release is an event deeply involved in PH-induced liver regeneration, and a possible target for the adverse effects that some drugs, such as ethanol, exert on the normal liver capacity to regenerate after surgical loss of functional liver mass.

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ARTICULO 3 EFECTOS DEL ETANOL EN LA ULTRAESTRUCTURA HEPATOCELULAR

Recientemente se demostró que una dosis baja de etanol produce una constante inhibición de la regeneración hepática inducida por remoción quirúrgica de la masa hepática. Este efecto fue estrechamente dependiente del tiempo y de la ruta de administración del etanol y se encontró que la ruta intragástrica produce más efectos deletéreos que la ruta intraperitoneal en la inhibición del proceso proliferativo. Por otra parte, la administración de etanol durante la regeneración hepática no induce necrosis hepatocelular ni incremento de las enzimas marcadoras de daño hepático (Morales-González y cols. 1999).

Es claro que las diferencias en el efecto inhibitorio del etanol al proceso regenerativo hepático, es dependiente de la ruta de administración, lo cual fue comprobado por estudio morfológico (microscopia de luz) y por la capacidad funcional del hígado (determinaciones bioquímicas).

Se ha reportado que los cambios morfológicos en las células hepáticas ocurren pocas horas después de la HP. Existen, excelentes reportes del curso temporal de la regeneración hepática inducida por HP en algunos aspectos ultraestructurales por estudio de microscopia electrónica (Virágh y Bartók, 1966; Stenger y Confer, 1966). Dentro de las principales características reportadas se incluyen modificaciones en el retículo endoplásmico (RE) y la mitocondria, disminución de glucógeno e incremento en el contenido de lípidos. Los cambios en la mitocondria y en el RE se han correlacionado con cambios drásticos en su función, incluidas alteraciones en la actividad específica de algunas enzimas (Pearson y cols. 1959; Von der Decker y Hultin, 1960). Por otra parte, se considera que en el proceso de regeneración hepática se produce un mínimo de modificaciones ultraestructurales en los compartimentos sub-celulares, como ocurre en la membrana plasmática durante las primeras 16 horas posteriores a la HP (Grisham y cols. 1975; Murray y cols. 1981).

Durante el segundo día post-operatorio (Figura 2), la estructura citoplasmática se encuentra desorganizada lo cual es seguido por una actividad de reparación y regeneración, mientras que a los 2 a 4 días, los cambios ultraestructurales en los organelos retornan a niveles normales (Figuras 4A y 5A). Los resultados obtenidos con marcaje radioactivo de proteínas y de fosfolípidos de membrana, indican que la

mitocondria tarda más tiempo en retornar a la morfología normal en comparación con otros organelos durante la regeneración hepática en la rata (Díaz Muñoz y cols. 1998).

Las principales manifestaciones de proliferación celular en el hígado normal en regeneración son: incremento perinuclear del retículo endoplásmico rugoso (REr), incremento de los depósitos de glucógeno en los hepatocitos (principalmente después de las 24 horas de la HP) (Figura 2). Además, existen cambios en el núcleo y en el nucleólo (Tabla 1), los cuales se han considerado que predicen la actividad celular con relación al aumento de la síntesis de proteínas y de la proliferación celular (Miller, 1966) y correlacionan con el incremento de la proporción de los ribosomas libres (Figura 2B), indicando un aumento en la síntesis de proteínas (Loeb y Yeung, 1978). Por otra parte, el retículo endoplásmico liso (REl) contiene las principales enzimas que metabolizan drogas las que se han sugerido que se activan para el metabolismo de xenobióticos. En contra parte, la actividad del REr indica que la célula se encuentra activa para la síntesis de proteínas (Phillips y cols. 1993).

Se ha reportado previamente que los hepatocitos modifican su actividad fisiológica y presentan alteraciones estructurales atribuibles al proceso proliferativo (Gutiérrez-Salinas y cols. 1996; Morales-González y cols. 1999). Se conoce que los cambios bioquímicos y fisiológicos que acompañan a los hepatocitos durante la proliferación, constituyen el inicio de eventos que conducen a la mitosis y a la replicación celular (Michalopoulos, 1990). La administración de etanol a animales sujetos a hepatectomía parcial inhibe la regeneración hepática, hecho que se pone en evidencia por la disminución en la síntesis de DNA y en la actividad específica de las enzimas relacionadas con el proceso de duplicación (timidín cinasa, timidilato sintasa y ornitín descarboxilasa) (Pösö y Pösö, 1980; Yoshida y cols. 1997). Los mecanismos exactos por medio de los cuales el etanol inhibe la regeneración hepática aún no están dilucidados.

Los presentes datos muestran que el etanol induce un daño ligero en el tejido regenerante, con un mínimo de cambios en el perfil normal de las estructuras subcelulares de los hepatocitos regenerantes (Figuras 1C y 1D). Particularmente, la ruta intragástrica de administración de etanol produce un mayor bloqueo a las modificaciones ultraestructurales del hígado inducidas por la HP, excepto por un incremento notable de gotas de grasa en el hígado de estos animales (Figuras 1C, 3A, 4B y 5B). Los cambios en la morfología del núcleo y del nucléolo que reflejan actividad de los mismos (por ejemplo distribución de la cromatina) y en la cantidad del REr, fueron

prácticamente ausentes en las ratas con HP que recibieron etanol intragástricamente (Tabla 1). Los hígados de estos animales no muestran signos de proliferación celular. El RE fue predominantemente liso (Figura 5B), indicando baja relación en la síntesis de proteínas en comparación con las ratas con HP no tratadas con etanol. Estos datos son significativos, considerando que la administración aguda de etanol produce cambios estructurales y bioquímicos en hígados de mamíferos (Gutiérrez-Salinas y cols. 1996; Sarphie y cols. 1997).

Por lo tanto, es claro que la administración de etanol por la ruta intragástrica induce incremento importante de los depósitos de grasa en el hígado en tiempos tardíos de regeneración (60 a 96 horas) en comparación con tiempos tempranos (Tabla 1). Los animales normalmente desarrollan esteatosis en el hígado después a la ingesta de etanol y este efecto se relaciona a cambios en el estado redox del citoplasma para reducir esta condición (Hernández-Muñoz y cols. 1978). El efecto del etanol en inducir acumulación de gotas de grasa, depende de manera importante de la actividad de la alcohol deshidrogenasa (ADH), tal como se demostró en animales que recibieron etanol y fueron tratados con 4-metilpirazola (un inhibidor específico de la ADH), donde se encontró una fuerte disminución de la grasa en el hígado (García Sáinz y col. 1980)

Por otra parte, los datos sugieren que la participación de la ADH en la oxidación de etanol no es la misma en todos los estadios de la regeneración hepática que siguen a la HP; se ha encontrado que esta enzima contribuye considerablemente al catabolismo del etanol a tiempos tardíos de regeneración, como previamente reportamos (Morales-González y cols. 1998; Gutiérrez-Salinas y cols. 1999).

Adicionalmente, la administración de etanol por la ruta intraperitoneal en ratas en regeneración hepática ocasiona ligeros cambios ultraestructurales que son inducidos por la HP durante el proceso proliferativo. Interesantemente, una dosis baja de etanol por la ruta intraperitoneal aplicada a tiempos tardíos de regeneración hepática induce una activación de la proliferación de los hepatocitos, en vez de la inhibición de este proceso (Figuras 4C, 4D, 5C y 5D, y Tabla 1). Datos que apoyan fuertemente este hallazgo se han reportado previamente (Morales-González y cols. 1999). Nosotros demostramos que, dependiendo de las condiciones, el etanol puede estimular más que inhibir la regeneración hepática.

Dependiendo de la ruta de administración, el etanol induce cambios en la organización ultraestructural del hígado regenerante que no habían sido descritos previamente. Los mecanismos involucrados en el efecto de bloqueo del etanol en la

progresión de la regeneración hepática inducida por HP son desconocidos. Nuestros datos sugieren que el etanol promueve un "desacoplamiento" de los inductores que responden a la proliferación en el hígado remanente, ya que la organización subcelular de los hepatocitos únicamente presenta cambios menores posteriores a la HP, en presencia de etanol. Por otra parte, la administración de etanol principalmente por ruta intragástrica, ocasiona un retorno de las células del hígado a un estado basal (estado de reposo), lo que hace suponer que el estímulo ocasionado por la cirugía disminuye, ocasionando inhibición del crecimiento de la masa hepática.

En conclusión, la administración de dosis bajas de etanol en ratas sometidas a HP ocasionan diferentes modificaciones en la ultraestructura del hígado regenerante, dependiendo fuertemente de la ruta de administración. La administración intragástrica de etanol disminuye prácticamente todos los cambios adaptativos de los hepatocitos regenerantes. La administración intraperitoneal de etanol promueve modificaciones ultraestructurales indicativas de proliferación celular.

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Effects of Ethanol Administration on Hepatocellular Ultrastructure of Regenerating Liver Induced by Partial Hepatectomy

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Acute ethanol administration partially inhibits DNA and protein syntheses during liver regeneration (LR) induced by partial hepatectomy (PH) in rats. Previous findings that the magnitude of ethanol's deleterious effects on LR are related to the route and timing of its administration led us to perform studies at the ultrastructural level, comparing ethanol effects on PH-induced LR, as a consequence of its administration route. PH promoted alterations on the endoplasmic reticulum and mitochondria, accompanied by decreased glycogen and increased lipid content in cytoplasm. Structural nuclear and nucleolar activities were also evident. Intragastric ethanol administration practically abolished the adaptative changes found in PH-promoted regenerating hepatocytes, whereas its administration through the intraperitoneal route induced later ultrastructural modifications, indicating cellular proliferation. These results suggest that ethanol, under certain conditions, could stimulate liver proliferation triggered by PH. The mechanism underlying this surprising effect of ethanol on LR remains to be elucidated. However, it is suggested that an altered ethanol metabolism by rats subjected to PH could be involved.

KEY WORDS: alcohol; nuclear activity; nucleolus; cell proliferation; ethanol metabolism.

Liver regeneration (LR) after partial hepatectomy (PH) is an useful experimental model to investigate the regulatory mechanisms of cellular proliferation *in vivo*. LR is a common mechanism which a patient recovers from liver injury due to trauma, infections, or hepatotoxins. This phenomenon has long been recognized (1) and is the subject of diverse biochemical, histochemical, and morphological studies (2–4).

The effects of ethanol on liver regeneration have been reported by several groups (5–8). We have demonstrated that ethanol can produce alterations in the PH-induced LR in the rat (3, 8). These alterations affect some physiological processes, such as levels of serum metabolites (glucose, triacylglycerols, albumin, and bilirubin); modifications in the serum activity of enzymes that reflect integrity of the liver (alanine and aspartate aminotrasferases, lactate dehydrogenase,

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ornithine carbamyltransferase, and glutamate dehydrogenase) (3, 8). Furthermore, ethanol administration to rats with regenerating liver inhibits DNA synthesis and the specific activity of enzymes closely related with this process (eg, thymidine kinase), and it decreases the mitotic index. The magnitude of these alterations is related to the route and timing of ethanol administration. Results showed that the gastrointestinal route of ethanol administration elicited more damage than the intraperitoneal route. These alterations involve physiological and structural disturbances, evidenced by morphological changes produced by ethanol administration, which were noted along the overall process of LR.

Ultrastructural studies in liver parenchymal cells, at the time of active cellular proliferation, have revealed changes in nuclei and in the smooth endoplasmic reticulum (SER) (9, 10). However, there are no reports of the temporal changes in the ultrastructural organization of the regenerating hepatocyte after acute ethanol administration and the influence of the timing and route of its administration.

The aim of the present study was to evaluate the changes produced in some intracellular components (by electron microscopy) of the hepatocyte during LR, and those in the presence of a low dose of ethanol given by two routes: intraperitoneal and intragastric.

MATERIALS AND METHODS

Animals. Male Wistar rats (230–270 g) were housed under a 12-hr light–dark cycle and allowed standard rat pellet chow (Purina de México SA) and water *ad libitum*, before treatment. All manipulations were made according to our Institutional Research Guide Criteria for Animal Care (National University of Mexico). Partial hepatectomy (PH; 70% removal of liver mass) was performed in the morning, according to that reported by Higgins and Andersen (1), under light diethyl ether anesthesia. As controls, sham-operated rats were subjected to surgery without tissue removal.

After surgery, animals were grouped as follows: (A) Sham-operated rats receiving intragastric or intraperitoneal administration of saline solution (0.9% NaCl); (B) rats subjected to PH receiving saline solution by either administration route (control of PH); (C) PH animals receiving a single intragastric ethanol administration (1.5 g/kg body wt) and, (D) PH rats treated with the same dose of ethanol, but through the intraperitoneal route.

Ethanol was administered at 0, 12, 24, 36, 48, 72, and 96 hr after PH, and animals were killed 8 hr thereafter (when blood ethanol level was no longer detectable) (4), by a lethal dose of anesthesia with sodium pentobarbital.

Liver Histology. Hepatic samples from each group were used for electron microscopy. Samples were fixed in 3% glutaraldehyde in 0.1 mol/liter phosphate buffer (pH 7.4) for 2 hr at 4°C, and then washed overnight with 0.1 M phosphate buffer containing 0.25 mol/liter sucrose. The samples were fixed in 1% OsO_4 in 0.1 mol/liter phosphate buffer (pH 7.4) for 2 hr at 4°C. The fixed samples were washed eight times (15 min each) with 0.1 mol/liter phosphate buffer (pH 7.4), containing 0.25 mol/liter sucrose and dehydrated with absolute ethanol. The samples were transferred to propylen oxide (two changes; 15 min each), infiltrated for 24 hr with a 1:1 dilution of complete Epon 812 resin, as described by Luft (11), and then transferred to BEEM capsules containing fresh Epon 812 resin for polymerization at 60°C for 35 hr. Thin sections were recovered on 200 mesh copper grids, stained with uranyl acetate and lead citrate, and examined with a JEM 1200 EXII electron microscope operated at 60 kV. Photomicrograph material was taken from representative areas in all groups tested. This photomicrographic material was coded and read blindly by two independent observers without knowledge of the applied treatments.

RESULTS

Parenchymal liver cells in sham-operated animals exhibited no significant morphological abnormalities. Abundant organelles were present at the periphery of a central nucleus surrounded by a double-layered membrane and containing a single nucleolus. Numerous glycogen depots were evenly distributed throughout the cytoplasm, usually accompanied by a few fat droplets. No alterations were oberved in any cytoplasmic structure (Figure 1A). However, the normal structure of the hepatocytes was substantially modified at the onset of PH-induced LR, and after ethanol treatment in both sham-operated and PH-treated animals. F1

Sham-operated rats receiving ethanol by either the intragastric or intraperitoneal routes did not show any significant ultrastructural change, except for an evident fatty accumulation in their liver cytoplasm (data not shown).

Rats subjected to PH showed no significant changes in hepatocyte architecture during the first 12 hr after surgery (Figure 1B). However, in samples from regenerating liver taken after 2 to 24 hr of PH, we found enlarged mitochondria, a slight increase in fat droplets, and a predominance of SER. At this stage, nuclear organization was unchanged (Figure 1C). F1

Ethanol administration immediately after PH only promoted fatty liver, quite similar to that induced in sham-operated animals. This effect was independent of the administration route. However, we noted a higher increase of liver fatty accumulation and predominance of SER, when animals subjected to PH-induced LR were treated with ethanol 12 hr after

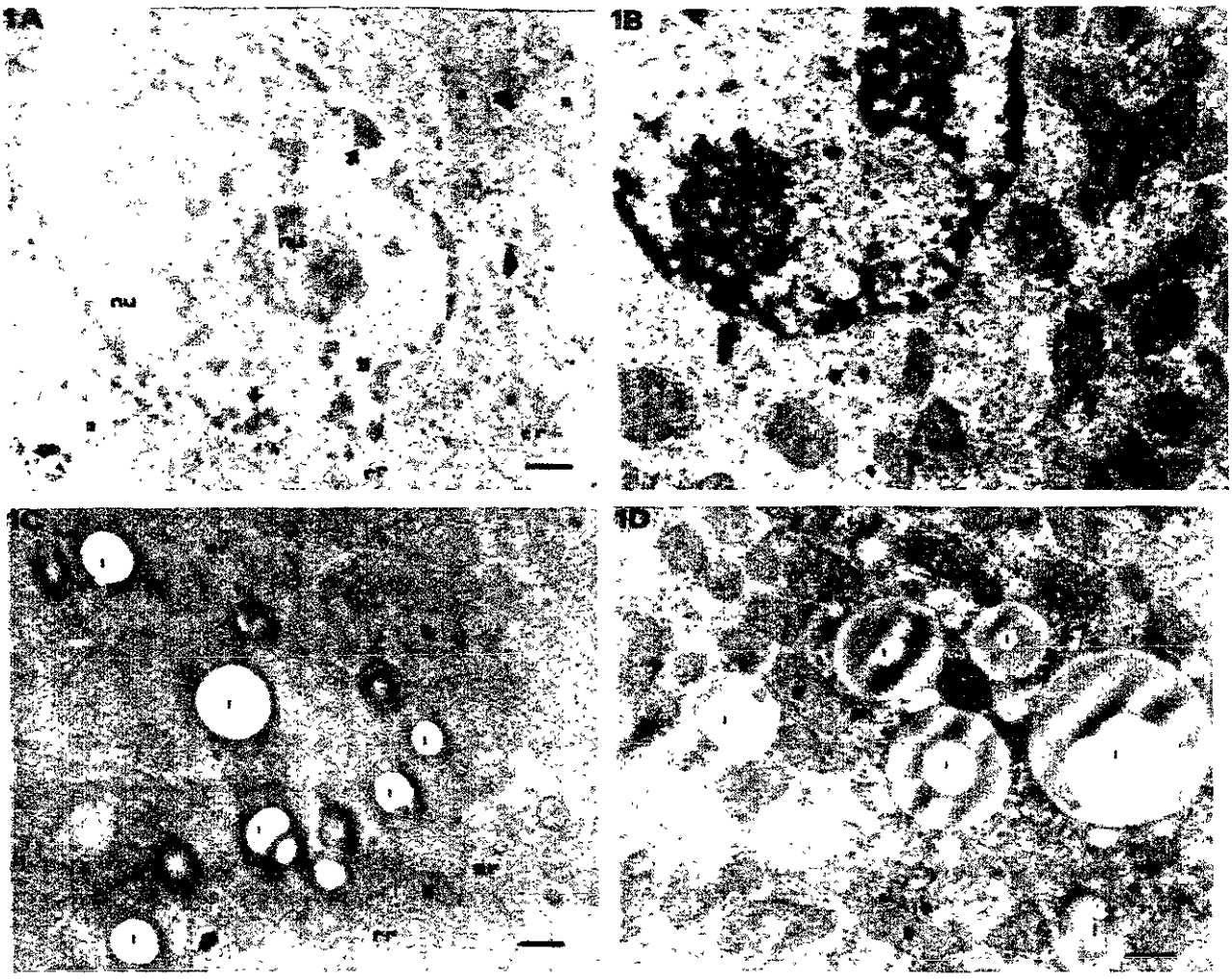

Fig 1. Electron micrographs of hepatocytes from rats at 12 hr of LR and treated with ethanol. (A) normal hepatocyte from sham-operated animals: nuclei with condensed chromatin (arrows); normal nucleolus (nu) without signs of activity. Glycogen deposits (arrowheads) were present in both mitochondria (m) and rough endoplasmic reticulum (rr). (B and C) Micrographs corresponding to proliferating hepatocytes after 12 hr of LR. (B) No nuclear activity was found, and only minor mitochondrial modifications are noted. (C) Lipid doplets (l) and a homogeneus distribution of both smooth endoplasmic reticulum (sr) and rough endoplasmic reticulum are shown. (D) Hepatocytes from rats 12 hr after PH and receiving intragastic ethanol administration. Here, lipid droplets were more abundant and mitochondria had reduced numbers and smaller cristae. No changes were recorded in the nuclei and nucleoli. Magnification: A, ×5000; B–D, ×7500. Bar represents 1 μm.

surgery. At this stage, the ethanol effects were more evident after intragastric administration (Figure 1D).

