Biochemical Pharmacology, Vol. 23, pp. 1021–1030. Pergamon Press, 1974. Printed in Great Britain.

EFFECTS OF MENADIONE ON THE METABOLISM OF GALACTOSE IN REGENERATING RAT LIVER*

DUTTA SESHACHALAM

Department of Medical Microbiology, Creighton University School of Medicine, Omaha, Nebr. 68131, U.S.A.

(*Received* 17 *October* 1972; *accepted* 24 *August* 1973)

Abstract—The effect of menadione sodium bisulfite (MSB) on the metabolism of (1-^{14}C)galactose was studied in regenerating and newborn rat liver slices. Incubation of liver slices with (1-^{14}C)galactose showed that the rate of $^{14}CO_2$ formation was faster in newborn and regenerating liver slices than that in adult sham-operated controls. MSB increased the formation of $^{14}CO_2$ from (1-^{14}C)galactose by approximately 150 per cent in regenerating liver, perhaps by increasing the ratio of NADP/NADPH. Such an increase was not observed with (1-^{14}C)glucose as substrate. Furthermore, MSB effectively reversed ethanol inhibition of galactose catabolism in regenerating and sham-operated rat liver slices. The results indicate that the reaction sequence involved in the conversion of galactose to glucose 6-phosphate operates at a faster rate than glucose to glucose 6-phosphate. Also, the accelerated metabolism of galactose in regenerating rat liver appears to occur via the hexosemonophosphate shunt (HMP) and thus the inhibitory effect of ethanol is attenuated. The effects of MSB are explicable on the basis of the hypothesis that the HMP is selectively stimulated in liver.

THE PHENOMENON of liver regeneration is associated not only with an increase in cell number but also with a burst of metabolic activity, as in embryonic and malignant tissues. The striking similarities between regenerating liver on the one hand, and neoplastic, neonatal and embryonic tissues on the other hand, prompted comparative metabolic studies in the past.[1] Whereas glucose is generally considered important in energy metabolism, diversity in galactose utilization attains special significance in the congenital disease, galactosemia,[2–4] and in benign and malignant tumors.[5,6] Moreover, recent studies indicate that the process of liver regeneration is closely correlated with changes in carbohydrate metabolism.[7–11] The metabolic responses of liver during regeneration are so altered that the inhibition of galactose oxidation by ethanol is markedly attenuated in comparison with that of other monosaccharides.[7]

The results of the present study show that the metabolism of galactose is accelerated in regenerating rat liver by the presence of menadione sodium bisulfite (MSB). It is postulated that MSB acts as an electron acceptor and promotes a high NADP/NADPH ratio and thus stimulates the operation of the hexosemonophosphate shunt (HMP).

MATERIALS AND METHODS

Preparation of the tissue and incubation. Male hooded Wistar rats, weighing 160–200 g and fed *ad lib.*, were partially hepatectomized by removing 70 per cent of the total liver according to the method described by Higgins and Anderson.[12] After the operation, the animals were fed *ad lib.* Sham-operated animals were used as controls.

* Part of this work was done at McGill University, Montreal, Canada.

The operations of animals were carried out between 9 and 10 a.m. to control the variations due to diurnal rhythm. The animals were caged individually and observed for their feeding habits. Animals which did not show normal activity or were not well fed were discarded. The animals were sacrificed by stunning and exsanguination for the preparation of liver slices. The methods of preparation of the adult rat liver slices and incubation procedures were adopted from Majchrowicz and Quastel.[13] Liver slices were also prepared from newborn rats (3-days-old) by using the McIlwain and Buddy chopper. Approximately 100 mg of liver tissue was used for incubation in Warburg vessels. The incubation medium contained Krebs–Ringer phosphate buffer (pH 7·4), (1-^{14}C)galactose, (1-^{14}C)glucose, (6-^{14}C)glucose or (U-^{14}C)fructose. The labeled compounds were added to the medium in a solution of 0·1 ml containing 0·1 μmole (0·5 μCi) and the nonlabeled carrier sugars were added to give a final concentration of 3–15 μmoles in 3·0 ml. The other compounds were present as indicated in the tables.

