Conversion of Glucose-^{14}C(UL) to $^{14}CO_2$, ^{14}C-Glycogen, and ^{14}C-Fatty Acids in the Partially Hepatectomized Rat (34795)

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Previous studies (1) have shown that the CO_2 production by the two-thirds hepatectomized rat is decreased in proportion to the plasma free fatty acid concentrations during the 1 hr to 7 days postoperative period. Simultaneously, the recovery of acetate-2-^{14}C as $^{14}CO_2$ from two-thirds hepatectomized rats is comparable to control values. These results have been interpreted to mean the two-thirds hepatectomized rat is proportionally deriving a greater amount of energy from fat than is the normal rat. Thus, it appears that any dilution of the acetate-2-^{14}C resulting from the breakdown of plasma free fatty acid into two carbon fragments is compensated for by an increased utilization of acetate through the TCA cycle.

In view of these findings, one would expect the conversion of glucose to CO_2 to be diminished in the two-thirds hepatectomized rat. Numerous studies (2, 3) have shown that the relative contribution of plasma fatty acids and glucose to the total energy production of an animal depends upon the respective concentrations of the two metabolites. However, it is possible for a decreased metabolic pool size to maintain it's proportional energy contribution to the whole animal by decreasing its turnover time (4), thus increasing its turnover rate. To determine the turnover time of endogenous glucose with ^{14}C-glucose requires measurements of plasma specific activity. This presents a technical problem of sampling and dilution in small animals that may interfere with their regenerative and metabolic processes. However, the relative utilization of glucose can be estimated from end products of ^{14}C-glucose, such as continuous $^{14}CO_2$ production, in conjunction with terminal measurements of other ^{14}C products. Through these measurements, it is possible to understand better the relative role of plasma free fatty acids and plasma glucose in contributing to the metabolic state and energy production in the two-thirds hepatectomized rat. These relationships are the subject of this report.

Methods. Approximately 67% of the liver of male Sprague-Dawley rats (155–180 g) was removed under ether anesthesia according to the procedure of Ralli and Dumm (5). Sham-operated control rats were exposed to the same surgical procedure, except their livers were handled and then returned intact to the abdominal cavity. The partial hepatectomies and sham operations were performed in pairs, one partially hepatectomized (PH) and one sham-operated (SO) rat of equal weight for each experiment. The study consisted of 48 such experiments over a 3-month period.

At various times (from 1 hr to 7 days) postoperative, a PH and an SO rat were each injected with 5 mg of glucose-^{14}C(UL) (10 μCi/5 mg *ip*) dissolved in 1.0 ml saline. Each animal was then placed in a continuous recording radiorespirometer (6) described in detail previously (7). The expired $^{14}CO_2$ and CO_2 were measured for 3 hrs by flushing the expired breath through the analyzer system with aged CO_2-free carrier gas (air) at flow rates of 1 liter/min. The flow rate was standardized at 760 mm Hg and 22° for each experimental run. The mean of the recorded output was taken at 2-min intervals for computer computational purposes. Upon removal from the radiorespirometer, the animal was decapitated, the plasma collected, and the whole liver weighed. Glycogen determinations were made on the gastrocnemius muscle and a portion of the liver as described by Montgomery (8). The ^{14}C-

glycogen content was determined on an aliquot of the final glycogen extract by liquid scintillation spectrometry using Cab-O-Sil as a suspension gel. A portion of the liver was saponified overnight with a solution of alcoholic-KOH over a steam bath. The nonsaponifiable liver lipid fraction was extracted with hexane prior to acidification, and the fatty acids (FA) were extracted with hexane after acidification at 4° with 6 *N* HCl. The ^{14}C-content of both lipid fractions was determined by liquid-scintillation spectrometry. The liver FA concentration was determined by evaporating a portion of the FA extract and weighing the dried residue.

Plasma levels of free fatty acid (FFA) (9), glucose (10), and cholesterol (11) were determined. A portion of the final heptane extract from the FFA extraction process was used to determine the ^{14}C-lipid content of the plasma (7).