When livers were examined 24 to 32 hr after PH, many hepatic structural modifications were recorded, consequent to the regenerating process. Nuclei were enlarged and irregular in outline, displaying many pores in the double perinuclear envelope. Chromatin masses were prominent and occasional large clumps of chromosomal material were irregularly distributed in the nuclei. Nucleoli were also enlarged and increased in number; several were located near the inner perinuclear membrane (Figure 2A). In these hepatocytes, rough endoplasmic reticulum (RER) was disorganized and reduced in amount. Most parallel tubules and cisternae of RER were broken into fragments and replaced by variably dilated vesicles containing electron-dense substance. Membranes of dilated RER were degranulated with partial or complete detachment of ribosomes, and a large number of free ribosomes was observed. Components of the Golgi complex were dilated and occasionally disorganized, and numerous SER vesicles were interspersed with groups of free ribosomes in the immediate vicinity of the Golgi complexes (Figure 2B).

At this time after PH, most mitochondria were swollen, rounded, or exhibited an irregular configu-

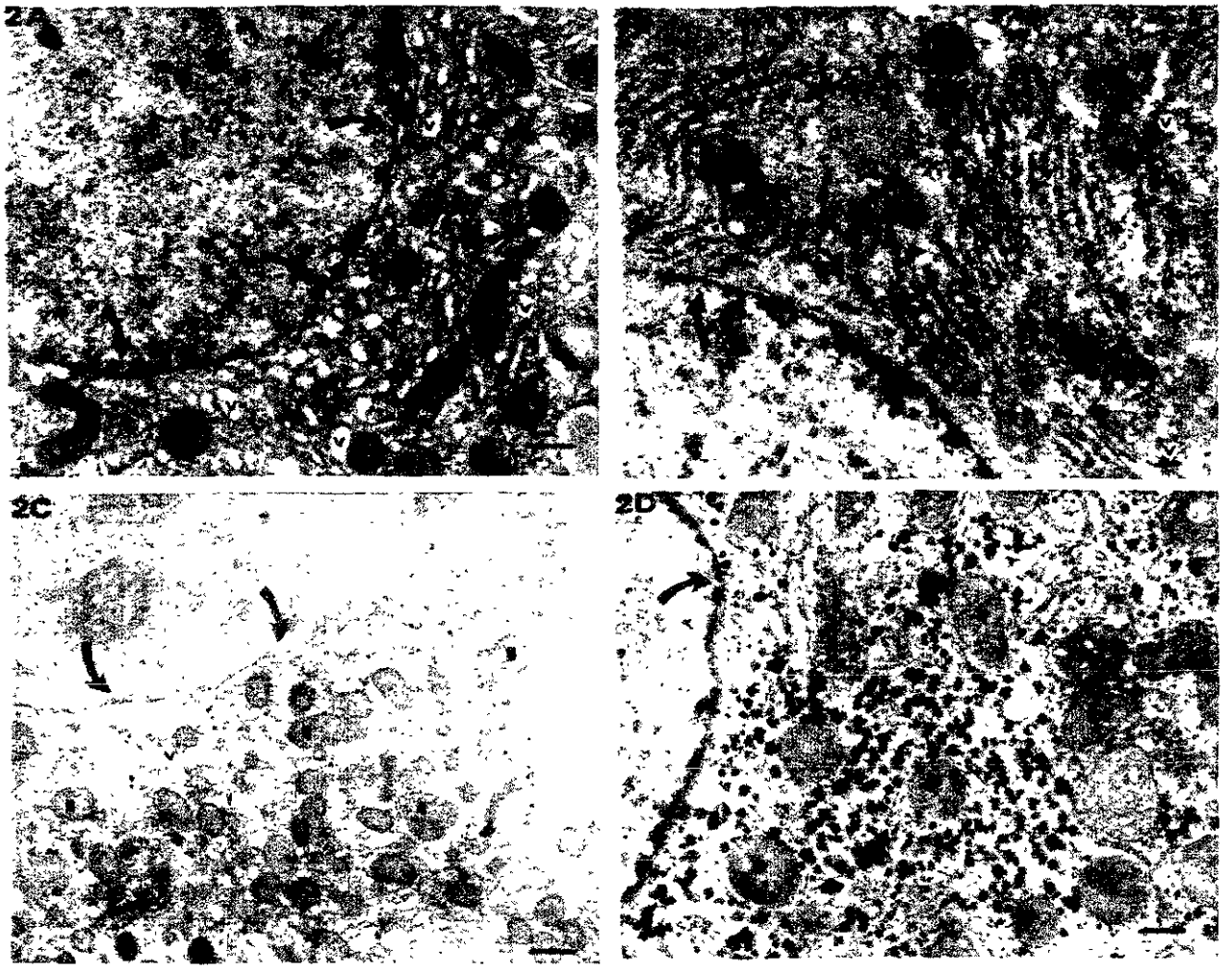

Fig 2. Electron micrographs of hepatocytes from rats at 24–36 hr of LR. (A) Nuclear condensed chromatin was largely diminished (large arrows) as compared with condensed chromatin (arrows). Cytosolic vesicles (v) of moderate density were observed. (B) Besides the diminution of nuclear condensed chromatin, the rough endoplasmic reticulum was more abundant and distributed in stratified layers. Micrograph C shows the integrity of the nuclear membrane and a normal nucleolus. Mitochondria were present in large numbers and were mostly enlarged. (D) There were abundant cytoplasmic glycogen deposits (arrowheads) in the cytoplasmic area. Magnification: A and D, ×7500; B, ×10,000; and C, ×5000. A, C, D: bar = 1 μm; B, bar = 500 nm.

ration. They seemed to be enlarged, while their cristae and intramitochondrial granules were reduced in number. Modifications of the hepatocellular plasma membrane were also noted (Figure 2C). In the cytoplasmic compartment, glycogen was almost entirely lost, and only a few scattered glycogen granules were observed among tubules and vesicles of the endoplasmic reticulum (ER). The hepatic cells also contained numerous lipid bodies (but less than at earlier surgical times) that did not appear to be membrane-limited. Lipid droplets were associated with myelin-like figures and closely related to mitochondria (Figure 2D).

The intragastric administration of ethanol to PH animals 24 hr after surgery induced a bigger fatty accumulation in the regenerating liver, whereas the amount of glycogen was practically unaffected by the hepatotoxin. Interestingly, the PH-induced changes on the distribution of the chromatin mass, as well as on the morphology and sizes of nuclei and nucleoli, were largely blocked by treatment with ethanol. In addition, the LR-promoted modifications of the relation between RER and SER were also strongly diminished by the hepatotoxin (Figure 3A). When ethanol was administered by the intraperitoneal route to these rats, the same effects were recorded but were of a lesser magnitude (Figure 3B). F3

During the 36- to 60-hr interval after PH, nuclei of the regenerating parenchymal cells continued displaying their characteristic alterations. Reorganization of

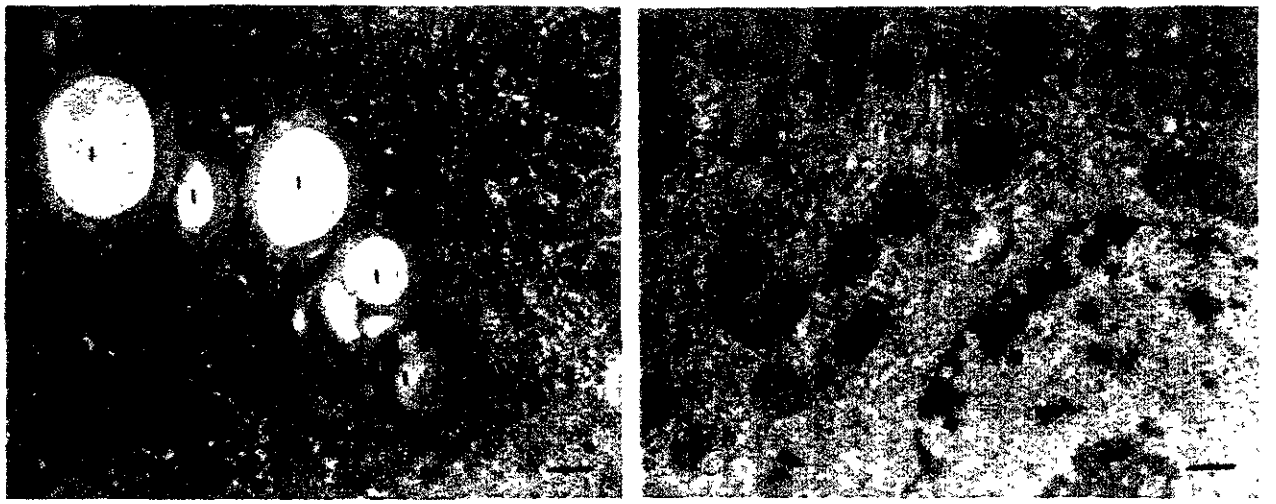

Fig 3. Electron micrographs of hepatocytes from rats at 24–36 hr of LR and treated with ethanol. (A) Regenerating hepatocytes after intragastric ethanol administration. Lipid droplets (l) were very abundant and larger.?? rough and smooth endoplasmic reticulum and glycogen were absent, and no nuclear activity was detected. (B) proliferating hepatocytes after intraperitoneal ethanol administration. Again, no nuclear activity was denoted, as assessed by the presence of condensed chromatin (arrows) and the nucleolus was unchanged. Both electron microscopy patterns revealed no proliferative activity in the presence of ethanol administered by either intragastric or intraperitoneal routes. Magnification of micrographs: ×7500; bar = 1 μm.

the RER was noted around hypertrophic Golgi zones, and free ribosomes appeared to be largely confined to paranuclear locations, while SER was still scanty during this period. Mitochondria gradually returned to normal size and structure, and cytoplasmic glycogen deposits started to increase within the cell. In addition, lipid droplets were sparse, but larger in number when compared with control livers (Figure 4A).

At this stage of LR (36–60 hr), ethanol administration through the intragastric route promoted accentuated fatty accumulation, whereas glycogen was practically absent. Nuclear and chromatin modifications were not observed in PH animals receiving ethanol intragastrically, and RER appeared organized, with a predominance of SER, quite similar to that found in livers from sham-operated rats (Figure 4B). Intraperitoneal administration of the same ethanol dose to animals subjected to PH (36–60 hr after surgery), did not reproduce the inhibitory effect of intragastric ethanol on LR. Surprisingly, these rats did show diminution of fat droplets, an increase in granules of glycogen, and changes in nuclear activity. For instance, chromatin mass was less dense, starting to display prominent chromosomal clumps (mainly after 60 hr post-PH). RER began to fragment, with few free ribosomes around Golgi complexes. In addtion, it was evident that mitochondria increased in number, which did not occur when PH animals were treated with the same dose of ethan, but by the intragastric route (Figure 4C and D).

Beyond the first 72-hr after PH, morphological alterations gradually ceased in the regenerating liver. Cytoplasmic structure resumed its normal appearance and organelles were restored. The RER swelling and fragmentation was gradually reduced, since dilated vesicles became narrow and formed parallel rows. The number of glycogen granules increased in the membrane network, and the SER frequently increased in predominance; fat content became similar to that found in control livers. In addition, nuclear activity was clearly diminished, as reflected by chomatin condensation and by a reduction in nucleolus size (Figure 5A). FS

Animals subjected to PH (72–96 hr) and receiving ethanol by the intragastric route had abundant fat deposits in the cytoplasmic compartment, while no other modifications in the organelle's structure were recorded. Indeed, activities of nuclear and RER were completely absent (Figure 5B). When the same dose of ethanol was administered intraperitoneally to PH rats at this stage (72–96 hr), changes in chromatin were more evident, nucleoli were enlarged, and there was definitive RER activity predominating over the SER (Figure 5C and D).

The time course of ultrastructural changes in parenchymal cells, which followed at the onset of PH-induced LR, is summarized in Table 1. Also shown is TI the differential effect of ethanol on structural features characteristic of LR that were directly dependent on its administration route *in vivo*.

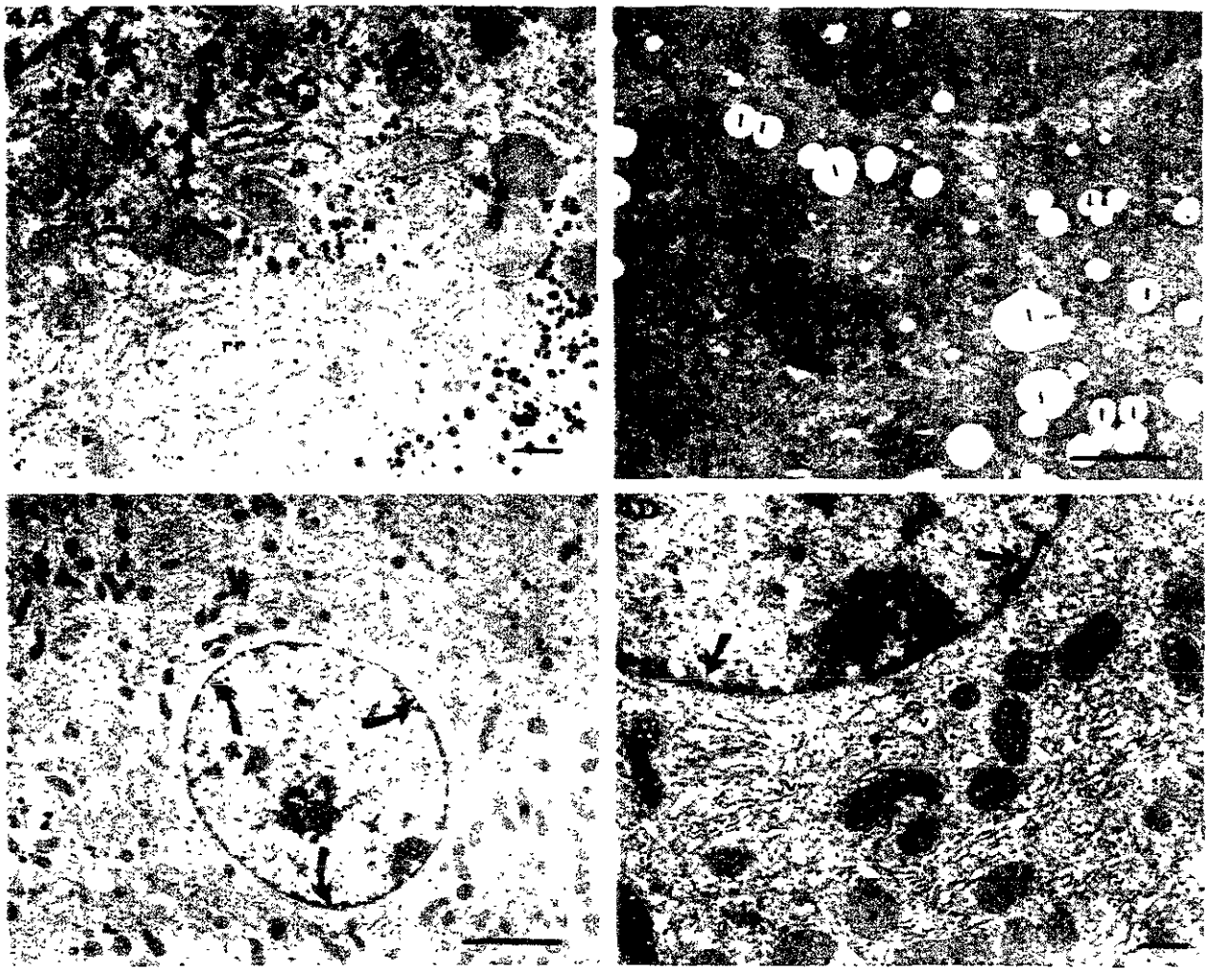

Fig 4. Electron micrographs of hepatocytes from rats at 36–60 hr of LR and treated with ethanol. (A) Regenerating hepatocytes show presence of glycogen deposits (arrowheads), as well as abundant rough endoplasmic reticulum, increased numbers of mitochondria were also noted. (B) Proliferating hepatocytes (36–60 hr after PH) from animals receiving intragastric ethanol administration are shown. There was striking fatty accumulation, and a clear absence of nuclear activity with condensed chromatin (arrows). (C) Hepatocytes from PH rats treated intraperitoneally with ethanol. Here, there was an increased number of mitochondria, as well as increased rough endoplasmic reticulum. Also evident were the enhanced size and number of nucleoli and the diminished amount of dense chromatin (large arrows). (D) Increased numbers of mitochondria and the presence of cytosolic vesicles of moderate density were evident. Changes in rough endoplasmic reticulum are in agreement with proliferative activity (compare with Figure 2A and B). Magnification of micrographs: A, ×7500; B and C, ×2500; and D, ×7500. Bar = 1 μm in A and D, 2 μm in B and C.

DISCUSSION

We showed recently that, in the PH-induced regenerating liver, ethanol metabolism is not directly implicated in the changes in the cellular redox state that occur during LR. These findings led us to propose that acute ethanol administration might minimize redox metabolic adjustments, probably leading to a decreased preparatory event culminating in the proliferative period that characterizes PH-induced liver regeneración (12).

We have also reported that a single low dose of ethanol produces a reliable and constant inhibition of the normal hepatic regenerative response induced by surgical removal of liver mass. This effect was strongly dependent on timing and the route of ethanol administration, and intragrastric administration of ethanol was more deleterious than the intraperitoneal route in inhibiting the regenerative process. However, ethanol administration to rats with regenerating liver did not induce hepatocellular necrosis, as evaluated by serum activities of hepatic marker enzymes (8).