The liver slices from normal, regenerating and neonatal rats were dried in a desiccator on microscope coverslips and weighed for determination of dry weight. The weights of the dry samples were found to be approximately 20 per cent of the fresh weights. Preliminary experiments with respiration and glucose catabolism have shown that the surface liver slices were metabolically less active than the rest of the tissue and, therefore, these were discarded. When this procedure was followed, uniform results were obtained with all major lobes of the liver. Routinely, oxygen uptake was measured manometrically and Q_{O_2} was determined as an additional control of each experiment.

The center wells of the Warburg vessels contained 0·2 ml Hyamine hydroxide in small tubes made to fit the center wells.[14] At the end of the incubation, the medium was acidified with 0·2 ml of 30% trichloroacetic acid tipped into the medium from the side arm of the vessel, and the released CO_2 was absorbed by Hyamine. The contents of the tubes were transferred into scintillation vials containing 15 ml of scintillation liquid and counted in a Packard Tri-Carb liquid scintillation spectrometer. The values were corrected for quenching, using an internal standard. The scintillation liquid contained 10 g 2,5-diphenyloxazole (PPO) and 0·6 g 1,4-bis-2(4-methyl 5-phenoloxazolyl)benzene (dimethyl-POPOP) in 2000 ml toluene. Values of the rates of $^{14}CO_2$ formation are given as natoms ^{14}C incorporated into $^{14}CO_2$/hr per 100 mg wet wt tissue. Preliminary experiments with KOH in center wells as an absorbent yielded similar results when allowance was made for quenching.

Extraction of glycogen. Glycogen from the liver slices was extracted at the end of the incubation period and purified for estimation of radioactivity incorporated from labeled sugars. Liver slices, after incubation, were transferred to 3 ml of cold 10% trichloroacetic acid in a Potter–Elvehjem homogenizer and homogenized for approximately 10 min. The homogenate was centrifuged and the supernatant collected. The residue was washed twice with 2 ml of cold 5% trichloroacetic acid, after which the residue was discarded. To the combined trichloroacetic acid fractions was added 5 mg of carrier glycogen contained in a solution of 1 ml of 5% trichloroacetic acid. To this extract was added 5 vol. of 95% ethanol; it was left overnight in the cold. Under these conditions the glycogen was precipitated. The precipitate was separated from the ethanol–trichloroacetic acid mixture by centrifuging, then dissolved in 2 ml of 10% NaOH and reprecipitated by the addition of 5 vol. of ethanol. The last step

was repeated four times and the precipitate was then washed with acetone and air dried. Finally, the glycogen was suspended in a small quantity of water and made up to a definite volume. An aliquot of this suspension was employed for colorimetric determination with anthrone reagent.[15] A similar aliquot was pipetted into a scintillation vial, dried and counted in the scintillation liquid mentioned earlier.

The amount of glycogen present in the regenerating liver slices was estimated after direct extraction from a large quantity of tissue incubated in Krebs–Ringer medium for 1 hr. It was found that the glycogen content of the slices, after incubation, was 0·01–0·02 mg/100 mg fresh wt. In the experiments in which glycogen was to be extracted, tissue slices weighing about 60 mg were used. The radioactivity associated with 5 mg glycogen in each sample was taken as corresponding to the total glycogen in the tissue used for the experiment, since endogenous glycogen in incubated slices was negligible relative to the carrier. The glycogen isolated from each sample was measured colorimetrically and the values were corrected for losses during experimental manipulations.

The results given in the tables are the mean values of at least six experiments and the standard errors of the means are given. Control experiments were always carried out in duplicate and the results are the mean values of at least 10 observations. Reagents for liquid scintillation were obtained from Packard Instrument Company Inc., Chicago, Ill.; radioactive chemicals from the Radiochemical Center, Amersham, England and Calbiochem, Los Angeles, California; and the rest of the chemicals from Merck, Sharp & Dohme, Montreal, Canada.

RESULTS

Preliminary studies were carried out to select an incubation period representative of CO_2 production from labeled carbohydrates. The production of $^{14}CO_2$ from various substrates was proportional with time from 15 min to more than an hr, when measured at 15-min intervals. Thus the experiments were carried out under steady state conditions. A low concentration of galactose (1·0 mM) was sufficient to obtain these conditions, because of the low K_m ($5{\cdot}8 \times 10^{-4}$ M) of galactokinase for galactose.[16] Thus the possible toxicity of the galactose metabolites resulting from the use of saturating concentrations can be avoided.[17] The rate of respiration was also constant during this period. Results obtained from a 60-min incubation time are taken to represent the rates of metabolism of sugars.