All rats were sacrificed between 11 AM and noon to control the effects of diurnal variation on metabolism and glucose utilization. Thus, surgery was performed at various times of the day and night to conform to this criterion and to obtain the desired postoperative time period. The maximum mitotic activity occurs approximately 28 hr after partial hepatectomy in similarly treated rats (12). The rats were maintained on a balanced diet containing 6–8% fat and were given food and water *ad libitum* except during the radiorespirometry procedure. In addition to the PH and SO rats, two groups of nonoperated (NO) control rats were run. These two groups were selected on the basis of weight, one group to approximate the weight of the PH and SO rats during the 1- to 3-day postoperative period (162 g average) and the other group, the 1-week postoperative period (211 g average). The data were analyzed with Student's *t* test comparing the PH rat with its SO control.

Results. Table I shows that the PH and SO rats weighed the same at the start of the experiment. During the postoperative time periods the SO rats continued to gain weight and by the 51-hr postoperative time period their final body weight was significantly greater ($p < .01$) than their presurgical weight. In contrast, the PH rats lost weight and their final body weight was significantly less than their corresponding SO controls for all postoperative time periods except the 4-hr period. Also, the PH rats' final body weight was significantly less than their own presurgical body weight for the 19-, 27-, 39-, and 51-hr postoperative time periods.

The regenerating liver when compared with the 4-hr SO control (Table I) had regained its original mass at the end of 1 week.

TABLE I. Rat Body Weight Prior to Sham Operation or Two-Thirds Hepatectomy and the Body Weight and Final Liver Weight at Various Times after Sham Operation or Two-Thirds Hepatectomy.

	Presurgical body wt (g)		Final body wt (g)		Final liver wt (g)	
Postoperative time period	Sham operated	Hepatectomized	Sham operated	Hepatectomized	Sham operated	Hepatectomized
4 hr	173 ± 5[g]	171 ± 5	169 ± 5	161 ± 5	7.77 ± 0.17	2.26 ± 0.16[c]
19 hr	162 ± 3	162 ± 4	163 ± 3	148 ± 3[bd]	7.20 ± 0.22	2.80 ± 0.13[c]
27 hr	159 ± 3	160 ± 3	159 ± 3	146 ± 2[bc]	6.96 ± 0.30	3.06 ± 0.13[c]
39 hr	167 ± 5	170 ± 4	171 ± 4	153 ± 3[bc]	8.01 ± 0.67	3.60 ± 0.34[c]
51 hr	160 ± 2	158 ± 3	168 ± 1[c]	141 ± 5[cd]	7.71 ± 0.25	3.98 ± 0.15[c]
75 hr	162 ± 5	162 ± 2	176 ± 1[d]	157 ± 5[b]	8.34 ± 0.35	5.24 ± 0.25[c]
1 week	160 ± 3	163 ± 2	213 ± 2[f]	197 ± 5[af]	10.06 ± 0.24	8.20 ± 0.22[c]
Nonoperated controls						
162 g			162 ± 1		6.97 ± 0.18	
211 g			211 ± 4		9.69 ± 0.36	

[a] $p < .05$, [b] $p < .01$, [c] $p < .001$ significantly different from sham-operated controls.
[d] $p < .05$, [e] $p < .01$, [f] $p < .001$ significantly different from own presurgical body weight.
[g] $N = 6 \pm$ SEM.

However, the regenerating liver had only 80% of the mass of its respective SO control at the end of 1 week as a result of the normal liver growth of these control rats during this period.

Plasma FFA (Fig. 1, upper) rose rapidly after two-thirds hepatectomy and reached a maximum of 1425 μeq/liter at the end of 39-hr postoperative period as compared to 333 μeq/liter for SO controls. This was followed by decreasing but still significantly higher values than SO controls during the 51-hr and 75-hr postoperative period.

Plasma glucose (Fig. 1, middle) levels for the PH rats were significantly lower than those of their SO controls during all postoperative time periods through the 75-hr period. Plasma glucose levels for the PH rats ranged from 80 to 89 mg/100 ml for the 19-, 27-, and 39-hr postoperative time periods as compared to 129 mg/100 ml for SO controls during these times. Plasma glucose levels for the PH rats had returned to SO control values at the end of the 1-week postoperative time period.

