Conversion of Acetate-2-^{14}C to $^{14}CO_2$ and ^{14}C-Fatty Acids in the Partially Hepatectomized Rat (33627)

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In vivo studies by Johnson and Albert (1, 2) have shown that the recovery of acetate-2-^{14}C in the ^{14}C-fatty acid portion of phospholipids in the regenerating liver of the rat is decreased during the premitotic phase and subsequently increased during the mitotic phase. Concomitantly, the recovery of the ^{14}C-fatty acid portion of glycerides is increased during both the premitotic and mitotic phases. Johnson and Albert suggested that the liver ^{14}C-fatty acids recovered during the premitotic phase are the result of lipid transport and esterification and that the high levels of fatty acids found in the regenerating liver are due to increased deposition of plasma free fatty acids originating from adipose tissue and not the result of an increased synthesis *in situ*. Bucher (3), in her review of their work, felt that the synthesis noted during the premitotic phase represents a decreased fatty acid synthesis in the regenerating liver which cannot be attributed to an isotopic dilution effect. This point can be ascertained only by measuring the endogenous acetate pool size, turnover rate, and specific activity.

The high levels of plasma free fatty acids resulting from two-thirds hepatectomy would influence acetate utilization since the metabolism of free fatty acids by muscle and liver is related to the plasma free fatty acid levels (4–6). The injected acetate must compete with the two carbon fragments originating from the breakdown of plasma free fatty acid, which would cause a dilution effect on acetate utilization similar to that found in centrifuged rats (7). To follow the specific activity of endogenous acetate in the rat presents a technical problem of sampling, which may interfere with the regenerative process. Thus, it becomes necessary to follow another end product of acetate-2-^{14}C that will not interfere with the regenerative process; namely, the formation of $^{14}CO_2$, to see if changes occurring in the oxidation of acetate are related to changes in fatty acid synthesis. This relationship is the subject of this paper.

Methods. Approximately 67% of the liver of male Sprague-Dawley rats (155–175gm) was removed under ether anesthesia according to the procedure of Ralli and Dumm (8). Sham-operated control rats were exposed to the same surgical procedure, except that 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 4-month period.

At various times (from 1 hr to 7 days) postoperative, a PH and an SO rat were each injected subcutaneously along the ventral midline of the abdomen with 1 mg of sodium acetate-2-^{14}C (10 μCi/mg) dissolved in 0.5 ml of saline. Each animal was then placed in a continuous recording radiorespirometer (9) described in detail previously (7). The expired $^{14}CO_2$ and CO_2· were measured for 3 hr 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 mean of the recorded output was taken at 2-min intervals for computational purposes. Upon removal from the radiorespirometer, the animal was decapitated, plasma collected, and the whole liver weighed. 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

TABLE I. Liver Weight and Fatty Acid Content of the Liver at Various Times after Sham Operation or Two-Thirds Hepatectomy.

Postoperative time period (hr)	Liver weight (g)		Liver fatty acid (g/100 g liver wet wt.)		Total liver fatty acid (mg)	
	Sham operated	Hepatectomized	Sham operated	Hepatectomized	Sham operated	Hepatectomized
4	7.22 ± 0.32[a]	2.12 ± 0.07[d]	3.81 ± 0.13	5.46 ± 0.37[b]	276 ± 18	115 ± 39[d]
19	7.17 ± 0.81	2.95 ± 0.14[d]	3.21 ± 0.11	5.92 ± 0.53[c]	230 ± 9	163 ± 17[c]
27	7.50 ± 0.42	3.40 ± 0.14[d]	3.51 ± 0.16	6.39 ± 0.58[c]	264 ± 11	214 ± 11[b]
39	7.46 ± 0.08	3.80 ± 0.08[d]	3.17 ± 0.16	9.02 ± 0.27[d]	246 ± 12	307 ± 10[d]
51	7.89 ± 0.29	4.48 ± 0.68[d]	3.18 ± 0.10	4.88 ± 0.56[b]	251 ± 11	217 ± 22
75	7.85 ± 0.20	5.81 ± 0.12[d]	3.14 ± 0.06	3.43 ± 0.34	247 ± 10	200 ± 20
1 week	10.04 ± 0.35	7.54 ± 1.40	3.05 ± 0.10	3.11 ± 0.05	300 ± 14	234 ± 44
Nonoperated controls						
168 g	7.52 ± 0.31		3.18 ± 0.04		240 ± 11	
207 g	9.85 ± 0.45		3.26 ± 0.11		320 ± 14	

[a] $N = 6 \pm$ SE.
[b] $p < .05$, significantly different from sham-operated controls.
[c] $p < .01$, significantly different from sham-operated controls.
[d] $p < .001$, significantly different from sham-operated controls.