Oxidation of (1-^{14}C)glucose and (1-^{14}C)galactose in normal adult and newborn as well as in regenerating liver shows the following features: (1) the production of $^{14}CO_2$ from (1-^{14}C)galactose is significantly greater than that from (1-^{14}C)glucose at 1 mM in all the tissues and (2) (1-^{14}C)galactose at 1 mM yields approximately 90 per cent more $^{14}CO_2$ in newborn and regenerating rat liver than in normal adult liver under similar experimental conditions. On the other hand, no similar increase is seen in the rates of production of $^{14}CO_2$ from (1-^{14}C)glucose in regenerating liver slices (Table 1).

Since glucose 6-phosphate is a common precursor in liver for CO_2 formation from glucose and galactose, it may be assumed that the difference in the rates of breakdown of these two sugars is due to their respective rates of conversion to glucose 6-phosphate. Since there is a possibility for dilution of the pool of glucose 6-phosphate by the pool of sugar phosphate formed by glycogenolysis, thus lowering the

TABLE 1. CATABOLISM OF (1-^{14}C)GALACTOSE AND (1-^{14}C)GLUCOSE TO $^{14}CO_2$ IN NORMAL, REGENERATING AND NEWBORN RAT LIVER SLICES*

Substrate	Normal (natoms ^{14}C)	Regenerating (natoms ^{14}C)	Newborn (natoms ^{14}C)
(1-^{14}C)galactose (1 mM)	20·0 ± 0·47(a)†	37·0 ± 0·72(c)	38·0 ± 0·15(e)
(1-^{14}C)glucose (1 mM)	10·0 ± 0·14(b)	11·0 ± 0·11(d)	15·0 ± 0·87(f)

* Rat liver slices were incubated in Krebs–Ringer phosphate medium (1·5 ml), described in Methods, for 1 hr in O_2 at 37°. The labeled glucose and galactose (1·0 mM each) were present at the start of the experiment. The final specific radioactivity of each substrate was 0·5 μCi/3 μmoles. The values of the rates of $^{14}CO_2$ formation are expressed as natoms (± S.E.) of ^{14}C incorporated into $^{14}CO_2$ hr per 100 mg wet wt.

† Significance by Student's *t*-test for a vs b, b vs f, c vs d, d vs f, e vs f, P = <0·001; d vs b = NS; n = 10.

production of labeled CO_2 from (1-^{14}C)glucose, incubations of regenerating rat liver slices were performed with the addition of unlabeled glucose to (1-^{14}C)galactose and alternately of unlabeled galactose to (1-^{14}C)glucose. The results (Table 2) show that there is no equivalent depression of $^{14}CO_2$ formation in either case, indicating that the isotopic dilution caused by glycogenolysis under the conditions of experimentation outlined does not materially alter the values for $^{14}CO_2$ obtained by the breakdown of (1-^{14}C)glucose and (1-^{14}C)galactose in regenerating rat liver. However, the possibility of dilution of the glucose 6-phosphate pool by glycogenolysis appears to be remote under conditions using tissue slices *in vitro*, because of the rapid depletion of glycogen during the preparation of the tissue.

The results obtained for the incorporation of labeled carbon from (1-^{14}C)galactose and (1-^{14}C)glucose into glycogen of the liver slices show that (1-^{14}C)galactose is incorporated into glycogen more rapidly than is (1-^{14}C)glucose (Table 3). Moreover, incorporation of (1-^{14}C)galactose into the glycogen of normal liver slices occurs at a faster rate than in that of the regenerating and the newborn liver. Menadione sodium bisulfite, which will be shown later to increase the rate of formation of $^{14}CO_2$ from these labeled sugars, decreased the rates of their incorporation into glycogen. In regenerating and newborn rat liver slices, the conversion of galactose 1-phosphate to CO_2 takes place at a higher rate than in liver of control animals. The effect of MSB on the rate of $^{14}CO_2$ from (1-^{14}C)galactose has been studied in normal adult and regenerating rat liver slices (Table 4). Higher concentrations (in excess of 1·5 mM) of MSB in these experiments had inhibitory effects on the production of $^{14}CO_2$ from (1-^{14}C)galactose.