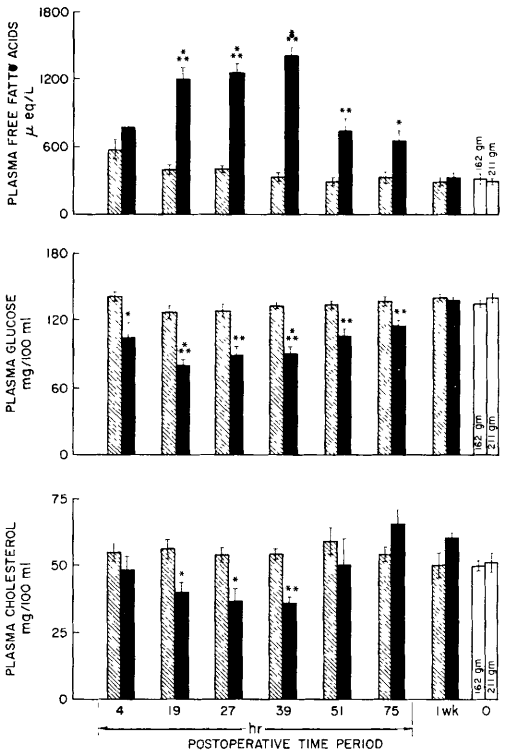

FIG. 1. Plasma free fatty acids (upper), glucose (middle), and cholesterol (lower) levels in rats after two-thirds hepatectomy. Each value represents the mean ±SE of 6 rats. *$p<.05$, **$p<.01$, ***$p<.001$, significantly different from sham-operated controls. Hepatectomized, solid block; sham-operated controls, shaded block; nonoperated controls, open block.

Plasma cholesterol (Fig. 1, lower) gradually decreased in the PH rats to a minimum of 36 mg/100 ml at the end of the 39-hr postoperative time period. This minimum was significantly ($p < .01$) less than SO control values of 54 mg/100 ml. Plasma cholesterol returned to SO control values by the end of the 51-hr postoperative time period.

The specific activity of the expired CO_2 (Fig. 2) was greater for the PH rats than for the SO controls during the descending portion of the respiratory curves for all postoperative time periods. The CO_2 specific activity for PH rats was significantly greater ($p < .05$) than for the SO controls at various points in time on the curve during all postoperative periods except 1 week. In general, the time of maximum specific activity was delayed for the PH rats.

The total percentage of recovery of glucose-^{14}C(UL) as $^{14}CO_2$ (Fig. 3) ranged from 21.9 to 26.2% for the PH rats as compared to 19.1 to 25.2% for the SO controls during all postoperative time periods studied with the exception of the 16- to 19-hr postoperative time, the PH rats excreted less $^{14}CO_2$ during the early part of the recovery period than did the SO controls. The PH rats showed a significant ($p < .05$) decreased production of $^{14}CO_2$ during the first hour of recovery for the 24- to 27-hr and 48- to 51-hr postoperative time periods. However, no significant difference was noted in the total percentage of recovery of $^{14}CO_2$ at the end of the 3-hr radiorespirometry period. The PH rats produced less CO_2 than the SO controls during the 3-hr radiorespirometry time for all postoperative time periods except the 1-week period. The CO_2 excretion by the PH rats became significantly less ($p < .05$) than that of the SO controls at the 16-hr postoperative time period and remained significantly less until the 1-week period.