These clearly established differential inhibitory effects of ethanol on the regenerating liver (dependent on its administration route), assessed by morpholog-

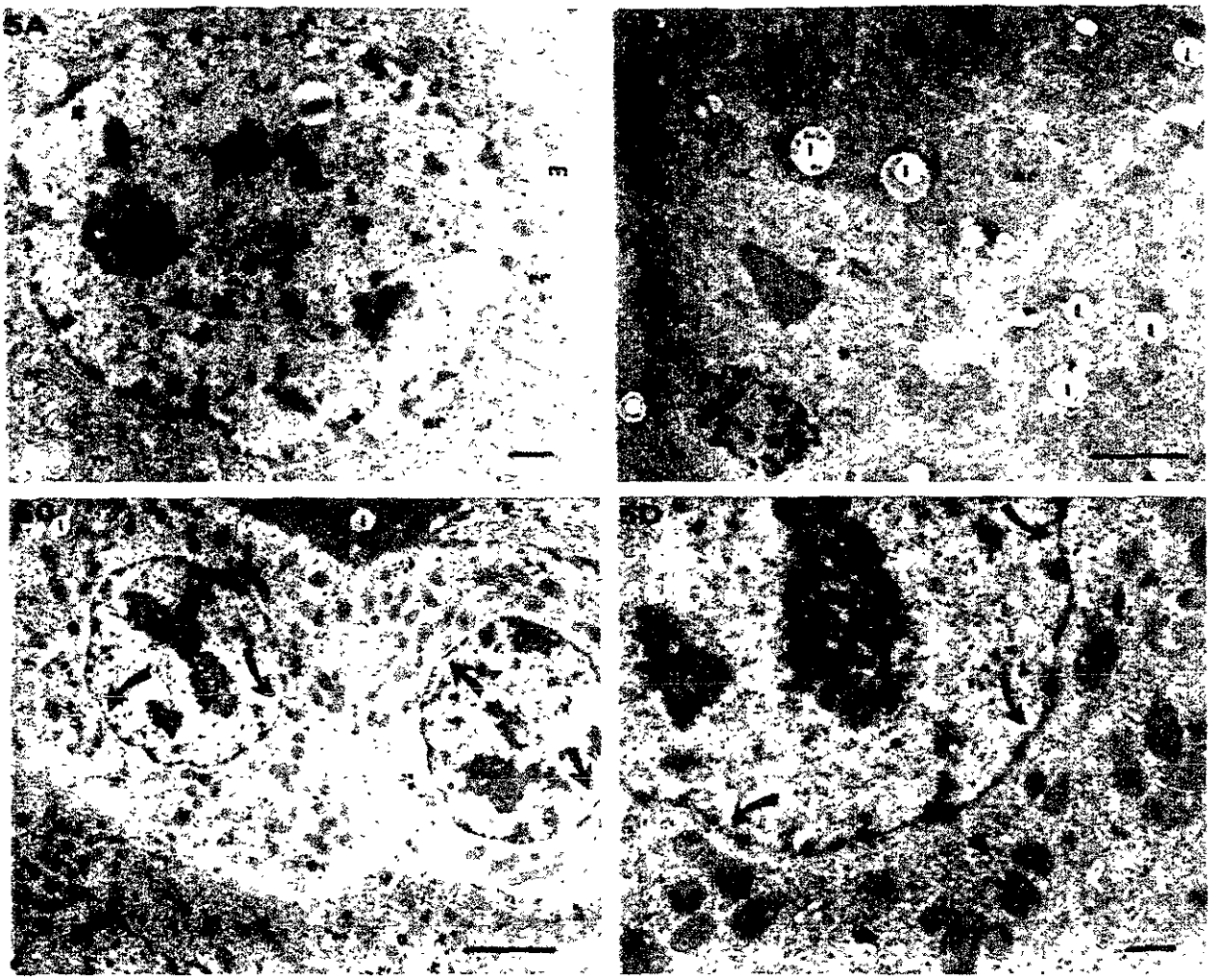

Fig 5. Electron micrographs of hepatocytes from rats at 72–96 hr of LR and treated with ethanol. (A) Hepatocytes at later LR stages, where restoration of the normal ultrastructural pattern is observed (compare Figure 1A). (B) Liver cells obtained from rats subjected to PH and receiving ethanol intragastrically showed essentially the same pattern observed along intragastric ethanol administration to rats subjected to PH (Figures 1D, 3A and 4B), mainly characterized by strong lipid accumulation and predominance of the smooth endoplasmic reticulum. (C) The intraperitoneal administration of ethanol resulted in increased size and number of nucleoli and of condensed chromatin (large arrows). Enhancement of mitochondria and rough endoplasmic reticulum was also evident, with restoration of glucogen deposits (arrowheads) (compare with Figure 4C). (D) Magnified picture of the latter groups shows the greater size of the nucleolus, and the diminution of dense chromatin. Magnification: A, ×5000; B and C, ×2500, and in D, ×7500. Bar = 1 μm (in A and D) and 2-μm in B and C.

ical studies (light microscopy) and by the liver's functional capacity (chemical determinations), were confirmed by the present findings extended to ultrastructural hepatocyte's organization studies.

It has been long recognized that morphological changes occur in the hepatic cells a few hours after PH. Indeed, there are excellent reports of the time-course of LR induced by PH at the ultrastructural level done through electron microscopy (9, 10). The main features include alterations on the ER and mitochondria, decreases in glycogen, and increases in lipid content. Mitochondrial and ER changes have been correlated with dramatic changes in their functions, including alterations in specific enzyme activities (13, 14). Nonetheless, it can be considered that the LR process produced only minor ultrastructural differences in subcellular compartments, as well as in plasma membranes during the first 16 hr after PH. Therefore, our results agree with those previously reported (15, 16).

On the second postoperative day, cytoplasmic structural disorganization was followed by reparative and regenerative activities, and from the second to fourth days, most organellar changes returned to normal levels. Mitochondria were the organelles that took more time for normalization, correlating with

TABLE 1. SUMMARY OF LIVER ULTRASTRUCTURAL FINDINGS IN PARTIAL HEPATECTOMIZED RATS AND TREATED WITH ETHANOL BY INTRAGRASTRIC (IG) AND INTRAPERITONEAL (IP) ROUTES*

Treatment (hr after surgery)	*Nuclear activity*	*RER activity*	*Lipid accumulation*	*Glycogen deposits*
Controls				
0–96 hr	(−)	(−)	(−)/+	+−−
PH rats + saline				
12 to 36 hr	++/+++	+++	++/+++	+
36 to 60 hr	+++	++/+++	+	+++
60 to 96 hr	+	+	+	++
PH rats + ethanol (IG)				
12 to 36 hr	(−)/+	+	++/+++	+
36 to 60 hr	(−)/+	(−)/+	+++	(−)
60 to 96 hr	(−)	(−)	++/+++	(−)/+
PH rats + ethanol (IP)				
12 to 36 hr	+	+	++/+++	+
36 to 60 hr	++	++	++	+/++
60 to 96 hr	++/+++	+++	+/++	++

*Parameters evaluated were: nuclear activity, (−) no change; (+) light; (++) moderate, or (+++) strong nuclear activity (enlarged nucleus, laxitude of chromatin mass and increased size and number of nucleoli); activity of the rough endoplasmic reticulum (RER): (−) no change, (+) light; (++) moderate, and (+++) complete RER activity (disorganization in fragments, dilatation, formation of vesicles, and appearance of free ribosomes in the Golgi vicinity); and fatty accumulation: (−) absent; (+) mild; (++) moderate, and (+++) severe. Presence of glycogen depots: (−) absent; (+) scanty; (++) mild, and (+++) abundant.

results obtained with radioactive labeling of proteins and membrane phospholipids, indicating that mitochondrial turnover lasts longer than other organelles in the regenerating rat liver (17).

Hence, the main manifestations of cellular proliferation in the normal regenerating liver were an increased perinuclear RER and a displacement of glycogen depots within the regenerating cells (mainly after 24 hr post-PH). These were accompanied by changes in the ultrastructure of the nucleus and nucleolus, which have been considered predictors of cell activity related to enhanced protein synthesis and proliferation (18), and they correlated well with a striking increase in the proportion of free ribosomes, indicating enhanced liver protein synthesis (19). Moreover, SER is the main source of drug-metabolizing enzymes and its predominance would suggest active metabolism of xenobiotics, whereas a predominant RER indicates that cells are subjected to an active protein synthesis (20). In any case, the hepatocytes seem to retain their functional activity and major structural attributes while proliferating, as reported previously (3, 8).

It is known that biochemical and physiological changes have to be accomplished in the regenerating hepatocyte, constituting the framework to drive mitosis and cell replication (21). Administration of ethanol to animals subjected to PH is able to inhibit LR, as evidenced by a diminution in DNA synthesis and in enzyme activity, closely related to the duplicative process (thymidine kinase, thymidylate synthetase, and ornithine decarboxilase) (22, 23). Nonetheless, the underlying mechanism in the inhibitory action of ethanol is still poorly understood.

The present results showed that ethanol, instead of inducing a sort of hepatocellular damage to the regenerating liver, seemed to minimize the normal profile of changes that occurred in subcellular structures of the regenerating hepatocyte. Particularly, the intragastric route of ethanol administration blocked most PH-induced modifications in the liver ultrastructure, except for the more pronounced fatty liver in these animals. The changes in liver nuclear and nucleolar activities (eg, chromatin distribution) and that of RER were practically eliminated when rats subjected to PH received ethanol intragastrically. Indeed, livers from these animals did not show signs of cell proliferation. Furthermore, ER was predominantly smooth, indicating a lower (normal) rate of protein synthesis, as compared to non-ethanol-treated PH rats. This is noteworthy, considering that acute ethanol administration produces structural and biochemical changes in mammalian livers, including alterations in the porosity of the sinusoidal endothelium, in endothelial fenestrations, and in some subcellular organelles, such as mitochondria (3, 24).

In addition, it was clear that intragastric administration of ethanol induced bigger fat deposits at later

postsurgery times (60–96 hr) than at earlier times of LR. Normal animals developed fatty liver after ethanol ingestion, an effect closely related to a shifted cytoplasmic redox state towards a reduced condition (25). This ethanol-induced effect in fat accumulation largely depends on liver alcohol dehydrogenase (ADH) activity, as demonstrated by a strong diminution of fatty liver in animals receiving ethanol and treated with 4-methylpyrazole, a specific ADH inhibitor (26).

Therefore, the data suggest that liver ADH participation in ethanol oxidation is not the same at all LR stages after PH and that this enzyme contributes to a considerable ethanol catabolism at later stages of LR, as previously suggested in previous communications by our group (4, 12).

In addition, intraperitoneal ethanol administration to rats undergoing PH-induced LR promoted a lesser blockade of the PH-induced ultrastructural changes in the regenerating liver. More interestingly, a single low i.p. dose applied to PH rats at later post surgery times induced activation of hepatocyte proliferation instead of inhibiting this process. These data strongly agree with those found in studies of liver function and morphological appearance previously reported by us (8). Hence, we are demonstrating that, depending on the conditions, ethanol could stimulate LR rather than inhibit it.

The route-dependence of ethanol-induced changes in the ultrastructural organization of regenerating livers has not been previously described. The mechanisms involved in the blocking effect of ethanol on the progression of PH-induced LR is still unknown. Data would suggest that ethanol somehow promotes an 'uncoupling' of the remnant liver to respond to proliferation inducers, since the subcellular organization of the hepatocyte only depicted minor changes after PH and in the presence of ethanol. However, ethanol administration (mainly by the i.g. route) was also able to return liver cells to baseline (quiescent stage), when they are supposed to be already stimulated by the surgical loss of hepatic mass. It would seem that a single ethanol dose can turn cellular or molecular mechanism activated by PH (switch on) to the level normally present in the intact liver (switch off). The nature of such mechanism(s) is currently being studied in our laboratory.

In conclusion, lower doses of ethanol administered to rats subjected to PH elicited different modifications in the ultrastructural framework of the regenerating liver, largely dependent on its administration route. Whereas the i.g. administration practically abolished all adaptative changes of regenerating hepatocytes, its administration by the i.p. route promoted later ultrastructural modifications, indicative of cell proliferation. The underlying mechanism involved in this surprising effect of ethanol on LR remains to be elucidated.

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ARTICULO 4 PAPEL DE LA ADH EN LA REGENERACIÓN HEPÁTICA

La regeneración hepática es un proceso altamente regulado en el que intervienen diversos cambios moleculares y ajustes metabólicos que resultan en un incremento en la síntesis de DNA y en la proliferación celular (Michalopoulos, 1990). La administración aguda de etanol inhibe la regeneración hepática inducida por HP, por disminución de los parámetros de regeneración celular en el hígado remanente (Frank y cols. 1979; Dugay y cols. 1982). El efecto inhibitorio del etanol sobre la regeneración hepática se ha atribuído al metabolismo de éste en el hígado, órgano en el que el etanol se oxida en acetaldehído por la ADH en el citosol (Gutiérrez-Salinas y cols. 1996). Recientemente reportamos en un estudio farmacocinético sobre biodisponibilidad del etanol que el hígado en regeneración presenta un manejo del etanol que es dependiente de la ruta y el tiempo de administración del etanol en animales sujetos a HP (Morales-González, y cols. 1998). Estos datos indican que durante la regeneración hepática se incrementa el metabolismo del etanol, particularmente en la primeras 24 horas posteriores a la cirugía y que el primer paso metabólico (FPM) dado por el estómago aunque transitorio juega un efectivo papel en las modificaciones farmacocinéticas del etanol a tiempos tempranos de regeneración hepática (primeras 3 horas posteriores a la cirugía). Por lo tanto constituye una barrera protectora contra los efectos adversos del etanol en estos animales. Quedan dos posibilidades: una concerniente a la participación de la ADH hepática en ratas HP que recibieron etanol y la segunda en la participación de vías alternas de oxidación de etanol que no sean mediadas por la ADH. La posible influencia de la participación de la ADH hepática en la farmacocinética del etanol a tiempos tempranos de regeneración se estudió utilizando un inhibidor específico de la ADH, la 4-Metilpirazola (4-MP) (Figura 1). Se puede observar que la 4-MP modifica significativamente las concentraciones máximas de etanol en sangre y las ABC (figuras 1 y 2), tanto en el grupo sham como en el grupo HP, lo que demuestra la gran participación de la ADH hepática para metabolizar etanol a tiempos tempranos de regeneración. Por otra parte, se puede observar en la Figura 1B la participación del estómago en el primer paso metabólico del etanol durante la regeneración hepática, ya que se mantienen niveles constantes de etanol en sangre durante las primeras 6 horas posteriores a la cirugía; después de este tiempo se puede observar como se incrementan estos niveles en sangre en el grupo HP lo que pone de manifiesto la participación del estómago en el FPM del etanol en tiempos tempranos de regeneración hepática.

Por otra parte, recientemente reportamos que durante la regeneración hepática se incrementa la capacidad de oxidar etanol por el hígado, la cual aumenta según se recupera la masa hepática (Morales-González y cols. 1998). En otro estudio se encontró que la actividad de la ADH disminuye en tiempos tempranos de regeneración hepática, posterior a una HP (Gutiérrez-Salinas y cols. 1999). Pösö and Pösö (1979) demostraron que la actividad de la ADH disminuye durante la regeneración hepática. Es difícil explicar el aumento en la oxidación de etanol por el hígado regenerante, siendo que el principal sistema de oxidación de etanol es parcialmente inhibido por la HP. Asimismo, la administración de etanol a tiempos tempranos posteriores a la HP protege contra la inhibición de la ADH hepática (Gutiérrez-Salinas y cols. 1999). En la figura 1B se puede observar que la administración de 4-MP ocasiona una gran inhibición de la ADH hepática que resulta en un incremento en las concentraciones de etanol en sangre durante la proliferación celular del hígado, lo que pone de manifiesto la participación que tiene esta enzima para metabolizar etanol durante la regeneración hepática y no otros sistemas oxidativos del etanol (catalasa y MEOS). En la Tabla 1 se puede observar que la actividad de la ADH hepática es dependiente de la concentración del inhibidor.

Los metabolitos séricos son indicadores de la integridad funcional del hígado. La administración aguda de etanol en la rata, inmediatamente después a la HP, aumenta la magnitud y la duración de la hipoglucemia. (Gutiérrez-Salinas y cols. 1996; Morales-González y cols. 1999). En el presente estudio, se encontraron modificaciones por el tratamiento utilizado. A las 24 horas posteriores a la HP, existe una disminución en la glucosa que tiende a recuperarse (Tabla 1; Morales-González y cols. 1999); por otra parte, cuando se utilizaron diferentes dosis de 4-MP inmediatamente después de la HP, la glucosa disminuye independientemente de la dosis (Tabla 1). Asimismo, se observa que tanto el etanol como la 4-MP ocasionan disminución de los niveles séricos de glucosa.

Durante la regeneración hepática inducida por HP, la acumulación de grasa es una característica constante que acompaña al progreso de la proliferación hepática, recientemente se ha atribuído a un incremento en la permeabilidad de los lípidos por el hígado, resultando en un aumento hasta de 20 veces en el hígado regenerante (Morsiani y cols. 1995). Por otra parte, los niveles séricos de triacilglicéridos no únicamente reflejan el estado funcional del hígado, sino también un balance entre la producción y su utilización (Hernández-Muñoz y cols. 1978). Recientemente, reportamos que la administración de etanol incrementa los niveles de triacilglicéridos por arriba de lo que

induce la HP, la cual se asocia con un acúmulo acentuado de gotas de grasa que se encontró en esos animales (Gutierrez-Salinas y cols. 1996; Morales-González y cols. 1999; Morales-González y cols. en prensa). Nuestros datos muestran que la HP induce producción de grasa y movilización de la misma. Por otra parte, la administración de 4-MP a diferentes dosis no modifica la esteatósis hepática, pero incrementa la hipertriacilgliceridemia (Tablas 1 y 2).

También nuestro grupo ha reportado que durante el proceso de regeneración hepática, existen ligeras modificaciones en los niveles séricos de albúmina y bilirrubina, mostrando que existe una disminución de albúmina en suero cuando se administró el etanol (Morales-González y cols. 1999). Al utilizar dosis bajas de 4-MP (25 y 50 mg/kg) en ratas HP, se encontró una disminución de albúmina y un incremento en los niveles séricos de bilirrubina. Sorprendentemente, al administrar dosis altas de 4-MP (100 y 200 mg/kg) a ratas HP, los niveles séricos de albúmina disminuyeron ligeramente y no fueron afectados los niveles de bilirrubina (Tabla 1).

Lo anterior sugiere que los efectos deletéreos del etanol dependen principalmente de la actividad de la ADH. Al administrar etanol se produce una inhibición de la regeneración hepática inducida por HP, que probablemente esté asociada al estado que guarde la actividad de la ADH, que se ve reflejada en los efectos adversos en la función del hígado y consecuentemente, en los ajustes metabólicos, los cuales aumentan la síntesis de DNA en la regeneración hepática.

En estudios morfológicos se encontró que durante la regeneración hepática se observa una acumulación transitoria de gotas de grasa, inflamación mínima y abundantes imágenes de mitosis. Así mismo, se observa desorganización hepatocelular la cual se considera una característica de los cambios morfológicos que evidencian actividad celular, que preceden a la restauración de la masa hepática (Morales-González y cols. 1999 y 2000). La misma interpretación ha sido reportada por varios autores (Jordan 1964; Trotter 1964; and Lane and Becker 1966). El alcohol modifica este patrón: el etanol administrado a ratas en proceso de regeneración hepática bloquea la desorganización hepatocelular que es inducida por la HP (Morales-González y cols. 1999). Por otra parte, los datos que se obtuvieron al administrar dosis bajas de 4-MP (25 y 50 mg/kg) son de un ligero infiltrado de gotas de grasa e inflamación mínima datos que son similares a los encontrados en las ratas con HP únicamente. Los cambios en la estructura hepatocelular fueron similares al grupo HP y se encontró una disminución ligera de las imágenes mitóticas en estos grupos, en comparación con los animales

sujetos a HP (Tabla 2 y figura 3). Dosis altas de 4-MP (100 y 200 mg/kg) ocasionan mínima infiltración de las gotas de grasa pero un incremento en el proceso inflamatorio, y abolición de las imágenes de mitosis. En estos grupos, la 4-MP bloquea la desorganización hepatocelular que induce la HP (Figura 3 y Tabla 2), efecto que es semejante a lo que ocasiona el etanol. Las diferentes magnitudes de inhibición que ocasiona la 4-MP en la regeneración hepática probablemente sean dependientes de la actividad de la ADH y esto pueda ser relacionado con el mantenimiento de la función del hígado regenerante. Por otra parte, un buen parámetro de regeneración, es la actividad especifica de la TK, la cual fue inhibida en una manera dosis dependiente de la 4-MP, fenómeno que ocurre al igual que con la ADH.

El incremento sérico de la actividad de las enzimas AST, LDH, ALT, GDH y OTC posterior a HP del 70% ha sido reportado previamente (Morales-González y cols. 1999). Este incremento en la actividad de estas enzimas en suero durante la regeneración hepática se ha interpretado de dos formas: en primer lugar como un evento de necrosis y en segundo lugar como un aumento en la permeabilidad de la membrana celular. Sorprendentemente, la administración de etanol a ratas HP, disminuye la actividad sérica de estas enzimas, lo que apoya fuertemente que la HP induce la elevación sérica en la actividad de estas enzimas y no está relacionado con la necrosis hepática dado que las primeras evidencias de que la regeneración hepática se acompaña de una liberación selectiva de estas enzimas (Morales-González y cols. 1999). Por otra parte, en estudios de microscopia electrónica realizados para conocer el efecto del etanol sobre la ultraestructura del hígado regenerante, no se encontraron datos de daño estructural del hepatocito por el etanol (Morales-González y cols. en prensa). Nuestros resultados demuestran que la administración de 4-MP durante la regeneración hepática inducida por HP disminuye la actividad sérica de las enzimas (AST y ALT), siendo esta inhibición dosis dependiente de la de 4-MP (Figura 4).