TABLE 2. INTERACTION OF GLUCOSE AND GALACTOSE IN REGENERATING RAT LIVER*

Substrate	$^{14}CO_2$ (natoms ^{14}C)	Inhibition (%)	P values†
(1-^{14}C)galactose	32·8 ± 1·03		
(1-^{14}C)galactose + glucose	29·4 ± 1·50	10	>0·05
(1-^{14}C)glucose	10·9 ± 0·17		
(1-^{14}C)glucose + galactose	8·8 ± 0·22	19	<0·01

* Rat liver slices were incubated in Krebs–Ringer phosphate medium (3·0 ml), described in Methods, for 1 hr in O_2 at 37°. Glucose and galactose (1 mM each) with a final specific activity of 0·5 μCi/3 μmoles each were present at the start of the incubation. The values of rates of $^{14}CO_2$ formation are expressed as natoms (± S.E.) ^{14}C incorporated into $^{14}CO_2$ hr per 100 mg wet wt.

† Statistical significance indicated by P values derived from Student's *t*-test; n = 6.

TABLE 3. INCORPORATION OF (1-^{14}C)GALACTOSE AND (1-^{14}C)GLUCOSE INTO GLYCOGEN OF NORMAL, REGENERATING AND NEWBORN RAT LIVER SLICES*

Substrate and additions	^{14}C incorporated†		
	Normal (natoms)	Regenerating (natoms)	Newborn (natoms)
(1-^{14}C)glucose	6·92 ± 0·21	2·56 ± 0·09	0·46 ± 0·01
(1-^{14}C)galactose	8·05 ± 0·17	4·94 ± 0·07	2·42 ± 0·05
(1-^{14}C)galactose + MSB	0·05 ± 0·01	2·04 ± 0·17	1·19 ± 0·08

* Rat liver slices were incubated in Krebs–Ringer phosphate medium (1·5 ml), described in Methods, for 1 hr in O_2 at 37°. Glucose and galactose (1 mM each) with a specific activity of 0·5 μCi/3 μmoles each were present at the start of the incubation. Menadione sodium bisulfite (0·5 mM) was added at the beginning of the experiment as indicated. Glycogen was isolated as described in Methods and the radioactivity assayed. The values of the rates of incoporation of labeled sugars into glycogen are expressed as natoms (± S.E.) ^{14}C incorporated into glycogen/hr/100 mg wet wt tissue.

† Significance by Student's *t*-test for vertical and horizontal comparisons: $P < 0{\cdot}001$; $n = 10$.

Effect of menadione sodium bisulfite on $^{14}CO_2$ *production from* (1-^{14}C)*glucose,* (6-^{14}C)*glucose and* (U-^{14}C)*fructose.* The results given in Table 5 show the effects of MSB on the formation of $^{14}CO_2$ from (1-^{14}C)glucose, (6-^{14}C)glucose and (U-^{14}C)fructose at 5 mM in regenerating rat liver slices. The rate of $^{14}CO_2$ formation from 5 mM (1-^{14}C)glucose in the presence of MSB (0·5 mM) increases nearly threefold. With (6-^{14}C)glucose (5 mM), MSB (0·5 mM) produces an increase in the rate of $^{14}CO_2$ formation of 67 per cent in normal and of 153 per cent in the regenerating liver tissues. On the other hand, with 5 mM (U-^{14}C)fructose as substrate there is no increase in the rate of $^{14}CO_2$ formation either in regenerating or in normal adult liver in the presence of MSB (0·5 mM), but these rates are much higher than those for (1-^{14}C)glucose and (6-^{14}C)glucose.