Figure 4 (upper) shows the recovery of

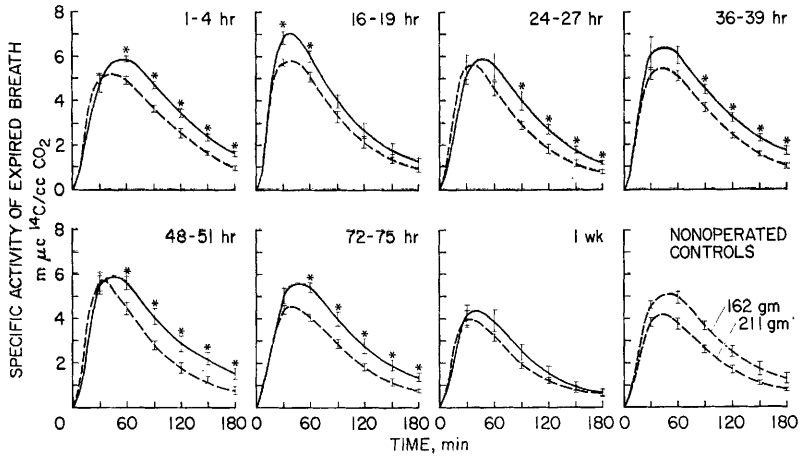

FIG. 2. Specific activity of the expired CO_2 from two-thirds hepatectomized rats after injection with glucose-^{14}C(UL) at various times postoperatively. Each value represents the mean ±SE of six rats. *$p<0.05$, significantly different from sham-operated controls. Hepatectomized, solid line; sham-operated controls, broken line; nonoperated controls, hatched line.

glucose-^{14}C(UL) as liver ^{14}C-fatty acids was significantly different for the PH rats and the SO control at the 51-hr postoperative time only. The PH rats at the end of the 51-hr postoperative period had 1024 dpm/g liver as compared to 2820 dpm/g liver for the SO controls. The liver FA concentration (Fig. 4, middle) in the PH rat was significantly ($p <$ .05) greater than in the SO controls at the end of the 4-hr postoperative time. The PH rats continued to increase their liver FA concentration to a maximum of 91.3 mg/g liver at the end of the 39-hr postoperative time. The liver FA concentration in the PH rats had returned to SO control values at the end of the 75-hr postoperative time. The liver FA specific activity (Fig. 4, lower) was decreased in the PH rats at the end of the 4-hr postoperative time. The specific activity level continued to decrease gradually to a minimum value of 52 dpm/mg FA for the PH rats at the end of the 51-hr postoperative

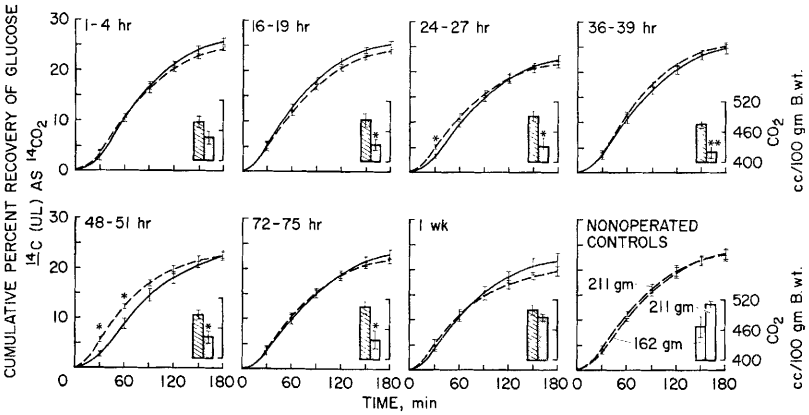

FIG. 3. Cumulative percentage of recovery of glucose-^{14}C(UL) as $^{14}CO_2$ and total CO_2 production from two-thirds hepatectomized rats after injection at various times postoperatively. Each value represents the mean ±SE of six rats. *$p<.05$, **$p<.01$ significantly different from sham-operated controls. Hepatectomized, solid line and solid block; sham-operated controls, broken line and shaded block; nonoperated controls, hatched line and open block.