El presente estudio demostró que la inhibición de la ADH hepática por la 4-MP induce alteraciones morfológicas y bioquímicas durante la regeneración hepática que correlacionan con la inhibición de la actividad de la ADH, datos que no habían sido descrito previamente. Esto sugiere que tanto la inhibición de la actividad de la ADH como las alteraciones en la regeneración hepática inducidas por la 4-MP ocurren secuencialmente y que la relación causa-efecto entre estos dos fenómenos es posible. Asimismo, el presente estudio sugiere una secuencia de eventos durante la inhibición de la regeneración hepática, que consiste en cambios en la actividad de la ADH hepática

seguidos por cambios histológicos y funcionales en los hepatocitos. Semejante perfil de alteraciones (farmacocinético, morfológico, bioquímico y fisiológico) ha sido descrito previamente por nosotros (Morales-González y cols. 1998, 1999, en prensa). Por lo tanto, será interesante estudiar el comportamiento de la ADH durante la regeneración hepática, tanto a nivel molecular como bioquímico.

Las conclusiones de los datos experimentales reportados en el presente estudio son: (1) la administración de dosis bajas de 4-MP (25 y 50 mg/kg) en ratas HP, se asocia con ligeros cambios en la actividad de la ADH hepática y en cambios metabólicos que disminuyen ligeramente la regeneración hepática. (2) la administración de dosis altas de 4-MP (100 y 200 mg/kg)en ratas sujetas a HP, disminuye de forma importante la actividad de la ADH hepática, la cual se asocia con cambios en la función y la estructura que precede aparentemente a los hepatocitos a la replicación celular y (3) estudios en el comportamiento de la ADH hepática se pueden utilizar para identificar la patogenia de la inhibición de la regeneración hepática que se conoce ocurren en la enfermedad hepática por alcohol en el humano

INIBITORY EFFECT OF 4-METHYLPYRAZOLE ADMINISTRATION ON LIVER REGENERATION INDUCED BY PARTIAL HEPATECTOMY IN RATS.

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ABSTRACT

Although acute and chronic ethanol treatment inhibits DNA synthesis in the regenerating rat liver after partial hepatectomy (PH), its inhibitory mechanism has not been fully elucidated, and also it is unknown whether this ethanol's effect is mediated by the molecule *per se*, or if through its liver metabolism. We have recently reported that remnant liver after PH shows enhanced ethanol metabolism *in vivo*, despite liver activity of alcohol dehydrogenase (ADH) was significantly diminished after PH. Therefore, the present study was aimed to assess the role of ADH of the remnant liver during ethanol metabolism *in vivo*. Thus, sham-operated and rats subjected to PH were treated with ethanol (1.5 g/kg b.w.) and several doses of 4-methylpyrazole (4-MP), a known specific inhibitor of ADH. *In vivo* administration of 4-MP revealed ADH as the main via oxidizing ethanol in both, control and PH-rats. However, the possible reliable participation of another ethanol-metabolizing in the remnant liver after PH was also evidenced. In the absence of ethanol, 4-MP treatment readily inhibited PH-induced liver regeneration in a dose-dependent manner. This 4-MP effect on PH-induced liver regeneration was not associated to evident structural alterations or to liver dysfunction, as assessed by histological assessment and serum metabolite and enzyme activities, markers of liver function integrity.

Therefore, these results strongly suggest a major role of liver ADH activity in the adaptative metabolic adjustments needed for the compensatory liver proliferation after PH, and its participation as possible target for the deleterious actions of ethanol catabolism on PH-induced liver regeneration.

Key words: Alcohol dehydrogenase, Mitotic index, Ethanol oxidation, Cell proliferation, Ethanol.

INTRODUCTION

Although the mechanism of its inhibitory effect is not defined, it is well known that acute and chronic ethanol treatment inhibits DNA synthesis in the regenerating rat liver after partial hepatectomy (PH) or toxic injury, as well as in cultured hepatocytes (1-4). Even though, PH-induced liver regeneration is inhibited by both acute and chronic ethanol treatment, evidence suggests that acute and chronic ethanol inhibits hepatocyte proliferation through different mechanisms. Indeed, it has been suggested that short- and long-term ethanol exposure could exert opposite effects at the onset of ethanol-induced inhibition of liver rat regeneration (5).

At present it is known, that chronic ethanol consumption disrupts induction of intracellular signaling pathways and growth-associated gene expression, thereby inhibiting the hepatotrophic and hepatoprotective actions of cytokines and growth factors (6-10), however, little is known about the acute ethanol-induced inibition of liver regeneration.

Acute ethanol induces a dose-dependent inhibition of ornithine decarboxylase and tyrosine aminotransferase activities (11). A single dose of ethanol is able to significant inhibit the accelerated synthesis of ornithine decarboxylase protein, but also causes inhibition of both ornithine decarboxylase and tyrosine aminotransferase degradation. These data led to suggest that ethanol acutely inhibits protein synthesis and activity in regenerating rat liver at the transcriptional level by interfering with the synthesis of RNA in nuclei (12,13). The same has been recently found for activities of thymidylate synthase and thymidine kinase, rate-determining enzymes in DNA synthesis. Indeed, it has been proposed that acute ethanol inhibits DNA synthesis by suppressing the gene expression of these dTMP-synthesizing enzymes in regenerating livers after PH (14).

In spite of the aforementioned, the mechanism underlying the inhibitory action of ethanol on liver regeneration remains elusive. It is neither known whether ethanol's effects on liver proliferation are mediated by the molecule *per se* or are due to its metabolism. In

this regard, we recently reported that the remnant liver after PH shows a very enhanced metabolism of ethanol *in vivo*, particularly during the first 24 hr after surgery, mainly attibutable to hepatic catabolism of ethanol, which would be the main factor affecting ethanol pharmacokinetics in rats subjected to PH (15). In addition, acute ethanol treatment to animals subjected to PH could minimize the PH-promoted diminution in alcohol dehydrogenase (ADH) activity and the metabolic adjustments mediated by redox reactions in the remnant liver, probably leading to an ineffective preparatory event that culminates in compensatory liver growth after PH (16).

Therefore, the present study was aimed at evaluating the net hepatic participation (through ADH activity) in ethanol pharmacokinetics, by inhibiting this enzyme *in vivo* in rats subjected to PH and administered 4-methylpyrazole (4-MP), a non-toxic and specific inhibitor of ADH (13). Results suggest that although ADH activity accounted for the main ethanol-metabolizing system in the remnant liver after PH, another pathway contributed largely to remove circulating ethanol in these animals. The 4-MP treatment to rats subjected to PH, in the absence of ethanol, induced a dose-dependent inhibition of mitotic index, without significantly altering the structural and functional integrity of the PH-induced proliferating liver.

MATERIAL AND METHODS

Materials. Yeast ADH, NAD+, semicarbazide hydrochloride and 4-MP were purchased from Sigma Chemical Co. (St. Louis, MO). Other chemicals of analytical grade were obtained from Merck (Merck de México, S.A.).

Animal Treatments. Male Wistar rats (230-270 g, body weight) were housed under a 12-h light/dark cycle and allowed to consume standard rat pellet chow and water *ad libitum* before treatments. Two-thirds partial hepatectomy was performed according to the technique of Higgins and Anderson (17), under light diethyl ether anesthesia. As controls,

sham-operated rats were subjected to the same surgical procedure, without removing of the liver mass. After surgery, animals were grouped as follows: (A) sham-operated rats receiving intraperitoneally (i.p.) administration of saline solution (0.9% NaCl; controls); (B) sham-operated animals receiving either 25 or 200 mg of saline-dissolved 4-MP/kg of body weight by i.p. route; (C) rats subjected to PH i.p. administered with saline solution (control of PH), and (D) animals subjected to PH and receiving a single i.p. dose of 4-MP (either 25, 50, 100 or 200 mg/kg of b.w.). 4-MP was administered immediately after surgery, in the absence or presence of ethanol. Animals were killed by decapitation, under general anesthesia with sodium pentobarbital (40 mg/kg of b.w.), 24 hr after PH. All procedures involving experimental animals were made according to our *Federal Regulations for Animal Experimentation and Care* (Ministry of Agriculture; SAGAR, Mexico).

Ethanol Bioavailabity in Rats subjected to PH and treated with 4-MP. In another set of fed animals grouped according to their surgical status (sham-operated or partially hepatectomized), ethanol only (1 5 g/kg b.w. in 40% w/v solution) was administered by intragastric intubation immediately after surgery, or concomitantly with a single dose of 4-MP (200 mg/kg of b.w.) administered by i.p. route. After treatments, animals were placed in restriction cages, without further access to water and food, and blood samples were collected from the wound of excised tips of the tail with 50 µl capillary pipettes. Blood sampling was scheduled every 15 min during the first 2 hr, every 30 min for the next 2 hours, and every hour thereafter until 9 hr post-administration (15). In the animals treated with 4-MP, sampling was done every 2 hr until 12-16 hr post-administration (not shown). Blood ethanol was quantified in neutralized acid-extracts by the enzymatic method described by Bernt and Gutman (18). The area under the curve (AUC) for ethanol elimination from the blood was calculated by the trapezoidal method, from the beginning of ethanol administration to the time it was no longer detectable (19).

Serum and Liver Samples: After decapitation, whole blood was collected and centrifuged to obtain serum, which was kept frozen at -70°C until use. Liver samples were taken for histological assessment and subcellular fraction. The cytosolic fraction was obtained by differential centrifugation and the purity of the fraction was evaluated as described by Aguilar-Delfín et al. (20). For the histopathological analysis, liver samples were prepared as described below.

Liver Histology: Hepatic samples from each group were used for light microscopy. Samples were fixed in formaldehyde (10% in buffered solution, pH 7.4), embedded in wax and stained with hematoxylin-eosin. Liver specimens were coded and read blindly without knowledge of the other data. Criteria for the analysis of morphological abnormalities were the same as those reported previously (21). Mitotic index was recorded with an optical microscope (Olympus, CH-30) and corresponded to the number of mitotic images found in 20 microscopic fields examined with a 40X objective and indicated as follows: (+), 1-3; (++), 4-7, and (+++), >7 mitotic cells/field, as described by Morales-González et al. (21).

Serum Enzyme Activities and Metabolite Assays: Serum activities of alanine (ALT; EC 2.6.1 2) and aspartate (AST; EC 2.6.1.1) aminotransferases, as well as that of lactate dehydrogenase (LDH; EC 1 1.1.27) were determined by methods reported elsewhere (21). Serum concentrations of triacylglycerols, glucose, albumin and bilirubin were quantified by using assay kits from Sigma Chemical Diagnostics Co. (St. Louis, MO).

Liver ADH Activity: Cytosolic ADH activity in livers from our experimental groups was measured spectrophotometrically at 25°C, as the increase in absorbance at 340 nm. The assay was done in a buffer containing 0.1 mol/l glycine/NaOH (pH 9.6), 2 mmol/l NAD+ and 100 µg of cytosolic protein. Reaction was started by the addition of 10 mmol/l of ethanol and followed for at least 5 min. ADH activity is reported as $nmol \cdot min^{-1} \cdot mg^{-1}$ of cytosolic protein (22).

Statistics: Results are expressed as the mean ± SEM. Significance of the differences among groups was analyzed by two-way ANOVA and, in case of significance, by Student's *t*-test ($p<0.01$).

RESULTS

Blood Ethanol Clearance in Animals subjected to PH and Treated with 4-MP. After intragastric administration of a single ethanol dose (1.5 g/kg of b.w.) sham-operated rats reached a maximum blood alcohol concentration (BAC) of 21.2 ± 3.9 mM (Fig. 1A), associated with an AUC of 11.7 ± 1.1 mM/hr (Fig. 2). As previously found (15), the same ethanol dose elicited a much lower peak during the first 12 hr after surgery in animals subjected to PH (BAC = 12.6 ± 2.5 mM, $p<0.01$; Fig. 1A), without significantly modifying the AUC, as compared with that of controls (15.8 ± 2.2 mM/hr; Fig. 2). However, administration of 4-MP significantly changed both BACs and AUCs in either sham-operated or PH-rats receiving the same ethanol dose (Figs. 1 and 2). Sham-operated animals treated concomitantly with ethanol and 4-MP showed a similar BAC (22.6 ± 3.9 mM; Figs. 1B and 2A), but the AUC for blood ethanol was significantly prolonged, as compared with control animals not-treated with 4-MP (39.4 ± 4.1 mM/hr, $p<0.01$ against control; Fig. 2). As expected, in rats subjected to PH and receiving 4-MP, ethanol administration produced an enhanced BAC (30.8 ± 2.8 mM, $p<0.01$ vs. untreated control and PH-rats. Figs 1B and 2A), and the AUC was largely increased ($p<0.01$) as compared to controls and animals subjected to PH without 4-MP treatment (51 8 ± 5.6 mM/hr; Fig. 2B). Therefore, treatment with 4-MP (200 mg/kg of b.w.) readily delayed blood ethanol clearance in both sham-operated and PH-rats, but the magnitude of the inhibitory effect of 4-MP on ethanol oxidation was not the same in both experimental groups (Figs. 1 and 2).

Effect of 4-MP on liver ADH activity and histological assessment. Since 4-MP administration to animals subjected to PH did not show a proportional effect (in relation to

the remaining liver mass after surgery) on blood ethanol clearance, when compared to intact (sham-operated) rats, we measured the extent of 4-MP-mediated inhibition of ADH in our experimental groups. At the 4-MP dose employed in studying ethanol pharmacokinetics, this compound promoted an *in vivo* inhibition of ADH activity of 82.5 and 78.6% in sham-operated and PH-rats, respectively. In addition, the presence of ethanol (1.5 g/kg b.w.) did not significantly modify the effect of a single 4-MP dose (200 mg/kg b.w.) in animals subjected to PH, since this pyrazole-derivate induced a 72.3% inhibition of liver ADH activity (against 78.6%, in animals without ethanol administration) in hepatectomized rats receiving ethanol plus 4-MP

In liver samples taken from ethanol-treated PH rats, 24 hr after surgery, PH-changes in hepatocellular organization and increased mitotic images were largely decreased by this low ethanol dose (not shown), confirming previous findings reported by us (21). Administration of 4-MP to these animals did not significantly modify the inhibitory effect of ethanol on PH-induced liver regeneration, as evaluated by histological assessment (not shown). This latter agrees with Frank et al. report (23), on pyrazole administration to PH-rats, in which they suggested that ethanol and not one of its metabolic end products is responsible for the inhibition of liver regeneration.

However, it was surprising to find that PH-rats treated with 4-MP at 200 mg/kg of b.w., in the absence of ethanol, showed a drastic reduction of the mitotic index, when evaluated at 24 hr after surgery (Fig. 3). In addition. these animals also presented lower content of fatty droplets, as well as evidence of moderate inflammation (Fig. 3) These findings led us to examine the effect of 4-MP *per se* on the PH-induced regenerating liver in the absence of ethanol.

Table 1 summarizes the effects of several 4-MP doses *in vivo* on the structural integrity assessed in liver samples from sham-operated and rats subjected to two-thirds PH, 24 hr after surgery. In sham-operated rats, 4-MP administered in the dose range of 25

to 200 mg/kg of b.w., only induced a slight fatty infiltration and hepatocellular disorganization; but this compound promoted quite different changes in regenerating livers, after PH (Table 1). Lower doses of 4-MP (25 to 50 mg/kg of b.w.) produced higher fatty liver, accompanied by a mild/moderate inflammation that seems to be associated to a reduction in one-third or inhalf the number of mitotic images (mitotic index) found in the untreated animals undergoing PH. Higher doses of 4-MP (100 to 200 mg/kg of b.w.) when administered to PH-rats, elicited a different histological pattern of the proliferating liver (Table 1). Whereas fatty deposition and hepatocellular disorganization were diminished, the degree of inflammation was readily enhanced, which seemed to culminate in an almost complete disappearance of mitotic images, indicating a strong inhibition of PH-induced liver regeneration by these used 4-MP doses in rats (Table 1).

The extent of inhibition of liver ADH activity achieved by 4-MP treatment also correlated well with the inhibitory effect of this compound on the PH-induced increase in the mitotic index (Table 2). Animals subjected to PH only had decreased ADH activity (23.7% of inhibition, as compared to sham-operated controls); lower 4-MP doses (25 to 50 mg/kg of b.w.) induced a 41 to 49% inhibition in ADH activity in the proliferating livers, whereas higher 4-MP doses produced a 63.9 to 78.6% inhibition at 100 and 200 mg/kg of b.w., respectively (Table 2). Therefore, a quite linear correlation was found between ADH activity and the magnitude of the mitotic index (Tables 1 and 2).

Effects of 4-MP on Serum Enzyme Activities and Metabolite Levels. Administration of 4-MP to rats subjected to PH inhibited liver proliferation, while increasing histological evidence of tisular inflammation (Fig. 3). Thus, to evaluate the effect of 4-MP on hepatocellular function, as possible contributing factor for the inhibitory action of 4-MP on liver regeneration, we measured serum activities of liver "marker" enzymes as well as the levels of metabolites considered to reflect hepatic function. Administration of the highest dose of 4-MP to sham-operated animals produced a marked hypoglycaemia, accompanied

by a drastic increase in serum levels of triacylglycerols. However, albumin and bilirubin levels were not affected by 4-MP in sham-operated rats (Table 2).

Rats subjected to PH showed decreased serum glucose level and elevated triacylglycerols, while serum levels of albumin and bilirubin were within the normal range, 24 hr after surgery (Table 2). 4-MP treatment to these animals (from 25 to 200 mg/kg of b.w.) induced a higher increase in serum triacylglycerols and potentiated the diminution of blood glucose; in addition, lower 4-MP doses administered to PH-rats also promoted a drop in serum albumin and a significant increase in bilirubin content (Table 2). Interestingly, the adverse effects of 4-MP on serum levels of albumin and bilirubin induced in PH-animals, were much less marked when using higher doses of 4-MP (100 to 200 mg/kg of b.w.; Table 2).

Although the aforementioned results would suggest liver dysfunction in animals subjected to PH and treated with 4-MP, serum enzyme activities (AST, ALT, and LDH) quantified 24 hr after surgery did not support this previous suggestion (Fig. 4). In sham-operated rats, 4-MP at the highest dose only induced a slight increase in serum LDH activity, while both AST and ALT were practically unaffected (Fig. 4). As previously reported (21), animals subjected to PH show increased levels of serum enzymes, being serum ALT levels one of the most increased serum enzymes after partial liver resection (Fig. 4). Treatment with 4-MP to PH-rats also increased serum LDH activity, being more notorious the effect achieved with the lowest 4-MP dose. However, an opposite effect of 4-MP was found in serum transaminase levels, since the ADH-inhibitor readily decreased the PH-induced augmentation of serum transaminase activities, this effect was much more evident at the highest 4-MP doses administered to PH-rats (Fig. 4).

As a whole, data would indicate that the inhibitory effect of 4-MP on PH-induced liver regeneration did not seem to be related with hepatocellular damage or markedly compromised hepatic function of the regenerating rat liver.

DISCUSSION

Liver regeneration consequent to two-thirds PH is a complex process, accompanied by changes in the major metabolic pathways, which maintain adequate energy availability for DNA replication, cell division, and restitution of the liver mass (24,25). Hence, several of these metabolic routes could be potential targets for the deleterious actions of acute ethanol administration on PH-induced liver regeneration, mainly those controlled by the NAD/NADH redox state (26).