Effect of MSB in the presence of ethanol. Ethanol (5 mM) inhibits the rate of $^{14}CO_2$ formation from (1-^{14}C)glucose by 41 per cent in the normal liver tissue and only 28 per cent in regenerating liver tissue. On the other hand, the degree of inhibition is much higher (approximately 68 per cent) in both tissues when (6-^{14}C)glucose is the substrate (Table 6). The highest rate of inhibition by ethanol is noted with galactose

TABLE 4. EFFECT OF MENADIONE ON $^{14}CO_2$ YIELDS FROM (1-^{14}C)GALACTOSE IN NORMAL ADULT AND IN REGENERATING RAT LIVER SLICES*

Menadione concn (mM)	Normal		Regenerating	
	$^{14}CO_2$ (natoms ^{14}C)	Increase (%)	$^{14}CO_2$ (natoms ^{14}C)	Stimulation†
Nil	21·0 ± 0·6		33·0 ± 0·2	
0·50	33·0 ± 0·1	57	83·0 ± 1·4	151
0·25	35·0 ± 1·3	67	97·0 ± 4·2	194
0·10	29·0 ± 0·3	38	77·0 ± 3·7	103

* Rat liver slices were incubated in Krebs–Ringer phosphate medium (3·0 ml), described in Methods, for 1 hr in O_2 at 37°. Galactose (1 mM) with a final specific radioactivity of 0·5 μCi/3 μmoles and MSB were present at various concentrations at the start of the incubation. The values of rates of $^{14}CO_2$ formation are expressed as natoms (± S.E.) ^{14}C incorporated into $^{14}CO_2$/hr per 100 mg wet wt; the number of observations is 6–10.

† Percentage increase in excess of the controls.

TABLE 5. EFFECT OF MENADIONE ON $^{14}CO_2$ FORMATION FROM LABELED GLUCOSE AND FRUCTOSE*

Substrate and additions	Normal		Regenerating	
	$^{14}CO_2$ (natoms ^{14}C)	Stimulation (%)	$^{14}CO_2$ (natoms ^{14}C)	Stimulation†
(1-^{14}C)glucose	46·0 ± 0·6(a)‡		50·0 ± 2·1(b)	
(1-^{14}C)glucose + MSB	135·0 ± 4·7	190	151·0 ± 5·5	200
(6-^{14}C)glucose	15·0 ± 0·5		19·0 ± 0·8	
(6-^{14}C)glucose + MSB	25·0 ± 0·5	67	48·0 ± 1·8	153
(U-^{14}C)fructose	1008·0 ± 36·7		912·0 ± 26·8(c)	
(U-^{14}C)fructose + MSB	900·0 ± 28·0(c)	nil	882·0 ± 13·0(d)	nil

* Rat liver slices were incubated in Krebs–Ringer phosphate medium (3·0 ml), as described in Methods, for 1 hr in O_2 at 37°. Each of the labeled sugars (sp. act., 0·5 μCi/15 μmoles) was present at a concentration of 5 mM in 3·0 ml medium. MSB was added when indicated at the start of the experiment to give a final concentration of 0·5 mM. The values of rates of $^{14}CO_2$ formation are expressed as natoms (± S.E.) ^{14}C incorporated into $^{14}CO_2$/hr per 100 mg wet wt.

† Per cent stimulation in excess of the control values (e.g. two-fold increase is represented as a 100 per cent increase).

‡ Statistical significance by Student's *t*-test for b vs a, c vs d and d vs c = NS; the differences for all other values, $P < 0{\cdot}001$; $n = 10$.

as the substrate in normal liver tissue, the inhibition being about 75 per cent. However, the inhibition is markedly attenuated in regenerating rat liver, as has been reported earlier.

MSB abolishes the inhibition of $^{14}CO_2$ production by ethanol to various degrees with each labeled sugar serving as a substrate, restoring the $^{14}CO_2$ yields at least to control values. The significant exception to this is the inhibition of $^{14}CO_2$ yields from (6-^{14}C)glucose in normal rat liver slices. With (6-^{14}C)glucose as a substrate in normal liver, inhibition of the formation of $^{14}CO_2$ is decreased by only 20 per cent in the presence of MSB. In regenerating liver, however, MSB reverses the inhibition of ethanol on the rate of production of $^{14}CO_2$ from (6-^{14}C)glucose.

DISCUSSION

Though the synthesis of nucleic acids is initiated in liver during the 24 hr after partial hepatectomy, protein synthesis occurs maximally between 40 and 48 hr.[18,19] If the alterations in enzymatic activity are a reflection of the *de novo* enzyme synthesis during regeneration, it is likely that these may occur during the latter period. Therefore, all the metabolic studies presented in this paper were performed using regenerating liver tissue obtained from animals 48 hr after partial hepatectomy.