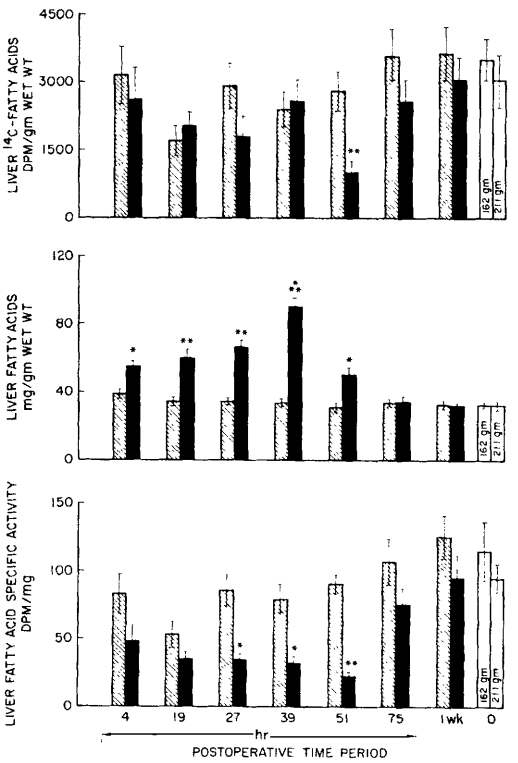

FIG. 4. Liver ^{14}C-fatty acids (upper), liver fatty acids (middle), and the specific activity of liver fatty acids (lower) in two-thirds hepatectomized rats 3 hr after injection with glucose-^{14}C(UL) at various times postoperatively. Each value represents the mean ±SE of six rats. $*p<.05$, $**p<.01$, $***p<.001$, significantly different from sham-operated controls. Hepatectomized, solid block; sham-operated controls, shaded block; nonoperated controls, open block.

time as compared to 90 dpm/mg FA for SO controls. After reaching the minimum value the specific activity increased to values comparable to those of the controls.

Figure 5 (upper) shows the recovery of glucose-^{14}C(UL) as liver ^{14}C-glycogen was decreased below SO control values in the PH rats during the postoperative times of 4, 19, and 27 hr. During the postoperative times of 39, 51, and 75 hr and 1 week, liver ^{14}C-glycogen in the PH rats was comparable to that in SO controls. The liver glycogen content (Fig. 5, middle) decreased significantly ($p < .001$) in the PH rats immediately after hepatectomy and remained significantly less ($p < .01$) than in SO controls for all postoperative time periods studied. The liver glycogen specific activity (Fig. 5, lower) was increased in the PH rats for all postoperative time periods except 1 week. The increased specific activity was primarily due to a lower liver glycogen content in the PH rats.

Figure 6 (upper) shows that the recovery of glucose-^{14}C(UL) as muscle ^{14}C-glycogen was generally less in the PH rats than in SO controls for all postoperative time periods studied. The muscle glycogen content (Fig. 6, middle) increased in both the PH rats and SO controls during the 19- and 27-hr postoperative time periods. However, no significant difference was found between the

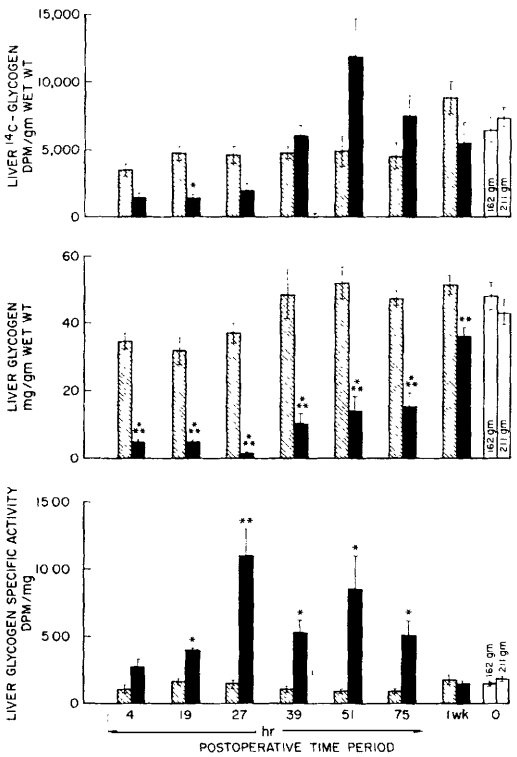

FIG. 5. Liver ^{14}C-clycogen (upper), liver glycogen (middle), and the specific activity of liver glycogen (lower) in two-thirds hepatectomized rats 3 hr after injection with glucose-^{14}C(UL) at various times postoperatively. Each value represents the mean ±SE of six rats. $*p<.05$, $**p<.01$, $***p<.001$, significantly different from sham-operated controls. Hepatectomized, solid block; sham-operated controls, shaded block; nonoperated controls, open block.