Although it is accepted that acute ethanol administration exerts a clear inhibitory effect on compensatory liver growth after PH, the question remains whether ethanol-induced inhibition of liver regeneration is mediated through the rate of ethanol metabolism (i.e. by acetaldehyde levels and/or changes in the NAD/NAH ratio) or its adverse effect depends on the amount of ethanol *per se* in the proliferating liver. In the presence of 4-MP, acute ethanol inhibited ^{3}H-TdR incorporation into hepatic DNA, 18 to 48 hr following PH (23). It has also been suggested that ethanol inhibits the synthesis of ornithine decarboxylase (27) and tyrosine aminotransferase activity (13) directly through ethanol molecules and not mediated via its by-products.

However, the regenerating liver has been shown to present abnormal ethanol and especially acetaldehyde metabolism (28), and acetaldehyde also diminished incorporation of ^{3}H-thymidine into liver DNA in regenerating rat liver, rat cells in culture, and rat fetal tissues (29). In addition, ethanol impairs hormonally induced amino acid transport system A in liver Golgi as well as in plasma membrane vesicles during hepatic regeneration, an effect probably mediated by acetaldehyde, by interacting with hepatic tubulin (30).

It has also been reported (31) that although ADH activity in the remnant liver does not significantly change immediately after PH, the rate of ethanol removal is significantly faster in PH-rats; hence, the authors suggested that the rate of ethanol elimination might

be limited by the ADH activity. Furthermore, we found recently in a pharmacokinetic study on ethanol bioavailability that the regenerating liver has a very enhanced capacity to metabolize ethanol, which seems to be the main factor affecting ethanol pharmacokinetics in rats subjected to PH (15). This latter effect occurred even though the proliferating liver promoted decreases in the ADH activity (the main ethanol-catabolizing pathway) and, to a much lesser extent, of cytochrome P4502E1 (16). Interestingly, ethanol treatment to animals subjected to surgery prevented the PH-induced diminution of liver ADH activity, as well as minimized the PH-promoted metabolic adjustments mediated by redox reactions, which are also involved in ethanol oxidation (16).

Taken as a whole, data suggest that ADH activity could be largely involved in the inhibitory mechanism promoted by ethanol administration on PH-induced liver regeneration. There are at least two possibilities to explain the dissociation between the diminished ADH activity found in the regenerating liver (16) and the enhanced blood ethanol clearance found in these animals,. Firstly, extra-hepatic tissues might show a more significant contribution in oxidizing administered ethanol in PH-rats than that present in control animals. This point is partially true, since we have shown that gastric metabolism of ethanol (first-pass metabolism) is increased in PH rats (15); however, since this PH-induced increase in gastric oxidation of ethanol is relatively slight and transient, could barely account for the increased ethanol metabolism in rats undergoing PH. The other possibility concerns the participation of non-ADH pathways for ethanol catabolism in the remnant liver after PH and ethanol administration.

Present results using *in vivo* administration of 4-MP, a specific inhibitor of ADH (32,33), demonstrate that liver ADH was the main via oxidizing ethanol in both sham-operated and rats undergoing PH. However, since the magnitude of the inhibitory effect of 4-MP on ethanol oxidation was not the same in both experimental groups (Figs. 1 and 2), it could indicate that other ethanol-metabolizing systems are actively participating in

removing ethanol from the systemic circulation. Further support for this possibility is the finding that 4-MP (at concentrations inhibiting up to 85% ADH) elicited a 80% and 55% inhibition of ethanol oxidation by isolated hepatocytes from control and rats subjected to PH, respectively (unpublished data).

On the other hand, it was quite surprising that 4-MP, *per se*, induced a dose-dependet inhibition of the mitotic index in remnant livers, when administered to PH-rats (Table 1). In addition, 4-MP effects, seen in remnant livers, on histological parameters such as fatty infiltration and hepatocellular organization, closely resembled those found in ethanol-treated rats subjected to PH (21). High pyrazole doses induce a hepatotoxic effect when given to intact rats (34,35), as well as decrease levels of brain noradrenaline (36). Similar doses of 4-MP seem to be safely used even in healthy human subjects (37-39). For instance, 4-MP use has been reported as a successful therapeutical approach, for the treatment of methanol or ethylene glycol poisoning (40,41).

Present results also indicate that 4-MP administration does not promote structural alterations in the quiescent (normal) liver (Fig. 3); however, this compound did modify liver function in control animals. The ADH inhibitor induced a marked decrease in serum glucose level and enhanced the level of serum triacylglycerols (Table 2). A slight but significant increase in serum LDH activity was also associated to 4-MP administration to sham-operated animals (Fig. 4). However, administration of 4-MP to animals subjected to PH had a quite different pattern of effects on the regenerating liver.

4-MP induced an even larger enhancement of serum triacylglycerols, while potentiating the PH-induced hypoglycaemia. In addition, at the higher 4-MP doses, serum albumin was significantly decreased and levels of bilirubin were lower than in control and PH-rats receiving saline (Table 2). In intact rats, 4-MP by itself produces a slight accumulation of liver triacylglycerols, leading to a mild fatty liver (42). However, in the regenerating liver, 4-MP decreased PH-induced fatty liver due to a drastic release of liver

triacylglycerols (Table 2 and Fig. 3). Although the mechanism underlying the decreasing effect of 4-MP in serum glucose and albumin levels is not known, the effects of the ADH inhibitor on serum metabolites present in PH-rats closely resembled those found in animals subjected to PH and treated acutely with ethanol (21). We have also shown that PH induces a selective increase in serum enzyme activities, considered as a "markers" of liver functional integrity (21). This PH-induced effect was not related to hepatocellular damage and agrees with the finding that the regenerative process did not lead to necrosis or inflammation, even in animals receiving alcohol (43). Indeed, in PH-rats, acute ethanol administration abruptly shortened the PH-associated selective increase in serum LDH, ALT, and AST activities (21). In the present study, 4-MP administered to PH-rats also evoked a drastic diminution in the serum activities of some liver marker enzymes (Fig. 4). This effect of 4-MP gives further support to the hypothesis that the selective increase of serum enzyme activities after PH, instead of being associated to cell necrosis is rather an event associated to the onset of liver regeneration, probably influencing the progress of the proliferative process. Hence, 4-MP by itself can mimic many effects associated to the inhibitory action of acute ethanol on PH-induced liver regeneration (16,21).

There is growing evidence that acute ethanol, as has been amply suggested for chronic feeding, could mute cellular responses to growth factor stimulation. For instance, ethanol treatment masked and/or delayed the increase in specific activity of DNAase II and RNAase II, decreasing calcium concentration and hepatic accumulation of cyclic AMP at early stages of liver regeneration (44,45) These effects could be involved in the ethanol-promoted blunted and delayed response of tyrosyl phosphorylation of insulin receptors (IRS-1), blockade of interleukine-6 (IL-6) induced Stat3 protein activation, as well as inhibition of prolongation of the activation of p42/44 MAPK (46-48). The latter might result in disruption of proto-oncogene expression near the restriction point at G_1/S boundary of the cell cycle in hepatocytes, leading to arrested compensatory proliferation after PH (49).

Although both acute and chronic ethanol exposure inhibit or delay liver regeneration induced by PH in the rat, it would be expected that the underlying mechanism(s) involved in ethanol's actions could differ according to the experimental model tested. We propose that ethanol oxidation by liver ADH activity is a major contributing factor in the inhibitory effect in liver proliferation during acute administration of ethanol to rats subjected to PH. Despite not having conclusive evidence, we believe that ethanol interferes with the adaptative changes in the flux of substrates and coenzymes through ADH, which could be a limiting-step in the metabolic adjustment required to drive compensatory cell proliferation after two-thirds PH.

In summary, through *in vivo* administration of 4-MP, a specific ADH inhibitor, to rats subjected to PH, it was found that the accelerated ethanol oxidation by the remnant liver cannot be accounted for only by ADH participation, but rather by the active participation of another ethanol-metabolizing pathway in ethanol elimination by the regenerating liver. In addition, 4-MP by itself was capable of inducing a dose-dependent inhibition of the mitotic capacity of the remnant liver after PH, which closely correlated with the magnitude of inhibition of the specific activity of ADH. The important role of liver ADH activity during liver proliferation after PH in the rat is further supported by the notion that 4-MP can mimic the inhibitory effect of ethanol on PH-induced liver regeneration.

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FIGURE LEGENDS

Figure 1. Effect of 4-methylpyrazole administration on blood alcohol concentrations (BACs) in sham-operated and rats subjected to PH after orally ethanol treatment.

Results are expressed mean ± SE of at least five animals per experimental group. Panel A depicts BACs obtained from sham-operated and in animals undergoing PH after intragastric ethanol administration and treated with saline solution. In panel B, control and PH-rats receiving the same i.g. ethanol dose (1.5 mg/kg b.w.) and treated with 4-MP (200 mg/kg b.w.). Note the participation of first-pass metabolism of ethanol (panel A) in rats subjected to PH and non-treated with 4-MP.

Figure 2. Calculated BACs and areas under curve (AUCs) of blood ethanol levels in sham-operated and rats subjected to PH and treated with 4-methylpyrazole.

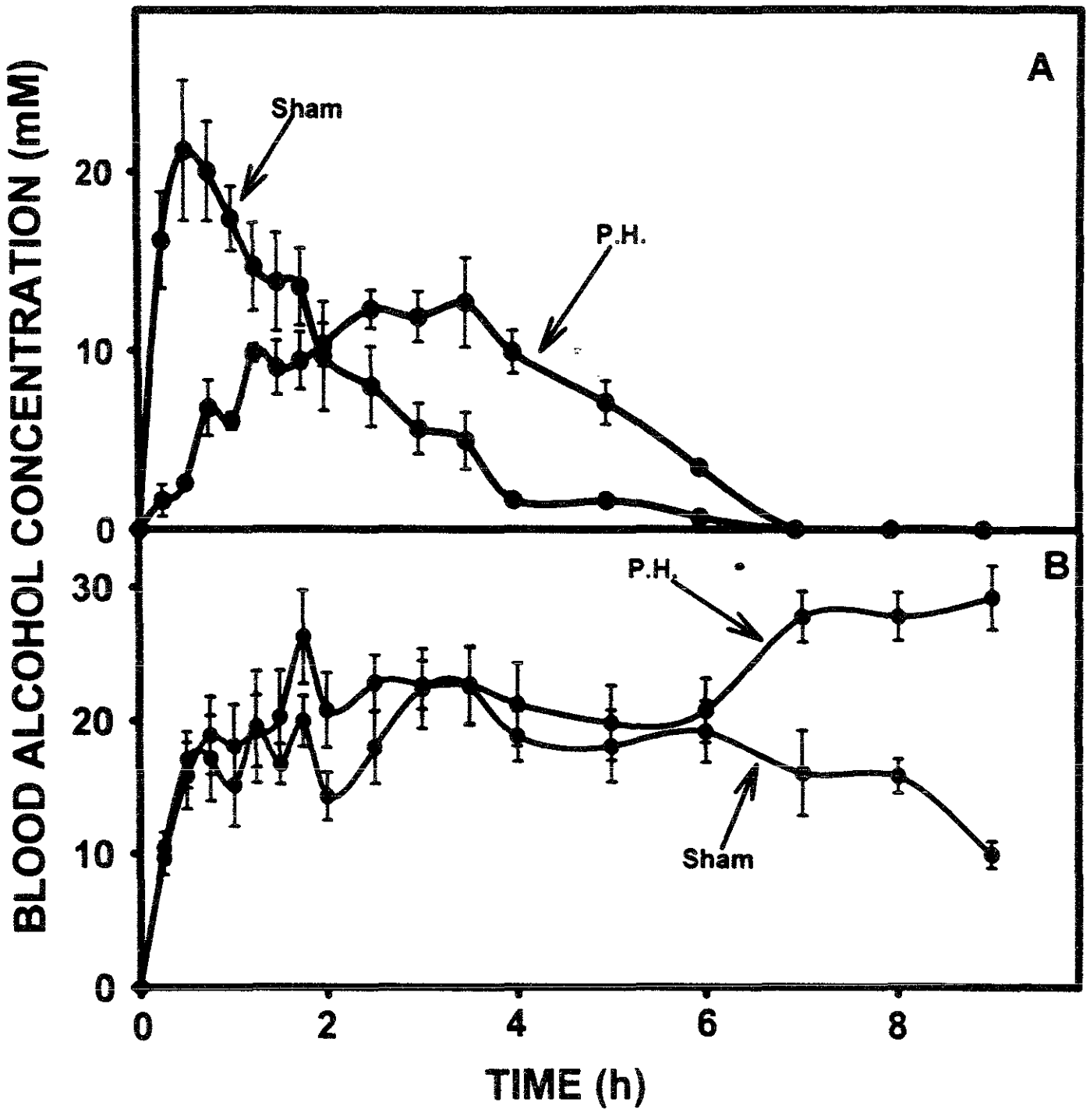
Maximal BACs and AUCs were calculated as described in the Material and Methods section with data taken from Fig. 1 for sham-operated rats (open bars) and for animals subjected to PH (dashed bars). Statistical significance as follows: **p<0.01 vs. control groups, and **p<0.01 against PH + saline group.

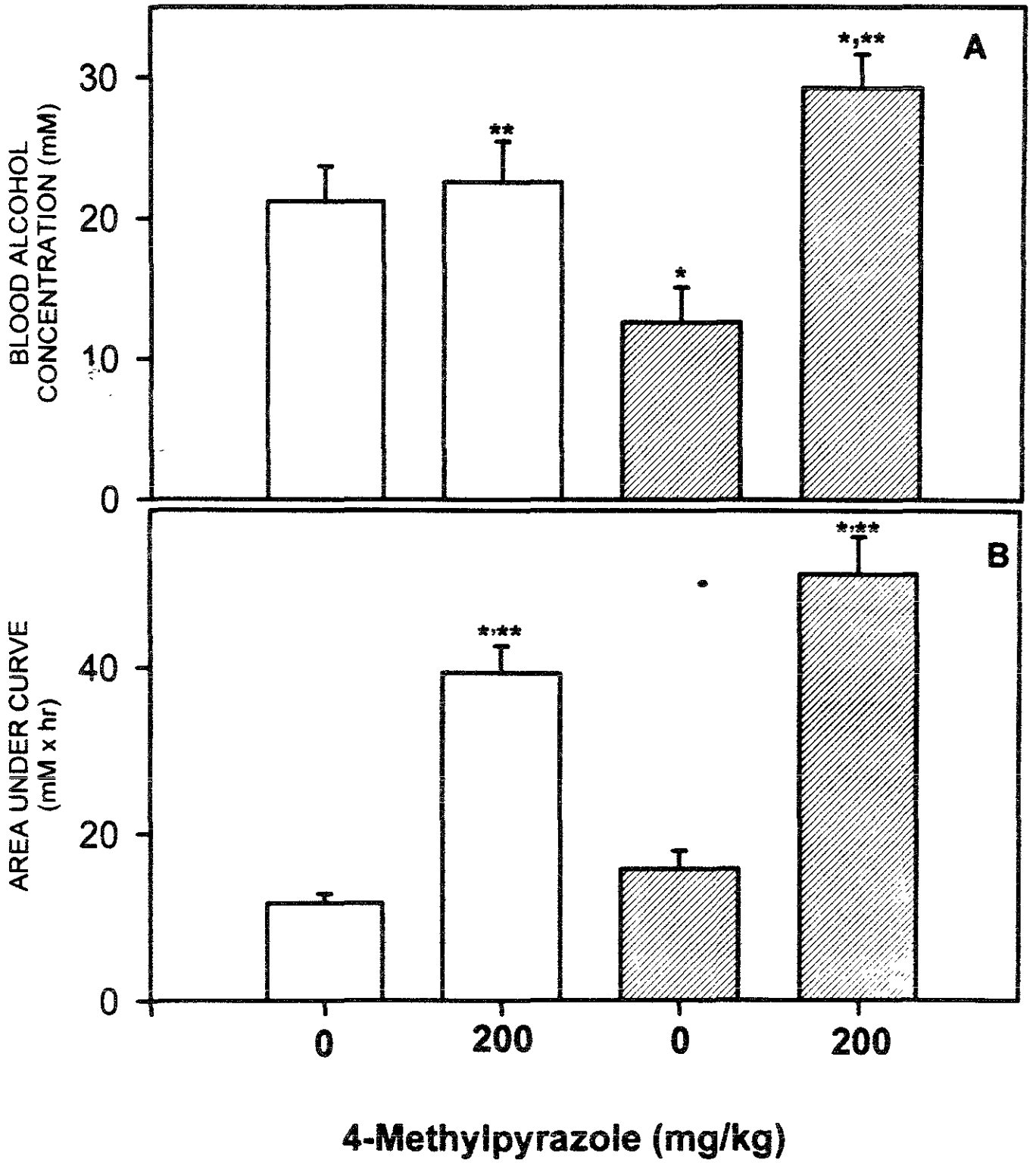
Figure 3. Histological assessment of livers from rats subjected to PH and treated with a high dose of 4-methylpyrazole.

Representative micrographs are shown of livers taken from control (A) and partial hepatectomized rats (B) treated with saline, and those of sham-operated (C) and rats subjected to PH (D) and receiving a single dose of 4-MP (200 mg/kg b.w.) Liver samples were obtained 24 h after surgery and treatments. Arrows indicate mitotic images, while arrowshead denote fatty infiltration.

Figure 4. Serum activities of aspartate (AST) and alanine (ALT) aminotransferases, and of lactate dehydrogenase (LDH) in hepatectomized rats treated with 4-MP.

Results are expressed mean ± SE of at least five animals per experimental group. Activities of ALT, AST and LDH were determined by spectrophotometric and colorimetric methods in sera obtained 24 h after 4-MP treatment in sham-operated rats (open bars) and in animals undergoing PH (dashed bars). Statistics as indicated in Fig. 2.