In regenerating rat liver, as well as in newborn rat liver, galactose catabolism is markedly increased. Segal *et al.*[3] made a similar observation in their studies using newborn rat liver slices. The metabolism of galactose is initially controlled by the activities of galactose 1-phosphate pyrophosphorylase, UDP-galactose transferase and galactokinase.[20] The activities of the first two enzymes are low in the livers of neonatal animals. Though the activities of these two enzymes steadily increase with maturity of the animal, the ratio of their activity remains constant.[20] However, not much is known about the developmental changes in galactokinase activity and it appears that the higher rate of oxidation of galactose in the tissue slices is due to this enzyme.[3] Since the overall metabolism of galactose is increased in the neonatal

TABLE 6. EFFECT OF MENADIONE SODIUM BISULFITE ON $^{14}CO_2$ FORMATION FROM LABELED GLUCOSE AND GALACTOSE IN THE PRESENCE OF ETHANOL*

Substrate	Additions	Normal		Regenerating	
		(natoms ^{14}C)	Inhibition (%)	(natons ^{14}C)	Inhibition (%)
(1-^{14}C)galactose	Nil	20·0 ± 0·5(10)	0	37·0 ± 0·7(10)	0
(1 mM)	Ethanol	5·0 ± 0·5(10)	75	30·0 ± 0·4(10)	19
	MSB	33·0 ± 0·6(6)	0	84·0 ± 1·0(10)	0
	Ethanol + MSB	18·7 ± 1·3(6)	0	76·5 ± 1·8(10)	0
(1-^{14}C)glucose	Nil	46·0 ± 0·6(10)		50·0 ± 2·1(10)	
(5 mM)	Ethanol	27·0 ± 0·5(10)	41	36·0 ± 2·5(10)	28
	MSB	135·0 ± 4·7(6)	0	151·0 ± 11·4(6)	0
	Ethanol + MSB	128·0 ± 4·7(6)	0	106·0 ± 2·8(6)	0
(6-^{14}C)glucose	Nil	15·0 ± 0·4(10)		19·0 ± 0·6(6)	
(5 mM)	Ethanol	5·0 ± 0·5(10)	67	6·0 ± 0·6(6)	68
	MSB	25·0 ± 0·5(6)	0	48·0 ± 3·7(6)	0
	Ethanol + MSB	8·0 ± 0·5(6)	47	18·1 ± 0·8(6)	5

* Rat liver slices were incubated in Krebs Ringer phosphate medium (3·0 ml), described in Methods, for 1 hr in O_2 at 37°. Labeled glucose (0·5 μCi/15 μmoles), galactose (0·5 μCi/3 μmoles), ethanol (5·0 mM) and MSB (0·5 mM) were present as indicated at the start of the experiment. The values of rats of $^{14}CO_2$ formation are expressed as natoms (± S.E.) $^{14}CO_2$/hr per 100 mg wet tissue. Number of observations is given in parentheses. The data presented in Tables 5 and 6 are derived from the same set of experiments.