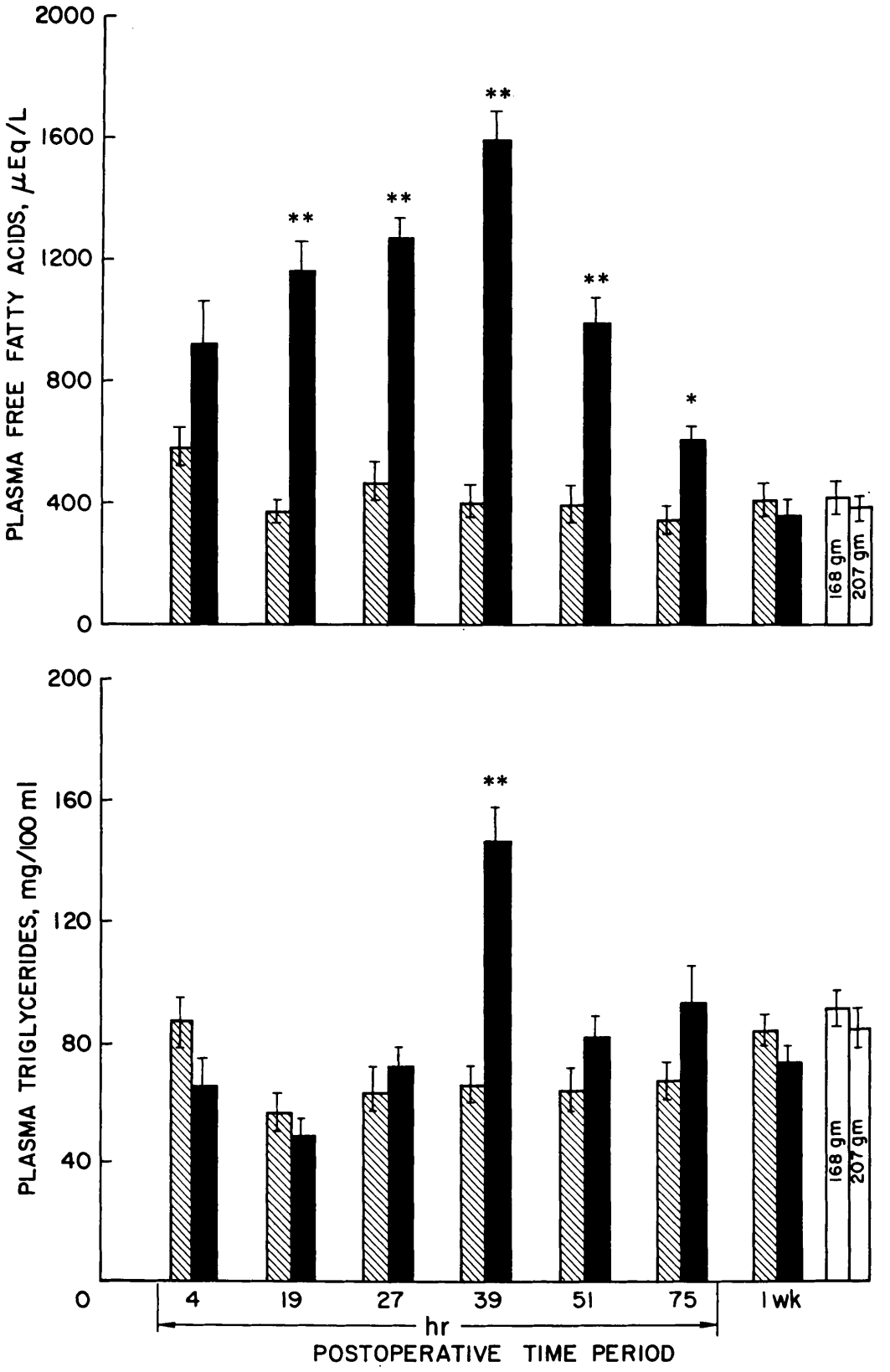
was determined by liquid scintillation spectrometry. The total tissue FA were determined by evaporating a portion of the FA extract and weighing the dried residue.