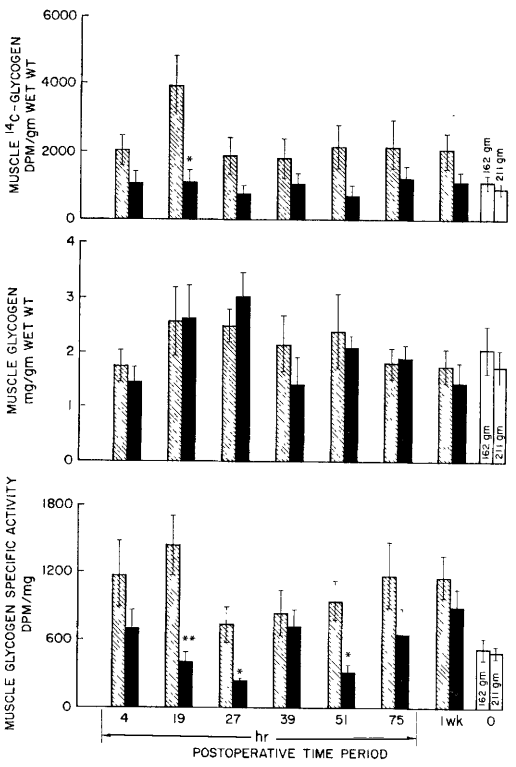

FIG. 6. Muscle ^{14}C-glycogen (upper), muscle glycogen (middle), and the specific activity of muscle glycogen (lower) in two-thirds hepatectomized rats 3 hr after injection with glucose-^{14}C(UL) at various times postoperatively. Each value represents the mean ±SE of six rats. $*p<.05$, $**p<.01$ significantly different from sham-operated controls. Hepatectomized, solid block; sham-operated controls, shaded block; nonoperated controls, open block.

rats and SO controls for all the postoperative time periods studied. In general, the muscle glycogen specific activity (Fig. 6, lower) was less in the PH rats than in the SO controls. This difference was significant ($p < .05$) at the 19-, 27-, and 51-hr postoperative periods. The decrease in the muscle glycogen specific activity is due to a decrease in ^{14}C-glycogen as the glycogen concentration is nearly in the PH and SO rats.

Figure 7 (upper) shows that the recovery of plasma ^{14}C-lipids from SO controls at the 4- and 19-hr postoperative time is significantly increased ($p < 0.5$) above that of the PH rats. This difference disappeared for later time periods as the plasma ^{14}C-lipids decreased in the SO controls from their earlier elevation.

The recovery of glucose-^{14}C(UL) as liver nonsaponified ^{14}C-lipid (Fig. 7, lower) gradually decreased in the PH rats to a value significantly less than SO control values at the 39- and 51-hr postoperative time. The nonsaponified liver ^{14}C-lipids returned to SO control values for the latter postoperative time periods.

Discussion. The data show an alteration of the metabolic pattern for glucose and FFA in the two-thirds hepatectomized rat. Hepatectomy causes a rise in plasma free fatty acids and a concomitant decrease in plasma glucose levels and CO_2 excretion. Hepatectomy also causes a decrease in liver glycogen and plasma cholesterol concentrations.