A
Sham
P.H.
20
10
0
B
P.H.
Sham
30
20
10
0
0
2
4
6
8
TIME (h)
BLOOD ALCOHOL CONCENTRATION (mM)


A
B
BLOOD ALCOHOL CONCENTRATION (mM)
AREA UNDER CURVE (mM x hr)
30
20
10
0
40
20
0
*,**
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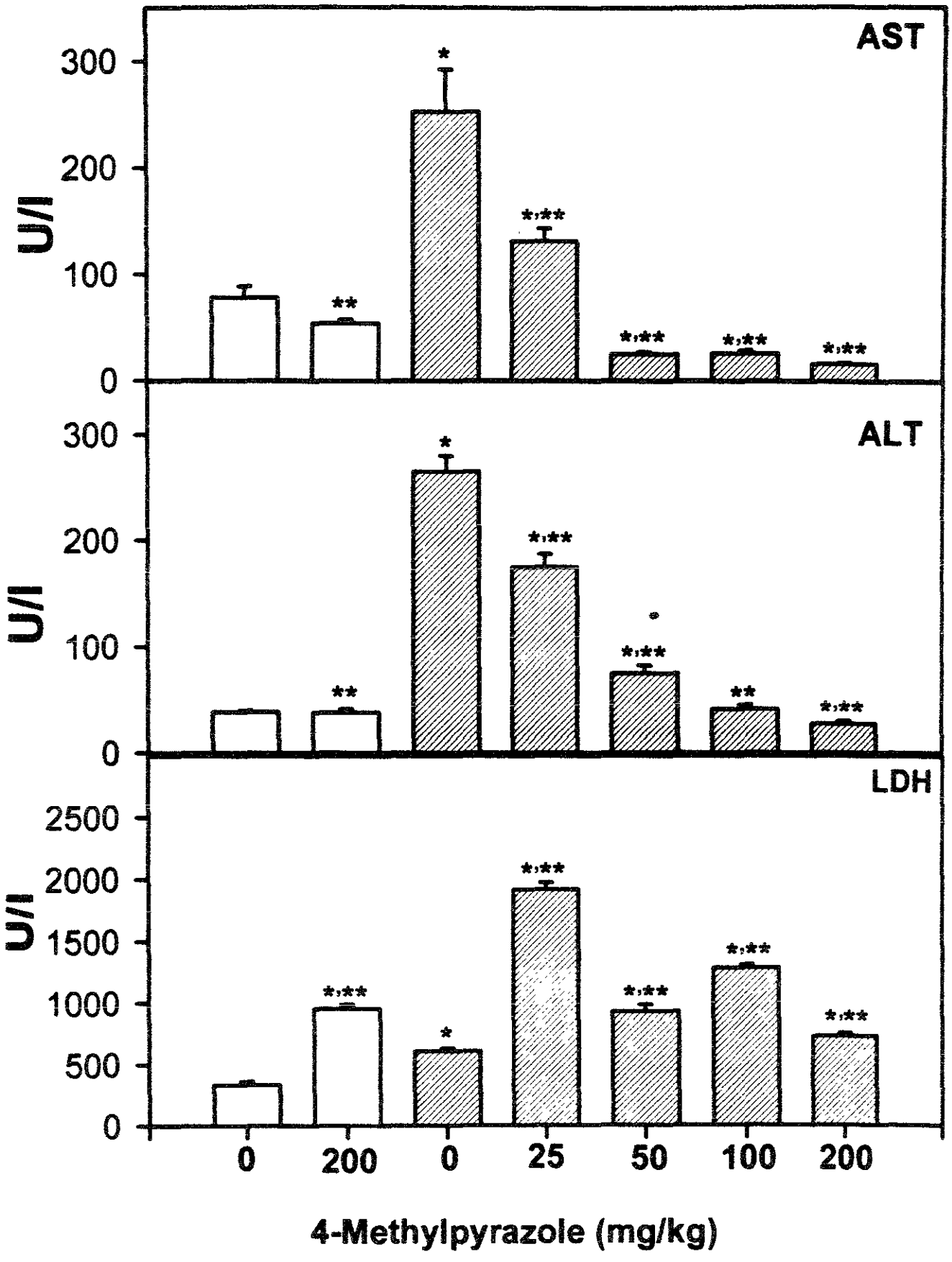
*
*,**
*,**
0
200
0
200
4-Methylpyrazole (mg/kg)


AST
ALT
LDH
U/l
300
200
100
0
2500
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0
200
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25
50
100
200
4-Methylpyrazole (mg/kg)

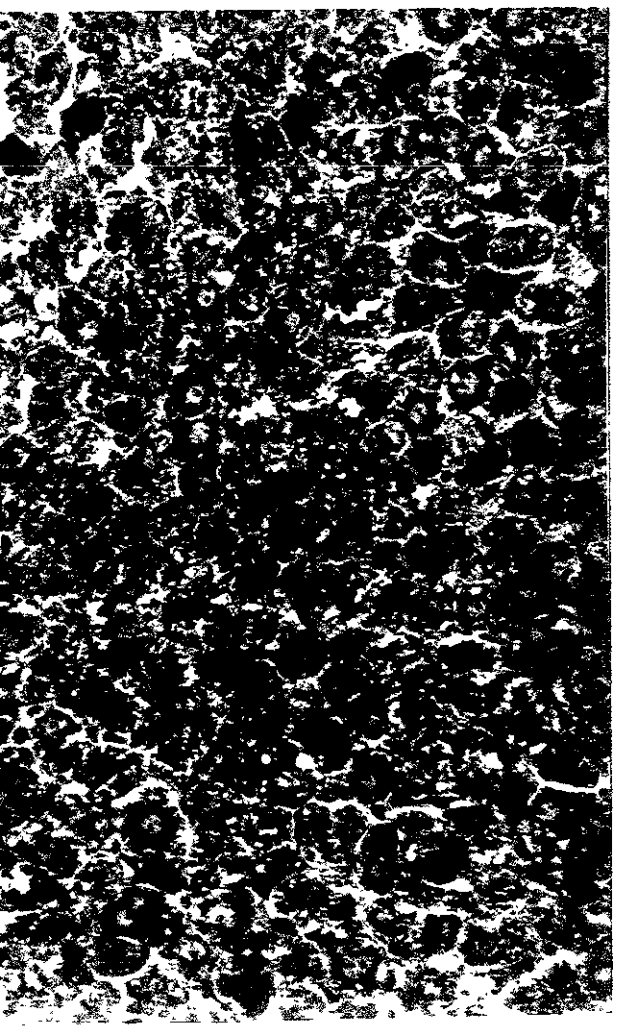

Table 1. Liver histopathological changes in rats subjected to partial hepatectomy and treated with 4-methylpyrazole (4-MP).

Treatment	Fatty change	Inflammation	Hepatocellular Disorganization	Mitotic Index
Controls				
-Plus saline	0	0	0	0
-Plus 4-MP (200 mg/kg)	+	0	+	0
Partial Hepatectomy				
-Plus saline	+	0	+/++	++
-Plus 4-MP				
25 mg/kg	++	0	+/++	+/++
50 mg/kg	++	+/++	+/++	+
100 mg/kg	+	+/++	+	0
200 mg/kg	+	++	+	0

Histopathological parameters were evaluated as follows: for fatty change, hepatocellular disorganization and inflammation, (0) absent; (+) mild; (++) moderate, and (+++) strong. Assessment of the mitotic index is described in Material and Methods.

Table 2. Effects of 4-methylpyrazole on liver ADH activity and serum metabolite levels in rats subjected to partial hepatectomy.

Treatment	ADH activity ($nmol \bullet min^{-1} \bullet mg^{-1}$)	Glucose (mg/dL)	T. G. (mg/dL)	Albumin (g/dL)	Bilirubin (mg/dL)
Controls					
-Plus saline	48.1 ± 1.8	102 ± 4	57 ± 7	4.7 ± 0.4	0.42 ± 0.04
-Plus 4-MP (200 mg/kg)	8.5 ± 1.0*	54 ± 6*	92 ± 6*	3.8 ± 0.3	0.32 ± 0.03
Partial Hepatectomy					
-Plus saline	36.7 ± 1.5*	83 ± 4*	92 ± 10*	4.1 ± 0.4	0.72 ± 0.06*
-Plus 4-MP					
25 mg/kg	24.5 ± 2.0*,**	70 ± 4*	136 ± 7*,**	3.1 ± 0.3*	2.91 ± 0.25*,**
50 mg/kg	28.3 ± 2.5*,**	68 ± 5*	116 ± 16*	3.2 ± 0.3*	1.25 ± 0.11*,**
100 mg/kg	17 4 ± 2.7* **	61 ± 5*,**	143 ± 12*,**	3.6 ± 0.4	0.29 ± 0.09**
200 mg/kg	10.3 ± 1.1*,**	45 ± 5*,**	111 ± 13*	3.5 ± 0.3	0.10 ± 0.02*,**

The results are expressed as mean ± SE of five individual determinations per experimental group. Liver ADH activity (per mg of protein) was determined by a spectrophotometric method, and serum metabolies by means of Diagnotic kits from Sigma Chemical Co. (St. Louis, MO). T.G.: Triacylglycerols. Statistics: *$p<0.01$ as compared to control rats; **$p<0.01$ vs. the hepatectomized group treated with saline solution.

CONCLUSIONES GENERALES

- En el primer paso metabólico (FPM) del etanol, el estómago tiene un papel transitorio, pero efectivo en las modificaciones farmacocinéticas del etanol a tiempos tempranos posteriores a la hepatectomía parcial en la rata, constituyendo una barrera protectora contra los efectos adversos del etanol en esos animales.
- El hígado en regeneración incrementa su capacidad de oxidar al etanol
- El aumento del metabolismo del etanol puede potenciar los efectos deletéreos que la droga ocasiona a la regeneración hepática.
- Los cambios coordinados en el metabolismo del etanol por el estómago y el hígado muestran una interesante interrogante de una comunicación interórganos que ocurre durante la proliferación celular del hígado, después a una hepatectomía parcial.

- Una dosis aguda de etanol es capaz de inhibir constantemente la regeneración hepática que se induce por la HP, la cual es dependiente del tiempo y ruta de administración.
- La administración intragástrica de etanol ocasiona más inhibición al proceso de la regeneración hepática que la ruta intraperitoneal; esto está probablemente relacionado con las diferencias hepáticas en el catabolismo del etanol durante la regeneración hepática, la cual varía de acuerdo a la ruta de administración.
- Se han dado las primeras evidencias de una liberación selectiva de enzimas por parte del hígado durante la regeneración hepática.

- La administración de dosis bajas de etanol en ratas sujetas a HP ocasiona diferentes modificaciones en la ultraestructura del hígado regenerante, dependiendo fuertemente de la ruta de administración.
- La administración intragástrica de etanol disminuye prácticamente todos los cambios adaptativos de los hepatocitos regenerantes.
- La administración intraperitoneal de etanol promueve modificaciones ultraestructurales, indicativas de proliferación celular.

- **La administración de dosis bajas de 4-MP (25 y 50 mg/kg) en ratas HP, se asocia con ligeros cambios en la actividad de la ADH hepática y en cambios metabólicos que disminuyen ligeramente la regeneración hepática.**
- **La administración de dosis altas de 4-MP (100 y 200 mg/kg)en ratas sujetas a HP, disminuye importantemente la actividad de la ADH hepática, la cual se asocia con cambios en la función y la estructura que precede aparentemente a los hepatocitos para la replicación celular.**
- **Estudios en el comportamiento de la ADH hepática se pueden utilizar para identificar la patogenia de la inhibición de la regeneración hepática que se conoce ocurren en la enfermedad hepática por alcohol en el humano.**

PERSPECTIVAS

I. Frank y cols. (1979), con sus experimentos demuestra el papel de la molécula *per se* del etanol como causante del daño a la regeneración hepática; mientras que, por otra parte, Duguay y cols. (1982) y Wands y cols. (1979), encuentran en sus trabajos que el metabolismo del alcohol es el causante de los efectos inhibitorios en la regeneración hepática. Lo anterior, aunado a nuestros datos, en donde se demuestra la importancia de la ruta y el tiempo de administración del etanol, hacen que sean necesarios más experimentos encaminados a explorar los probables mecanismos de inhibición de la regeneración hepática por el etanol.

II. Una de las interrogantes, de los resultados obtenidos es saber por que el etanol ocasiona diferentes efectos, dependientes de la ruta de administración, en la inhibición de la regeneración hepática, por lo tanto se plantean las siguientes hipótesis:

A. Se conoce que el etanol es capaz de alterar el estado metabólico de los tejidos y por otra parte, se conoce que el EGF es un mitógeno que participa en tiempos muy tempranos (0-8 hrs) de la regeneración hepática inducida por HP y es producido por las glándulas de Brunner que se localizan en el duodeno. De modo, que si la ruta intragástrica de administración del etanol inhibe más la regeneración hepática que la ruta intraperitoneal, ello probablemente se deba a que la ruta intragástrica daña a las glándulas de Brunner y por ende, la producción de EGF.

B. La ADH hepática, es el principal sistema de oxidación de etanol en el hígado y se ha relacionado con el catabolismo de las catecolaminas, específicamente con la noradrenalina, el cual es un conocido co-mitógeno, que participa en la proliferación celular hepática. Si durante la regeneración hepática, la administración de etanol intraperitonealmente ocasiona menor inhibición del proceso replicativo del hígado que la ruta intragástrica, esto probablemente se deba a que la ADH hepática por estar metabolizando etanol deja de catabolizar a la noradrenalina y esta continúa con su papel de co-mitógeno.

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ANEXOS

El primer anexo es un artículo que se publicó en la revista del Hospital Juárez de México, con algunos datos parciales obtenidos hasta ese momento, sobre la farmacocinética del etanol durante la regeneración hepática.

El segundo anexo, es un trabajo sobre el estado redox y energético del hígado durante la regeneración hepática y el efecto del etanol sobre estos parámetros. Si bien, no es un artículo directamente de mi protocolo de investigación del doctorado, si es un trabajo que se realizó en el grupo del Dr. Rolando Hernández Muñoz, en donde participé.

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La participación del estómago en el metabolismo del etanol

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RESUMEN

El hígado es el principal responsable del metabolismo del etanol (85-90%). El metabolismo extrahepático del etanol es realmente insignificante, a excepción del estómago en donde se ha encontrado un efecto de primer paso que constituye una barrera gástrica protectora cuyo proceso determina, en parte, la biodisponibilidad del etanol y modula su toxicidad sistémica y hepática.
Se utilizaron ratas Wistar que fueron hepatectomizadas parcialmente (HP) y se les administró etanol por vía intragástrica o intraperitoneal, a diferentes tiempos de regeneración hepática (0-96 h). Se determinó la concentración de etanol y se calculó el área bajo la curva del curso temporal de etanolemia, evaluándose la absorción y eliminación de etanol a partir de estas curvas.
Los resultados muestran una pérdida del componente de absorción y retardo en la eliminación de etanol en el grupo HP que lo recibió por vía oral; mientras que con la vía intraperitoneal no se modificaron los niveles máximos de etanol y sí se encontró un aumento en su eliminación. Se concluye que se modifica el componente de primer paso del estómago causado por la pérdida hepática, y que el hígado en regeneración posee una gran capacidad para oxidar etanol.

Palabras clave: Alcohol, farmacocinética, primer paso de oxidación, alcohol deshidrogenasa.

ABSTRACT

The liver is the main organ responsible for ethanol metabolism and accounts for 85 to 90% of its oxidation. Extra-hepatic ethanol metabolism is negligible, except for the stomach, where the first pass metabolism of ethanol accounts for the disposal of an important fraction of the ingested ethanol, modulating both bioavailability and sistemic effects of ethanol.
Male Wistar rats subjected to two-thirds hepatectomy were used. In these animals, ethanol was administered by either intragastric and intraperitoneal routes, at several times post-surgery. Ethanol concentration in blood was assayed and the area under curve (AUC) was determined.
The results show that both absorption and elimination of ethanol were changed after partial hepatetomy (PH), when ethanol was administered intragastrically to these rats. When ethanol was given intraperitoneally maximal levels of blood ethanol were unchanged, but its elimination was increased. Data strongly suggest that removal of liver mass exerts an important influence in the gastric first pass metabolism of ethanol, and regenerating liver has an enhanced capacity for oxidizing ethanol.

Key words: *Alcohol, farmacokinetics, first pass metabolism, alcohol dehydrogenase.*

INTRODUCCIÓN

El alcoholismo es uno de los problemas de salud más importantes en el mundo,[1] causa de multitud de complicaciones médicas y constituye un preocupante y creciente problema de salud pública en nuestra sociedad,[2-4] que ocupa uno de los primeros lugares de morbimortalidad entre la población

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económicamente activa de nuestro país.[5] Se sabe que el 75% de las muertes atribuibles al alcoholismo crónico es resultado de cirrosis hepática; por lo tanto la enfermedad hepática inducida por alcohol representa un problema médico de primera magnitud tanto por su frecuencia como por su gravedad.

El etanol es fácilmente absorbido en el estómago e intestino delgado y más del 90% es metabolizado por el hígado, ya que posee tres sistemas principales bien definidos de eliminación y metabolismo del etanol,[6-8] que son: sistema de las enzimas alcohol deshidrogenasas (ADH); sistema microsomal de oxidación del etanol (MEOS); y el sistema de la catalasa.[8,9] El resto del etanol se metaboliza por otros tejidos (riñón, músculo, estómago, pulmón, etc.), principalmente por la acción de la ADH.

El metabolismo extrahepático del etanol es realmente insignificante, a excepción del estómago en donde se ha encontrado a la enzima ADH en la mucosa gástrica.[10-12]

La ADH gástrica contribuye a la oxidación del etanol ingerido y por ende disminuye los niveles séricos de etanol, lo que se considera como el primer paso metabólico del etanol, que constituye una barrera gástrica protectora contra los efectos adversos del mismo.[13-15]

Este primer paso del metabolismo del etanol por el estómago es una determinante importante en las concentraciones sanguíneas de alcohol (etanolemia) y de las áreas bajo la curva (ABC) de alcohol en la circulación sistémica alcanzados por la ingesta de etanol, tanto en humanos[13-16] como en animales experimentales.[13,14]

La actividad de la ADH gástrica se encuentra afectada por el género,[12,17] edad,[17] ayuno,[10] alcoholismo crónico,[10,12] algunas drogas[18-20] y por microorganismos,[21] esto da como resultado el aumento en los niveles de la etanolemia y de las ABC posteriores al consumo de alcohol[18-20,22] que produce un incremento en la susceptibilidad de daño hepático en relación a los niveles de etanol alcanzados.

Estudios recientes demuestran diferencias en las concentraciones de etanol en sangre después de administrarlo por vía oral en comparación con la vía intravenosa, donde se puede observar que las concentraciones de alcohol en sangre son más bajas después de administrarlo intragástricamente en comparación con la administración por vía intravenosa con una misma dosis de alcohol tanto en humanos[10] como en ratas.[13,14] Se concluye que esta diferencia se debe al primer paso metabólico del alcohol, debido a la actividad de la ADH gástrica, que tiene una isoenzima con una alta Km para el etanol que no exhibe una inhibición de sustrato con altas concentraciones de etanol, como ocurre con la ADH hepática.[15]

Más aún, Roine y cols.[23] al utilizar diferentes vías de administración de etanol (intravenosa, intraduodenal, intraportal), en un análisis comparativo de la farmacocinética de la vía intragástrica con estas diferentes rutas, encontraron que en la vía intragástrica, las etanolemias son inferiores que cuando se administra por otras rutas. De aquí que el primer paso metabólico se encuentra asociado con concentraciones inferiores de etanol en la circulación sistémica, así como una menor biodisponibilidad del etanol. Por otra parte Smith y cols.[24] sugieren que un lento índice de absorción podría realizar el primer paso del metabolismo hepático y concluyó que el metabolismo del etanol por el estómago es en realidad insignificante. Por lo tanto, el presente estudio se diseñó para determinar la participación del estómago en la farmacocinética y farmacodinamia del etanol en ratas hepatectomizadas parcialmente (HP), donde se elimina el componente hepático; y evaluar la capacidad del hígado para metabolizar el alcohol basado en las concentraciones de etanol alcanzados en la circulación sistémica (etanolemia) tanto para la vía oral como para la vía intraperitoneal.

MATERIAL Y MÉTODOS

Animales. Se utilizaron ratas macho de la cepa Wistar (obtenidas del bioterio del Instituto de Fisiología Celular, UNAM), con un peso promedio de 250 ± 20 gramos. Son alimentadas con dieta balanceada para roedores (# 5001, PMI Feeds Inc., St.Louis, MO) y agua *ad libitum*, excepto el día del experimento.

Procedimiento quirúrgico. Se realizó hepatectomía parcial (HP) del 68 ± 2% acorde a la técnica clásica de Higgins y Anderson.[25] Se utilizó como anestésico el éter etílico por el tiempo necesario para realizar la HP (15 minutos aproximadamente). Se realizó una incisión en la parte media del abdomen de aproximadamente 2.5-3 cm, retirando los lóbulos medio y lateral izquierdo del hígado. Las cirugías se realizaron entre las 09:00-10:00 a.m.

Grupos. Los animales experimentales se dividieron en dos grupos dependiendo la vía de administración del etanol. Al primer grupo con hepatectomía parcial se le administró etanol por vía intragástrica (HP + EtOH IG), a una dosis de 1.5 g/kg de peso en una

ución al 40%, a diferentes tiempos de regenera-
n hepática (0-96 h). Al segundo grupo con
patectomía parcial se le administró el etanol por
ección intraperitoneal (IP) (HP + EtOH IP) a la
sma dosis y concentración, así como a los mis-
s tiempos de regeneración que al grupo uno.
mo controles se utilizaron ratas falsamente ope-
las (sham), a las cuales únicamente se les mani-
a el hígado, regresándolo a la cavidad abdomi-
y administrándoles la misma dosis de etanol.