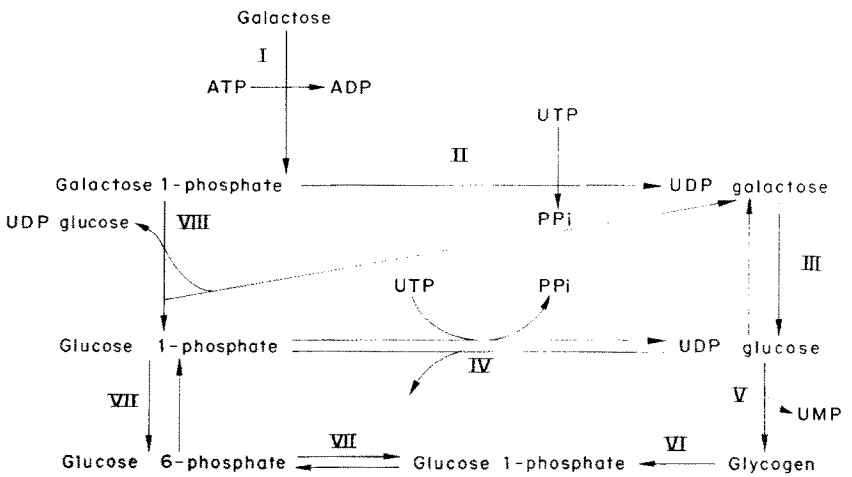

FIG. 1. Scheme showing conversion of galactose to glucose 6-phosphate. The individual reactions are catalyzed by the following enzymes: I, galactokinase, EC 2.7.1.6; II, UDP galactose pyrophosphorylase (UTP:D-galactose 1-phosphate uridylyl transferase), EC 2.7.7.10; III, UDP-glucose 4-epimerase, EC 5.1.3.2; IV, UDP glucose-pyrophosphorylase, EC 2.7.7.9; V, glycogen synthetase, EC 2.4.1.11; VI, glycogen phosphorylase, EC 2.4.1.1; VII, phosphoglucose mutase, EC 2.7.5.1; VIII, hexose 1-phosphate uridylyltransferase, EC 2.7.7.12.

rat liver, it is probable that galactokinase is the rate-limiting enzyme (Fig. 1, step 1).

During the development of an animal from fetus to adult, changes involving glycogen metabolism and the synthesis of enzymes affecting carbohydrate breakdown appear to take place.[3,21,22] In agreement with these findings *in vivo*, the synthesis of glycogen *in vitro* appears to proceed at a higher rate in normal livers than in newborn and regenerating livers. Incorporation of (1-^{14}C)galactose proceeds at a higher rate than that of (1-^{14}C)glucose at 1 mM indicating that UDP-glucose is formed at a faster rate from galactose than from glucose at equivalent concentrations (Table 3). Under conditions favourable for the oxidation of galactose, it appears that incorporation of galactose into glycogen is generally lowered, as indicated in Table 3. Nevertheless, due to the large increase in the metabolism of galactose in regenerating rat liver in the presence of MSB, incorporation into glycogen occurs marginally.

Incorporation of (2-^{14}C)galactose into the glycogen of liver was shown to have been recovered as the glucose moiety labeled in the C-2 position to the extent of 75-92 per cent, indicating that the galactose molecule is carried intact into the metabolic pathway until it is converted to UDP-glucose.[23] Though galactose and glucose show common intermediates in their conversion to glycogen or to CO_2, it appears that the higher rate of metabolism of galactose as compared to that of glucose is due to the phosphorylation of the latter being self-limiting. As shown in Fig. 1, galactose is metabolized to glucose 6-phosphate before it is converted to CO_2 through the HMP and glycolysis. At present, no simple and precise method exists to estimate the extent of the operation of these pathways in such a complex condition as that in liver.[24] However, the relative quantitative importance of these pathways can be ascertained by the use of specifically labeled glucose under identical conditions. Labeled CO_2 is derived from (1-^{14}C)glucose through the HMP and glycolysis, whereas $^{14}CO_2$ from (6-^{14}C)glucose is mainly derived from glycolysis and the citric

acid cycle. Thus the excess of $^{14}CO_2$ from (1-^{14}C)glucose is a simple indication that the HMP is operative in a tissue. However, excessive operation of the HMP will result in recycling of (6-^{14}C)glucose through transaldolase and transketolase reactions and may contribute to the $^{14}CO_2$ derived through the HMP, thus complicating the picture. Then one may not be able to observe any increase in the HMP by the mere ratio of $^{14}CO_2$ derived from (1-^{14}C)glucose and (6-^{14}C)glucose.[24] A specific inhibitor or stimulator may be useful to ascertain the relative operation of these pathways. The use of ethyl alcohol as an inhibitor acting at the NADP level had been suggested for the study of the HMP[25] which was, indeed, found to be inappropriate.[26] Majchrowicz and Quastel[13] have shown that $^{14}CO_2$ yields from the metabolism of (1-^{14}C)glucose are not suppressed by ethanol, but yields from (6-^{14}C)glucose are stoichiometrically suppressed by isotopic dilution. When the effect of ethanol on the oxidation of various substrates has been examined in liver, that of galactose is markedly inhibited.[7] The inhibition is, however, much attenuated in regenerating rat liver. Similar results were obtained in the present study. Formation of $^{14}CO_2$ from (1-^{14}C)glucose is inhibited by ethanol by about 41 per cent in the controls, in contrast with 28 per cent in the regenerating tissue slices. The inhibition of $^{14}CO_2$ formation from (6-^{14}C)glucose is much higher (68 per cent), the net yields in the presence of ethanol being not significantly different ($P > 0{\cdot}05$). This is in conformity with the hypothesis that ethanol exerts its effect by isotopic dilution of the acetyl CoA pools derived from labeled hexoses. Accordingly, the diminished inhibition of galactose oxidation by ethanol in regenerating rat liver is due to its channeling to the HMP pathway.[7] The mechanism of action of MSB can be conjectured from this background.