The total percentage of recovery of glucose-^{14}C(UL) as $^{14}CO_2$ in the PH rat is not significantly different from that in the SO

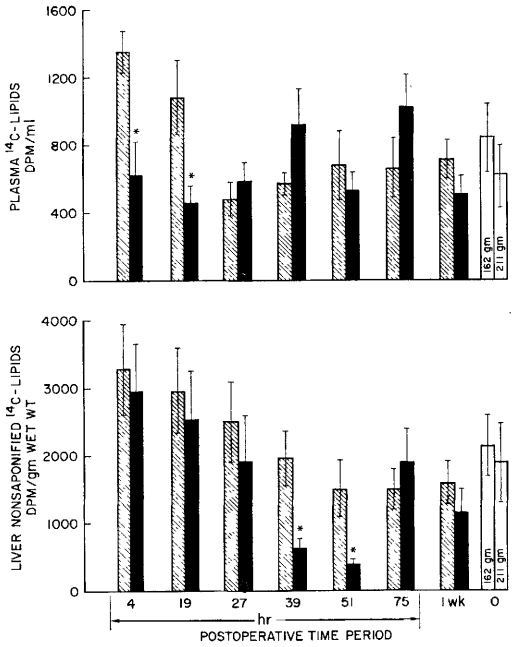

FIG. 7. Plasma ^{14}C-lipids (upper) and liver nonsaponified ^{14}C-lipids (lower) in two-thirds hepatectomized rats 3 hr after injection with glucose-^{14}C(UL) at various times postoperatively. Each value represents the mean ±SE of six rats. $*p<.05$ significantly different from sham-operated controls. Hepatectomized, solid block; sham-operated controls, shaded block; nonoperated controls, open block.

controls, although the plasma glucose level of the PH is significantly decreased. However, the recovery rate of $^{14}CO_2$ is decreased during the first hour of the recovery period for the PH rats and the time of maximum specific activity of the expired CO_2 occurs later in the PH rat than in the SO controls. This would indicate that the utilization of glucose is less in the PH rat than in the SO control. Figure 1 (upper) shows the plasma glucose level for the PH rat is only 65% of the SO controls at times. If the low levels of plasma glucose in the PH rat are due to an increased utilization of glucose, then the turnover time of plasma glucose would be decreased. Concomitantly, a decreased turnover time would decrease the time of maximum specific activity in the expired CO_2 by the PH rats which is not the case as shown in Fig. 2. Likewise, the total percentage of recovery of glucose-^{14}C(UL) as $^{14}CO_2$ would be increased above SO controls which is not the case.

Although the total percentage of recovery of glucose-^{14}C(UL) as $^{14}CO_2$ for the PH rat is not significantly different from that for the SO controls, the specific activity of the expired CO_2 is significantly increased in the PH rats. This is due partially to the lower CO_2 production by the PH rats and partially to a dilution effect upon the injected glucose. The injected glucose must be absorbed and transported to various target sites by the plasma. Also, the injected glucose must compete for the target sites with the endogenous plasma glucose. Thus, a standard injection of glucose-^{14}C(UL) given to two rats having different plasma glucose levels would cause a higher specific activity of plasma glucose in the rat with the lower plasma glucose level, notably in this case, the PH rat. Since the PH rat has a higher plasma glucose specific activity, it is possible for the PH rat to produce as much $^{14}CO_2$ as the SO controls even though the turnover of glucose is greater in the SO rat. Furthermore, any adjustment of the data on the basis of differences in the specific activity of plasma glucose between the PH and SO rats would influence the results of all ^{14}C measurements. The difference between the PH rats and SO controls would decrease where the PH rat has a greater incorporation of glucose-^{14}C(UL) into the measured end products than does the SO controls. Contrarily, the difference would increase where the PH rat incorporates less glucose-^{14}C(UL) into the measured end products than does the SO control.