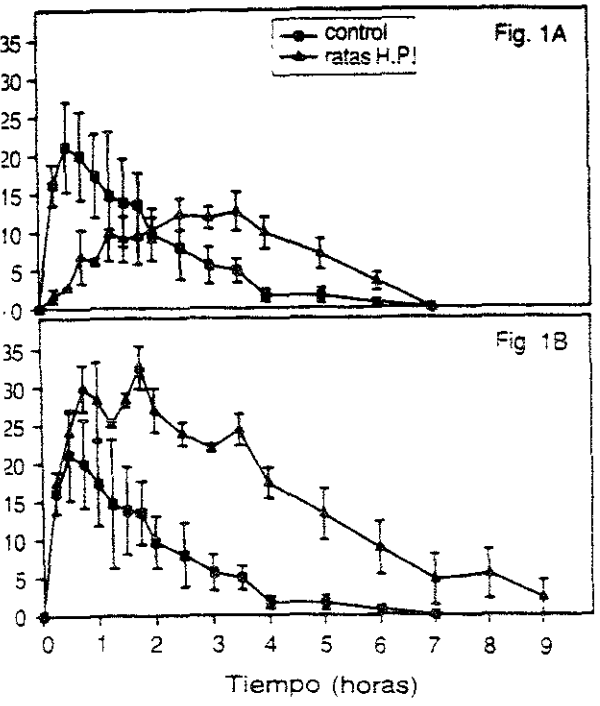
ocedimiento experimental. Las ratas se coloca-
en jaulas de restricción donde se realizó una
nción de la punta de la cola para tomar muestras
sangre en tubos capilares de 50 µL cada 15 mi-
os las primeras dos horas, cada 30 minutos las
uientes dos horas y cada 60 minutos hasta que el
ohol no fue detectable. Las muestras de sangre
desproteinizaron con ácido perclórico (6% p/v) y
cantidad de alcohol se determinó por medio de un
tema enzimático con alcohol deshidrogenasa pu-
cada, tal como lo describió Bert y Gutmann.[26]

ocedimiento computacional. Las áreas bajo la
rva (ABC) fueron calculadas por el método del
pezoide, desde la administración de etanol hasta
iempo en donde no fue detectable.[27] La cantidad
de alcohol absorbido en función de tiempo (Qt) se calculó desde el tiempo t = 0 hasta t = T (donde el etanol ya no fue detectable en la sangre).

Análisis estadístico. Los resultados se expresan como el promedio ± EE. Las diferencias significativas entre los grupos fueron analizadas por Student's *t* test. Los efectos significativamente diferentes de las concentraciones de etanol o ABCs fueron sujetas a análisis siguiendo t Benferroni's test.[28]

RESULTADOS Y CONCLUSIONES

Los resultados muestran que en la administración de alcohol por vía intragástrica, el grupo HP al tiempo inicial (0 h), presentó una absorción y eliminación de etanol más lenta en comparación con el grupo control, como se muestra en la *figura 1A*. En la *figura 1B* se puede observar que a tiempos tempranos de regeneración (12-24 h), la absorción fue más rápida y la eliminación seis veces más lenta en comparación al grupo control; mientras que a tiempos tardíos de regeneración (48-96 h), se recuperó el componente de absorción y la eliminación fue 2.5 veces más lenta en relación con el control *(Figura 2A)*. La concentración de etanol se afectó en los di-



ura 1. Etanolemias en la vía intragástrica, durante la re-
eración hepática. En **A**, tiempos iniciales 0 horas, y en **B**
ipos tempranos de 12-24 horas, postquirurgicos, HP *vs*
trol

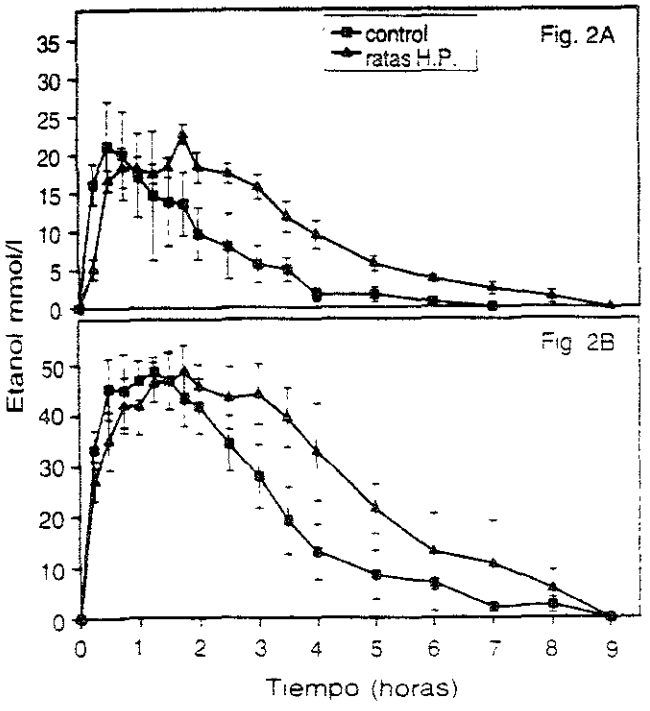


Figura 2. En **A**, etanolemias de tiempos tardíos (48-96 horas) de regeneracion hepatica via intragastrica HP *vs* control. En **B** etanolemias de tiempo inicial (0 horas) de regeneración, vía intraperitoneal HP *vs* control

ferentes tiempos de regeneración hepática cuando se administró por vía intragástrica, como lo demuestran las siguientes áreas bajo la curva (ABC): 0 h: 51.6 ± 0.95; 12 h: 130.9 ± 2.90; 24 h: 150.4 ± 1.85; 48 h: 80.6 ± 2.07; 72 h: 68.9 ± 2.03; 96 h: 75.0 ± 1.65; con una n = 4 en cada tiempo.

Así mismo, las concentraciones máximas de etanol en sangre (BAC) en los diferentes tiempos de regeneración fueron las siguientes: 0 h: 12.6 ± 2.5; 12 h: 35.4 ± 1.8; 24 h: 29.7 ± 2.0; 48 h: 24.0 ± 1.5; 72 h: 24.2 ± 0.4; 96 h: 20.7 ± 2.7. El control para este grupo tuvo los siguientes datos ABC 45.7 ± 1.87 n = 6, y BAC de 21.1 ± 5.9.

Se obtuvieron diferencias significativas importantes en las ABC en cada uno de los diferentes tiempos de regeneración en comparación con el control: 0 h: $p < .05$; 12 h: $p < .001$; 24 h: $p < .001$; 48 h: $p < .001$; 72 h: $p < .001$; 96 h: $p < .001$

Al cambiar la vía de administración de intragástrica a intraperitoneal, se obtuvieron los siguientes resultados: en el tiempo inicial de HP (0 h), no hubo diferencias en los niveles máximos de etanol y la eliminación fue más lenta en comparación con el grupo control; sin embargo, si se realiza el cálculo por gramo de hígado existente, el hígado en regeneración eliminó más rápidamente el alcohol circulante *(Figura 2B)*. A tiempos tempranos de regeneración (12-24 h), se alcanzaron niveles inferiores de etanol, pero la eliminación se retrasó en el grupo HP *(Figura 3A)*. Por último, en los tiempos tardíos de regeneración (48-96 h) la eliminación fue 50% más rápida en relación con el control *(Figura 3B)*. Por otra parte las ABC y BAC que se obtuvieron al utilizar la vía intraperitoneal (IP) fueron las siguientes, ABC: 0 h: 225.2 ± 3.55; 12 h: 180.3 ± 2.76; 24 h: 246.8 ± 2.40; 48 h: 104.4 ± 3.88; 72 h: 105.6 ± 2.53; 96 h: 81.6 ± 3.46; con una n = 4 en cada tiempo.

Las BAC alcanzadas en los diferentes tiempos de regeneración en el grupo que recibió el etanol por vía IP son las siguientes: 0 h: 49.5 ± 3.3; 12 h: 40.2 ± 6.3; 24 h: 48.4 ± 6.4; 48 h: 44.5 ± 3.2; 72 h: 39.2 ± 2.6; 96 h: 38.3 ± 3.4.

En el control para estos tiempos de regeneración hepática se obtuvo una ABC de 163.2 ± 4.42 n = 6, con una BAC de 48.7 ± 2.8

Las diferencias en las ABC resultaron significativas en comparación con el control: 0 h: $p < .001$; 12 h: $p < .025$; 24 h: $p < .001$; 48 h: $p < .001$; 72 h: $p < .001$; 96 h: $p < .001$

Se concluye que el hígado en regeneración posee una gran capacidad para oxidar etanol, lo que exacerbaría los efectos tóxicos de dicho fármaco. Además, es de notar que la pérdida de la masa hepática influye gradualmente en la absorción gastrointestinal del alcohol, poniendo de manifiesto la comunicación que existe entre el estómago y el hígado, modificando el componente de primer paso del estómago causado por la hepatectomía parcial.

Por último, el primer paso metabólico participa importantemente en las modificaciones de la biodisponibilidad del etanol posterior a la hepatectomía parcial, y presumiblemente en el catabolismo del etanol que se realiza en el estómago. Por otra parte, el metabolismo del etanol que se muestra durante la regeneración hepática puede deberse a la existencia de diversos factores que regulan la farmacocinética del etanol en ratas sujetas a hepatectomía parcial.

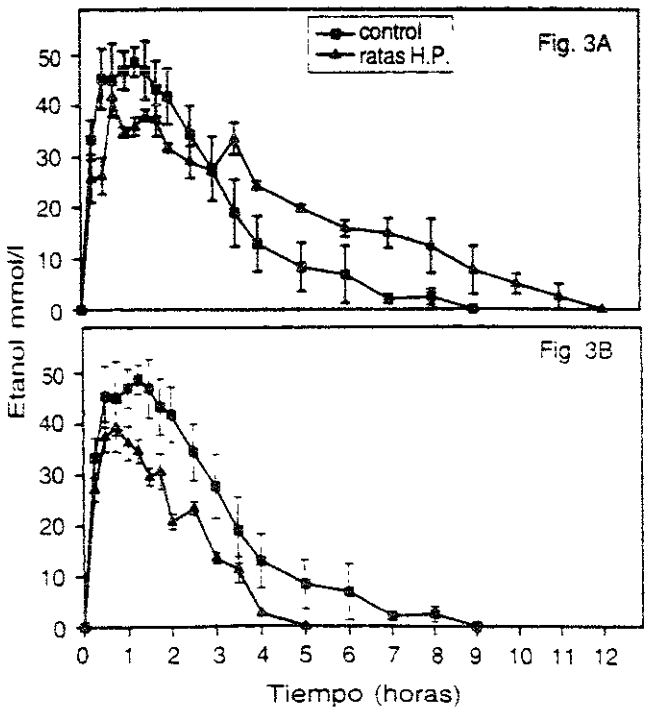


Figura 3. Etanolemias en la vía intraperitoneal, durante la regeneración hepática. En **A**, tiempos tempranos de 12-24 horas, y en **B**, tiempos tardíos de 48-96 horas, postquirúrgicos. HP *vs* control.

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Redox State and Energy Metabolism during Liver Regeneration

ALTERATIONS PRODUCED BY ACUTE ETHANOL ADMINISTRATION

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ABSTRACT. Ethanol metabolism can induce modifications in liver metabolic pathways that are tightly regulated through the availability of cellular energy and through the redox state. Since partial hepatectomy (PH)-induced liver proliferation requires an oversupply of energy for enhanced syntheses of DNA and proteins, the present study was aimed at evaluating the effect of acute ethanol administration on the PH-induced changes in cellular redox and energy potentials. Ethanol (5 g/kg body weight) was administered to control rats and to two-thirds hepatectomized rats. Quantitation of the liver content of lactate, pyruvate, β-hydroxybutyrate, acetoacetate, and adenine nucleotides led us to estimate the cytosolic and mitochondrial redox potentials and energy parameters. Specific activities in the liver of alcohol-metabolizing enzymes also were measured in these animals. Liver regeneration had no effect on cellular energy availability, but induced a more reduced cytosolic redox state accompanied by an oxidized mitochondrial redox state during the first 48 hr of treatment; the redox state normalized thereafter. Administration of ethanol did not modify energy parameters in PH rats, but this hepatotoxin readily blocked the PH-induced changes in the cellular redox state. In addition, proliferating liver promoted decreases in the activity of alcohol dehydrogenase (ADH) and of cytochrome P4502E1 (CYP2E1); ethanol treatment prevented the PH-induced diminution of ADH activity. In summary, our data suggest that ethanol could minimize the PH-promoted metabolic adjustments mediated by redox reactions, probably leading to an ineffective preparatory event that culminates in compensatory liver growth after PH in the rat. BIOCHEM PHARMACOL 58;11:1831–1839, 1999. © 1999 Elsevier Science Inc.

KEY WORDS. cellular metabolism; energy charge; redox potential; alcohol; cell proliferation

Human and rodent livers have the remarkable capacity of regenerating in response to various stimuli, including massive destruction of hepatic tissue by toxins, viral agents, or surgical resection [1, 2]. Liver regeneration depends on the ability of hepatocytes to undergo cell division, which is controlled tightly by intra- and extrahepatic factors [1, 2]. The proliferative process requires an oversupply of metabolic energy to match increased DNA synthesis, which occurs during liver regeneration [1–4]. In addition, cellular redox potential, another metabolic control system, is subjected to modifications (as the NAD^+/NADH redox pair) in both cytosolic and mitochondrial compartments in the regenerating liver [4, 5]. Both cellular parameters are closely linked, and energy parameters (i.e. ATP/ADP ratio and phosphorylation potential) and the free NAD^+/NADH ratio depend on activities of the major liver metabolic pathways, in turn reflecting the metabolic integrity of the liver cell [6, 7].

Acute ethanol administration to animals subjected to (PH‡) readily inhibits the resultant liver regeneration after surgery, as assessed by a reliable diminution of parameters of cell proliferation in the remnant liver [8, 9]. Although the exact mechanisms underlying ethanol-induced inhibition of liver regeneration are not well understood, it is reasonable to assume that this hepatotoxin could alter the overall metabolism of the regenerating liver. Indeed, it has been reported that ethanol causes disturbances in the redox state, energy charge, and mitochondrial oxidative capacity of the remnant liver early after PH [10–12].

The effects of ethanol on the regenerating liver could be attributed to its hepatic metabolism, which comprises cytoplasmic ethanol oxidation to acetaldehyde, catalyzed

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‡ Abbreviations: PH, partial hepatectomy; ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase; CYP2E1, cytochrome P450 2E1; K_{eq}, equilibrium constant; E_h, redox potential; E_{h_c}, cytosolic redox potential; and E_{h_m}, mitochondrial redox potential.

by ADH, and further conversion of acetaldehyde to acetate by mitochondrial ALDH. Both enzymatic reactions implicate the reduction of a molecule of NAD^+. The excess of NADH production in both cytosol and mitochondria readily modifies the cellular redox state and, consequently, energy availability, leading to alterations of metabolic pathways such as glycolysis, and oxidative mitochondrial function in liver tissue [13–15]. Ethanol administration can affect the regenerating liver at the metabolic level [14, 15]; however, little information is available about the effects of this drug on cellular metabolic systems, such as the redox state and energy availability in the proliferating liver.

Therefore, the present study was aimed at assessing whether energy availability and redox state are major controlling factors in the onset of liver regeneration and possible targets for the deleterious actions of acute ethanol on rat liver regeneration. Results show that liver regeneration *per se* induces modifications in the liver redox state, without significantly changing the energy status. Acute ethanol administration largely shifted the PH-induced modifications in the hepatic redox state, whereas liver energy availability was unaffected by this hepatotoxin.

MATERIALS AND METHODS

Reagents

Enzymes, coenzymes, and substrates were obtained from the Sigma Chemical Co. Standard analytical grade reagents were purchased from Merck.

Animals and Treatments

Male Wistar rats weighing 220–260 g were maintained on a 12-hr light–dark cycle, with standard rat pellet chow and access to water *ad lib*. All procedures were conducted following our Institutional Animal Care, Selection and User's Guide for Laboratory Animals. Rats were subjected to two-thirds PH under light diethyl ether anesthesia, according to a technique reported by Higgins and Anderson [16]. Sham-operated animals were used as controls.

Animals were grouped according to their surgical status and treated as previously reported [15]. They received a single dose of ethanol (5 g/kg body weight; 63% solution in water) given immediately after surgery through an oral cannula, or an equivalent volume of saline solution. At the times indicated, animals were killed by a blow to the back of the head; around 300 mg of liver tissue was removed as rapidly as possible (less than 10 sec) and frozen in liquid nitrogen. Frozen samples were extracted in 4 vol. of cold perchloric acid for determination of redox metabolites (0.6 N $HClO_4$) and adenine nucleotides (0.5 N $HClO_4$) as indicated previously [17]. The extracts were centrifuged to get rid of denatured protein, and the supernatant was aliquoted in plastic tubes and frozen at −70° until processing.

Determination of Metabolites

Perchloric acid extracts were neutralized with 5 mol/L of K_2CO_3 and used for metabolite determinations by enzymatic methods: lactate [18], pyruvate [19], β-hydroxybutyrate [20], and acetoacetate [21]. Adenine nucleotides (ATP, ADP, and AMP) were quantified by HPLC, according to the method of Hoffman and Liao [22].

Definition and Calculation of Redox and Energy State

Cytosolic and mitochondrial redox states were calculated from lactate/pyruvate and β-hydroxybutyrate/acetoacetate ratios, respectively, in accordance with Stubbs *et al.* [23]. Estimation of the NAD^+/NADH ratio from both subcellular compartments was done using the following equation: $NAD^+/NADH = [\text{oxidized substrate}]/[\text{reduced substrate}] \times 1/K_{eq}$, taking into account the K_{eq} of lactate dehydrogenase (1.11×10^{-4} M) for the cytosolic fraction, and the K_{eq} of β-hydroxybutyrate dehydrogenase (4.93×10^{-2} M) for the mitochondrial compartment [23, 24]. Redox potential (E_h), defined as the ability to dissect an overall electron transfer into two half-reactions [24, 25], was calculated based on the Nernst equation, as follows: $E_h = E_0' + 0.03 \log NAD^+/NADH$, where $E_0' = 0.314$ V [25]. The difference between cytosolic and mitochondrial redox potentials (ΔE) was calculated as $\Delta E = E_{hm} - E_{hc}$.

Liver energy charge (E.C.) was calculated from the adenine nucleotide concentrations by the method reported by Atkinson [26], as follows: E.C. = (ATP + 0.5 ADP)/(ATP + ADP + AMP).

Enzyme Assays

Liver samples were homogenized in a buffer containing 0.25 mol/L of sucrose, 10 mmol/L of Tris–HCl, 0.3 mmol/L of EGTA, and 0.2% BSA, pH 7.4. The homogenate was centrifuged at 800 *g* for 10 min, at 4°, and the resulting supernatant was spun for 15 min at 8500 *g* to pellet the mitochondrial fraction. The second supernatant was centrifuged at 25,000 *g* for 60 min to obtain the peroxisomal fraction (pellet). The supernatant was spun further at 100,000 *g* for 60 min, to obtain the cytosolic and microsomal fractions. The specific activities of ADH (EC 1.1.1.1) and ALDH (EC 1.2.1.5) were measured in the cytosolic and mitochondrial fractions, respectively, by standard procedures [27, 28], and expressed as nanomoles per minute per milligram of protein. The specific activity of catalase (EC 1.11.1.6) was determined in the peroxisomal fraction and expressed as nanokatals per gram of liver, as reported by Aebi [29]. In addition, the specific activity of CYP2E1 was measured using the method reported by Salmela *et al.* [30]. Protein was quantitated by the method of Lowry *et al.* [31].