In regenerating rat liver slices, as well as in those of normal animals, MSB (0·5 mM) increases the formation of $^{14}CO_2$ from (1-^{14}C)glucose to an equal extent (three-fold). Such an increase has not been observed with (6-^{14}C)glucose in normal liver (Table 5). Menadione has been shown by Marki and Martius[27] to oxidize reduced NADP by the liver enzyme, NADP-methyl-1,4-napthoquinone-oxidoreductase (EC 1.6.99.2). The resultant high NADP/NADPH ratio is likely to allow metabolism of glucose and galactose (the latter via glucose 6-phosphate) to proceed maximally through the HMP pathway. Similar rates of glucose catabolism in normal and regenerating liver tissues in the presence of MSB indicate that the pools of glucose are of identical size in both. On the other hand, an increase noted in the yields of $^{14}CO_2$ from (6-^{14}C)glucose in regenerating rat liver suggests an extensive recycling of C-6 of glucose through the transaldolase and transketolase reactions of the shunt pathway. This increase from C-6 cannot possibly be due to stimulation of glycolysis and the citric acid cycle because MSB seems to have no effect on the path of carbon channeled through them, as evidenced from the studies on fructose. MSB is specific for the oxidation of NADPH in tissue slices because it does not increase the formation of $^{14}CO_2$ from (U-^{14}C)fructose either in regenerating rat liver tissue or in the controls. Fructose is metabolized in liver as fructose 1-phosphate into glyceraldehyde and dihydroxyacetone phosphate, and NADP is not involved in $^{14}CO_2$ formation by this pathway.[28] Whereas MSB increases the formation of $^{14}CO_2$ from (1-^{14}C)galactose by 151 per cent in regenerating rat liver, the increase is only 57 per cent in normal adult liver (Table 4). The difference in MSB stimulation of yields of $^{14}CO_2$ observed in the two tissues is again a reflection of the relative rates at which galactose

is converted into the pools of glucose 6-phosphate which presumably would be channeled into the HMP in each tissue. The addition of unlabeled glucose to labeled galactose, and *vice versa*, does not significantly alter the amount of labeled CO_2 produced from the respective precursor. Thus the endogenous pool of glycogen, which is so small under the select experimental conditions, does not appear to alter the rate of galactose oxidation. Any minor contribution made to the pool of glucose 1-phosphate by the endogenous glycogen of the liver slices would not alter the net $^{14}CO_2$ yields, because, as has been elegantly demonstrated by Majchrowicz and Quastel[13] in a similar study, an increase in concentration of glucose would result in a proportional increase in the rate of metabolism over a wide range of concentrations (0·5-50·0 mM).

In summary, the results presented show that the metabolism of (1-^{14}C)galactose to $^{14}CO_2$ proceeds at a more rapid rate in regenerating rat liver than in that of controls. The formation of $^{14}CO_2$ from labeled galactose is stimulated by MSB (0·1-0·5 mM). Also, the inhibition of the production of $^{14}CO_2$ from labeled galactose by ethanol is counteracted by MSB. This action of MSB appears to be due to its ability to oxidize NADPH and thus, presumably, accelerate the operation of HMP. The role of vitamin K derivatives on the metabolism of carbohydrates *in vivo* is not known. It would be of value to study their role in ethanol intoxication and impairment of carbohydrate metabolism in the intact animals.

Acknowledgement It is a pleasure to acknowledge the interest and guidance of Professor J. H. Quastel, F.R.S., University of British Columbia, Vancouver, Canada, throughout the course of this work.

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