The utilization of muscle glycogen by the PH rat appears to be less in magnitude than in SO controls. The muscle glycogen content in the PH rats was comparable to that in the SO controls while the ^{14}C-glycogen content was consistently less than that of the SO controls. If the utilization of muscle glycogen in the PH rats had been increased while maintaining the same concentration as in the SO controls, then the ^{14}C-glycogen content would have been increased in the PH rats. Perhaps this decreased utilization of muscle glycogen can be attributed to an increased utilization of plasma free fatty acids (13, 14). On the other hand, the turnover of liver glycogen by the PH rat is increased over that by SO controls until the end of the 75-hr postoperative period. The liver glycogen content of the PH rat is significantly decreased during all postoperative periods. However, the conversion of glucose-^{14}C(UL) to ^{14}C-glycogen is increased in the PH rats as shown by a high specific activity of liver glycogen. It cannot be ascertained from the data presented here if the increased specific activity of liver glycogen in the PH rats is truly representative of an increased liver glycogen turnover or is an artifact of increased plasma glucose specific activity in the PH rats. If an adjustment is made for the liver glycogen specific activity on the basis of a difference in the plasma glucose specific activity between the PH rats and SO controls, then the specific activity of liver glycogen in the PH rat would suggest a decreased turnover of liver glycogen in the premitotic phase until the initiation of mitotis when turnover increases. It is interesting that an increased turnover of liver glycogen does not occur during the 1-week postoperative period. This is a time when the plasma glucose level and liver glycogen content of the PH rat and the SO controls are comparable. However, the PH rat at the end of the 1-week postoperative time period has a lower liver glycogen

specific activity than does SO controls. The Crabtree effect has been noted in liver slices from PH rats during the 4- to 7-day postoperative period whereby the addition of glucose to the incubation media causes a respiratory inhibition in the liver slices (15). Simek and Sidláček (16) felt that the regenerative liver during this postoperative time period is not able to utilize glucose as readily because of the liver's previous adaptation to a predominantly lipid metabolism and utilization.

A comparison of the data presented here with a similar experiment using acetate (1) indicates a decreased conversion of glucose to lipid material. The liver nonsaponified ^{14}C-lipid in the PH rat originating from glucose-^{14}C(UL) gradually decreased in dpm until it was significantly less at the end of the 39- and 51-hr postoperative time periods. In the case of acetate, liver nonsaponified ^{14}C-lipids were uniform. Also, the specific activity of the liver FA arising from glucose in the PH rats gradually decreased to a minimum value at the end of the 51-hr postoperative period and at no time exceeded the specific activity of SO controls. With acetate, the time of minimum specific activity occurred at the end of the 39-hr postoperative period and was followed by an increased specific activity above that of the SO controls. Since the liver nonsaponified ^{14}C-lipids decreased significantly from their earlier level in the PH rat, whereas, with acetate they remained constant, a likely explanation is that glucose is not being reduced to acetyl CoA in the PH rat as fast as in the SO controls. This in turn causes a prolonged decrease in the liver FA specific activity of PH rats.

Summary. Partially hepatectomized (67%) rats and sham-operated controls were injected with glucose-^{14}C(UL) at various times postoperative from 1 hr to 1 week and the expired $^{14}CO_2$ and CO_2 was measured for 3 hr. At the end of the radiorespirometry procedure, plasma free fatty acids, glucose, cholesterol, and ^{14}C-lipids were determined. Fatty acids, ^{14}C-fatty acids, nonsaponified ^{14}C-lipids, glycogen, and ^{14}C-glycogen were determined in liver. Glycogen and ^{14}C-glycogen were also determined in muscle. Upon partial hepatectomy plasma free fatty acids increased while glucose and cholesterol decreased. The cumulative percentage of recovery of $^{14}CO_2$ decreased in the partially hepatectomized rat during the first hour of the radiorespirometry period. However, no significant difference between the partially hepatectomized rats and the sham-operated controls was noted at the end of the 3-hr radiorespirometry period. The specific activity of the expired CO_2 was increased in the partially hepatectomized rats because of their decreased CO_2 production. The specific activity of muscle glycogen was decreased in the partially hepatectomized rats while liver glycogen showed an increase which is interpreted to mean the turnover of muscle glycogen is decreased while the turnover of liver glycogen is increased. The recovery of liver ^{14}C-fatty acids and nonsaponified ^{14}C-lipid was decreased, indicating that a decreased conversion of glucose to acetyl CoA occurs in the partially hepatectomized rat. The effect of isotopic dilution resulting from a difference in plasma glucose levels between the two groups of rats is discussed.

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