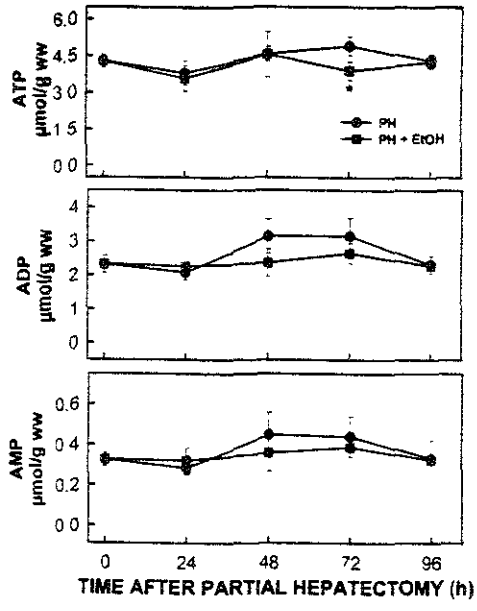

FIG. 1. Concentration of adenine nucleotides in the regenerating liver after ethanol administration. Rats were partially hepatectomized, and a single intragastric ethanol dose was administered immediately. Adenine nucleotides were determined in the remnant liver by HPLC. Each point is the mean ± SEM of 6–15 independent experiments. Key: (*) indicates statistical significance ($P < 0.01$) vs control.

Statistical Analysis

All results are expressed as means ± SEM, and statistical significance of differences among groups was assessed by Student's *t*-test.

RESULTS

Liver Energy Parameters

Figure 1 shows the time–course profile of liver adenine nucleotides (ATP, ADP, and AMP) from animals subjected to PH, with or without acute ethanol administration. Hepatic adenine nucleotides were not modified significantly in sham-operated and intact rats that received ethanol (not shown). Moreover, neither PH nor ethanol administration elicited changes in these parameters, except for ATP, which was lower in the PH + EtOH group at 72 hr post-surgery. Despite the ethanol-induced diminution of liver ATP in the animals subjected to PH, control energy parameters such as total nucleotides (6.93 ± 0.59 µmol/g liver), the ATP/ADP ratio (3.69 ± 0.15), and energy charge (0.78 ± 0.02) were not changed significantly by the treatments tested.

Effect of PH and Acute Ethanol Treatment on Redox State of Liver Cells

While neither PH nor acute ethanol administration promoted significant changes in liver energy parameters, the redox state was modified profoundly under these experimental conditions. Determinations of liver levels of lactate and pyruvate, as well as β-hydroxybutyrate and acetoacetate, were used as redox-pair metabolites for calculating cytosolic and mitochondrial (NAD^+/NADH) redox potentials, respectively.

Cytosolic Redox-Pair Metabolites

Liver lactate and pyruvate levels from experimental animals are shown in Table 1. Sham-operated rats receiving ethanol did not present any significant change in the liver lactate level 24 hr post-treatment (3.20 ± 0.12 vs 3.30 ± 0.15 µmol/g liver in controls). An increased level of hepatic lactate was found in animals subjected to PH at 24 hr post-surgery, which was followed by a decrease in this metabolite at 72 hr post-PH and normalized thereafter. Ethanol administration to PH animals showed the same initial peak of liver lactate, but the subsequent decrease of this metabolite found in animals undergoing PH was earlier and lasted longer under ethanol treatment (Table 1). Hepatic pyruvate in controls (0.32 ± 0.02 µmol/g) was not modified significantly in sham-operated animals after ethanol administration (at 24 hr, 0.31 ± 0.03 µmol/g). PH induced a significant decrease of liver pyruvate during the first 48 hr after surgery, returning to the control level thereafter. Ethanol administration to PH rats practically abolished the effect of the partial removal of the liver on the pyruvate concentration, at all times tested (Table 1). The changes elicited by PH and ethanol administration in liver concentrations of lactate and pyruvate induced modifications of the lactate/pyruvate ratio and of the liver redox NAD^+/NADH potential (Fig. 2). In both groups, PH and PH + ethanol animals, the lactate/pyruvate ratio increased significantly at 24 hr after surgery. Animals subjected to PH showed a gradual diminution in this ratio starting at 48 hr after PH and normalizing thereafter, while PH rats receiving ethanol showed a sudden decrease of this ratio at 48 hr post-surgery, returning the lactate/pyruvate ratio within normal range at 96 hr after surgery (Fig. 2A). Partial removal of the liver promoted a decrease in the cytosolic NAD^+/NADH ratio, indicating a reduced cytosolic redox state, which lasted for 48 hr and normalized thereafter. Acute ethanol administration to this group modified the effects of PH on the cytosolic NAD^+/NADH ratio, since at 48–72 hr post-PH, ethanol promoted a significantly more oxidized cytosolic redox state (Fig. 2C). This action was not associated with the presence of ethanol, since ethanol is cleared 10 hr after its administration [15]. At later times post-surgery (96 hr), both experimental groups presented a normal cytosolic redox state (Fig. 2C).

Mitochondrial Redox-Pair Metabolites

Table 1 also presents the hepatic levels of β-hydroxybutyrate and acetoacetate as redox-pair metabolites indicative of the mitochondrial redox state. Again, sham-operated animals receiving acute ethanol administration did not have significant changes in the liver content of acetoace-

TABLE 1. Concentration of metabolites from livers of partial hepatectomized (PH) rats with or without a single ethanol dose

Metabolite (μmol/g liver wet weight)	Time after partial hepatectomy (hr)			
	24	48	72	96
Lactate (3.30 ± 0.15)				
PH	4.40 ± 0.38*	2.80 ± 0.26	1.85 ± 0.15*	3.15 ± 0.16
PH + EtOH	5.26 ± 0.42*	1.37 ± 0.25*†	1.79 ± 0.16*	3.20 ± 0.18
Pyruvate (0.32 ± 0.021)				
PH	0.24 ± 0.038*	0.20 ± 0.020*	0.25 ± 0.030	0.35 ± 0.025
PH + EtOH	0.31 ± 0.022	0.31 ± 0.021	0.25 ± 0.031	0.32 ± 0.025
Acetoacetate (0.13 ± 0.009)				
PH	0.19 ± 0.010*	0.17 ± 0.009*	0.14 ± 0.013	0.13 ± 0.016
PH + EtOH	0.17 ± 0.008	0.16 ± 0.010*	0.16 ± 0.010	0.13 ± 0.020
β-Hydroxybutyrate (0.22 ± 0.005)				
PH	0.17 ± 0.007*	0.18 ± 0.008*	0.20 ± 0.009	0.22 ± 0.018
PH + EtOH	0.21 ± 0.008*†	0.19 ± 0.009*	0.17 ± 0.009*†	0.21 ± 0.007

Metabolites were determined according to Materials and Methods. Each point is the mean ± SEM of 5–10 independent experiments. Mean control concentrations ± SEM of each metabolite are shown in parentheses.

* $P < 0.01$ vs controls

† $P < 0.01$ vs the PH group

tate at any of the times tested (0.12 ± 0.01 vs 0.13 ± 0.01 μmol/g in controls). PH induced a significant increase of acetoacetate, which lasted until 48 hr after surgery and declined to normal values thereafter; ethanol administration to these animals did not modify the PH-induced changes in liver acetoacetate concentration significantly. In regard to the liver β-hydroxybutyrate level, PH also elicited a significant decrease within the first 48 hr post-surgery, an effect that was initially blocked by ethanol administration (24 hr post-PH), and indeed was shifted to later times post-surgery (48–72 hr; Table 1). Again, ethanol administration to sham-operated rats did not change the hepatic β-hydroxybutyrate level significantly (0.21 ± 0.02 μmol/g) after 24–96 hr of treatment.

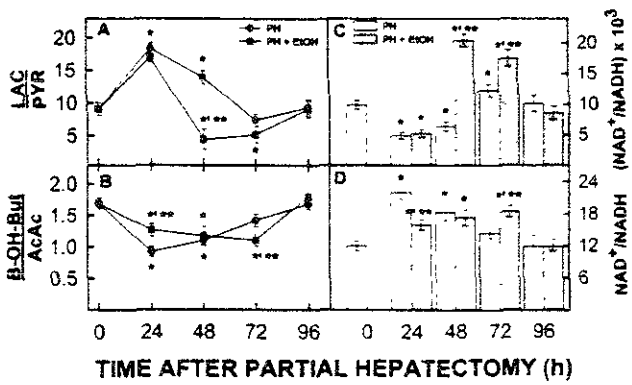

FIG. 2. Redox parameters in cytosol and mitochondria from the regenerating liver. Panel A shows the lactate/pyruvate ratio, and panel B shows the β-hydroxybutyrate/acetoacetate ratio. Panels C and D depict the calculated $NAD^+/NADH$ ratio in cytosol and mitochondria, respectively. The relationships were calculated as described in Materials and Methods. Each point is the mean ± SEM of 6–15 independent experiments. Key: (*) indicates statistical significance ($P < 0.01$) vs control; and (**) indicates statistical significance ($P < 0.01$) vs PH rats.

Thus, derived parameters of the mitochondrial redox state were modified significantly by the treatments tested. PH promoted a diminution in the β-hydroxybutyrate/acetoacetate ratio in the first 48 hr after surgery, followed by a complete recovery of this ratio. Ethanol administration partially blocked the PH-induced diminution of the liver β-hydroxybutyrate/acetoacetate ratio, but a decrease of this ratio was noted at a later time post-PH (72 hr) in the PH + ethanol group (Fig. 2B). Hence, PH animals presented a more oxidized mitochondrial redox state, as indicated by an increased $NAD^+/NADH$ ratio in the first 48 hr after surgery, whereas ethanol treatment of PH rats shifted the highest mitochondrial $NAD^+/NADH$ ratio to 72 hr post-surgery (Fig. 2D). Again, at 96 hr after PH no modifications in the mitochondrial redox state were recorded in the different experimental groups (Fig. 2D).

Effect of PH and Acute Ethanol Administration on Cytosolic and Mitochondrial Redox Potentials

Table 2 shows the calculated redox potential (E_h; expressed in mV) from the cytosolic and mitochondrial NAD^+ NADH ratios, and the difference (ΔE) between the two subcellular compartments. No significant changes were found in the cytosolic redox potential (E_{hc}) as a consequence of PH, but ethanol co-administration elicited a more positive redox potential after 48 hr post-surgery. However, the mitochondrial redox potential (E_{hm}) was virtually unaffected by the experimental conditions (Table 2). Although ΔE was a constant

TABLE 2. Redox potential (E_h) in cytosol and mitochondria and the differences between mitochondrial and cytosolic redox potential (ΔE) from livers of rats after partial hepatectomy (PH) with or without a single ethanol dose

Parameter	Time after partial hepatectomy (hr)			
	24	48	72	96
E_{h_c} (224.1 ± 4.6)				
PH	−233.3 ± 3.3	−229.7 ± 4.2	−221.5 ± 3.3	−224.3 ± 5.6
PH + EtOH	−232.3 ± 4.5	−214.7 ± 3.1*	−216.8 ± 1.6	−224.1 ± 5.6
E_{h_m} (281.1 ± 6.6)				
PH	−273.7 ± 6.6	−276.1 ± 6.3	−279.4 ± 5.6	−281.7 ± 6.8
PH + EtOH	−277.8 ± 3.5	−276.9 ± 7.2	−276.0 ± 2.3	−281.6 ± 8.6
ΔE (57.5)				
PH	40.4†	46.4†	57.9	57.4
PH + EtOH	45.5†	62.2†	59.2	57.5

Parameters were calculated from metabolites shown in Fig. 1 according to Materials and Methods. Each point is the mean ± SEM of 5–10 independent experiments. The control values ± SEM for E_h (mV) and ΔE are shown in parentheses.

* $P < 0.01$ vs PH group.

† $P < 0.01$ vs control.

value maintained within a narrow range in control livers (52–58 mV), there were significant changes due to PH. In these animals, a decrease in ΔE was found during the first 48 hr after surgery, mainly attributed to a more negative (reduced) cytosolic redox potential, which was transient and was readily normalized thereafter. Ethanol treatment of rats undergoing PH promptly shortened the PH-induced effect on ΔE, which was noticeable 24 hr after surgery in the PH + ethanol group (Table 2).

Effect of PH and Acute Ethanol Administration on the Activities of Ethanol-Metabolizing Enzymes

To correlate the changes in both cytosolic and mitochondrial redox states induced by PH and PH + ethanol with liver ethanol metabolism, activities of ethanol-metabolizing enzymes were measured in several subcellular compartments. Figure 3 shows the changes elicited by PH and ethanol in NAD^+-dependent enzymes (cytosolic ADH and mitochondrial ALDH), as well as in the NAD^+-independent peroxisomal enzyme catalase, which are involved in ethanol catabolism [32].

Acute ethanol administration to sham-operated rats did not modify significantly the activity of the enzymes tested. However, surgical removal of the liver promoted a gradual inhibition of the ADH activity, reaching a minimal activity at 24 hr post-PH and recovering its activity thereafter. Ethanol treatment reduced the PH-induced inhibition of liver ADH activity, readily increasing ADH activity with a peak after 48 hr post-surgery (Fig. 3A).

Mitochondrial ALDH activity was readily increased by PH (starting from 48 up to 96 hr). Ethanol administration promoted an earlier peak of ALDH activity (12 hr), without further modification of the PH-induced pattern of ALDH activity (Fig. 3B).

On the other hand, peroxisomal catalase activity greatly responded to the loss of liver mass. PH induced an early decrease in catalase activity (3–12 hr after surgery), which was recovered at 24 hr post-PH and followed by a further diminution of its activity up to 72 hr post-surgery (Fig. 3C). Acute ethanol co-administration elicited minor changes in the PH-induced liver pattern of NAD-independent catalase

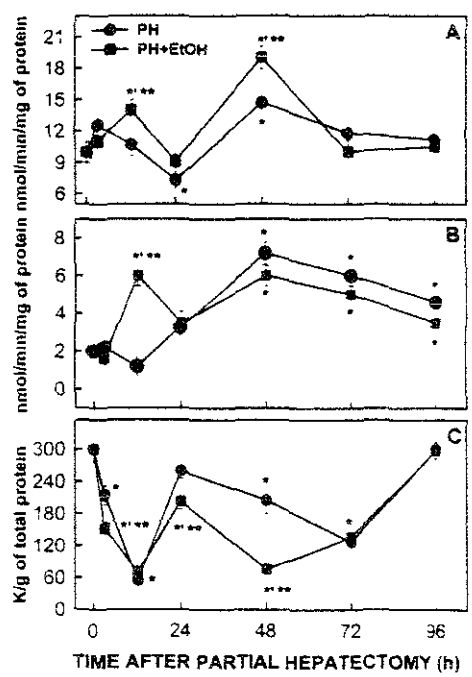

FIG. 3. Specific activities of ADH, ALDH, and catalase after PH and ethanol administration. The activities of ADH (A), ALDH (B), and catalase (C) were determined in the remnant livers of PH rats receiving ethanol. Results are expressed as the means ± SEM of 6–15 independent experiments. Statistical significance is as indicated in the legend of Fig. 2.

The effects of ethanol on the NAD^+/NADH ratio occur during the time that it remains detectable in the blood [10, 11]. In the PH-induced regenerating liver, modifications of the redox state pattern by ethanol administration lasted up to 72 hr post-surgery, indicating that ethanol metabolism is not directly implicated in the changes in the cellular redox state during liver regeneration. These findings lead us to propose that acute ethanol administration might induce inhibition of liver regeneration by blocking adaptive changes in metabolic pathways occurring in the proliferating liver. Hence, ethanol seems to minimize the metabolic adjustments mediated by redox reactions, probably leading to a decreased preparatory event culminating in the proliferative period that characterizes PH-induced liver regeneration.

In the same context, the process of liver regeneration was accompanied by changes in the specific activity of some enzymes [33–35]. Here, our interest was focused on the activity pattern of ethanol-metabolizing enzymes during liver regeneration, and the putative action of acutely administered ethanol. We recently reported that regenerating liver shows an enhanced capacity to oxidize ethanol, which increases as long as hepatic mass is being restored [44]. In the present study, we found that ADH activity was decreased early after PH; hence, it is difficult to explain the enhanced ethanol oxidation by the regenerating liver when the main oxidative pathway for catabolizing ethanol is partially inhibited in PH animals.

However, ethanol administration early after PH readily protects ADH activity against PH-induced inhibition. Although the underlying mechanism of the PH-induced inhibition of ADH activity is still not elucidated, the decreased ADH activity found in regenerating liver has been reported previously [10]. Thus, the present data showing that ethanol protected against the PH-induced decrease of ADH, as well as against the decrease of cytosolic NAD^+ availability, will support the statement that the proliferating liver can actively oxidize the administered ethanol mainly through the participation of cytoplasmic ADH. This statement is supported further by the decreased activity found in microsomal CYP2E1, which is another ethanol-metabolizing system, mainly after chronic alcohol consumption. In our conditions, PH promoted a drastic reduction of CYP2E1 activity from 24 to 72 hr post-surgery, which closely agrees with that reported recently [45], an effect that was unchanged by the administration of ethanol.

Mitochondrial ALDH activity was increased readily at the post-PH times (48–72 hr) when a higher mitochondrial NAD^+/NADH ratio was found in the regenerating liver. This enzyme also is involved in ethanol metabolism by removing acetaldehyde, which in turn is converted to acetate. Ethanol administration to PH rats did not significantly modify the mitochondrial profile of ALDH activity, despite the fact that treatment with this hepatotoxin promoted a long-lasting enhancement of the oxidized redox state in mitochondria.

The PH-induced changes already described for the two NAD-dependent enzymes (ADH and ALDH) that are involved in ethanol metabolism could be related to utilization of endogenous substrates potentially involved in liver proliferation. Thus, ethanol administration could modify the activities of these enzymes toward their putative endogenous substrates, altering the metabolic adjustments for cell proliferation.

Catalase is another enzyme involved in ethanol catabolism; this NAD-independent enzyme is limited by the endogenous amount of hydrogen peroxide required to carry out ethanol oxidation to acetaldehyde [46]. This enzymatic activity was also modified by PH, but in a different manner than ADH and ALDH. The role of catalase in ethanol oxidation by the regenerating liver has not been evaluated; however, the presence of high levels of hydroperoxides, found in the cytoplasm of the proliferating liver during the first 36 hr after surgery [47], might favor the participation of this enzyme in the oxidation of ethanol through a non-ADH pathway. The involvement of each metabolic pathway for ethanol catabolism in the regenerating liver is currently being studied in our laboratory.

The effects of ethanol on the PH-induced changes in cellular redox state and activity of cytoplasmic ADH resulted in a blockade in the adaptive modifications of the redox state in the proliferating liver, whose pathophysiological significance remains to be elucidated.

In summary, partial removal of the liver mass did not affect energy availability in the remnant liver, but did modify considerably the cellular redox state in a transient manner, which would suggest its involvement in the preparatory period for cell proliferation. Administration of ethanol, known to readily alter the cellular redox state through its catabolism, in turn blocked the PH-induced pattern of cytosolic and mitochondrial redox reactions without modifying energy parameters. The specific activity of ethanol-metabolizing enzymes also changed during liver regeneration, and ethanol prevented the PH-induced effect mainly on ADH activity. The data suggest that ethanol could minimize the PH-promoted metabolic adjustments mediated by redox reactions, probably leading to a decreased preparatory event that culminates in the compensatory liver growth after PH in the rat.

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