Plasma free fatty acids (FFA) were determined by the method of Trout *et al.* (10). 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). Plasma triglycerides (TG) were determined with a chloroform–methanol extraction as described by Jover (11). A portion of the final chloroform–methanol extract from the triglyceride extraction procedure was used to determine plasma phospholipids by measuring inorganic P after digestion and oxidation of the extract (12).

All rats were sacrificed between 11:00 AM and noon to control the effects of diurnal variation on metabolism and acetate 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 (unpublished data). 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 (168 gm average) and the other group, the 1-week postoperative period (207 gm average). The data were analyzed with Student's *t* test comparing the PH rat with its SO control.

Results. Table I shows the regrowth of the remaining liver in PH rats and the liver FA concentrations. The liver weight at the time of sacrifice shows that the regenerating liver had grown to its original mass at the end of the 1-week postoperative period. However, the regenerating liver had only 75% of the mass of its respective SO or NO controls at

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


PLASMA FREE FATTY ACIDS, μEq/L
2000
1600
1200
800
400
0
**
**
**
**
*
168 gm
207 gm
PLASMA TRIGLYCERIDES, mg/100 ml
200
160
120
80
40
0
**
168 gm
207 gm
4
19
27
39
51
75
1 wk
hr
POSTOPERATIVE TIME PERIOD

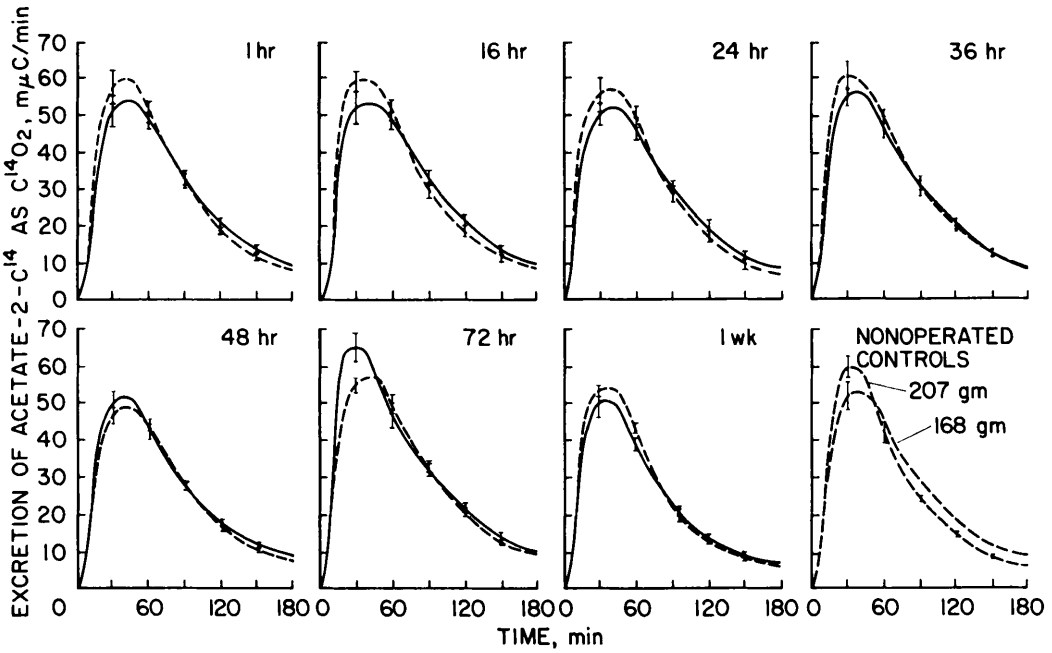


FIG. 2. $^{14}CO_2$ excretion patterns of two-thirds hepatectomized rats after injection of acetate-2-^{14}C. Each value represents the mean ±SE of 6 rats. Hepatectomized ——, sham-operated - - -, non-operated — — —.

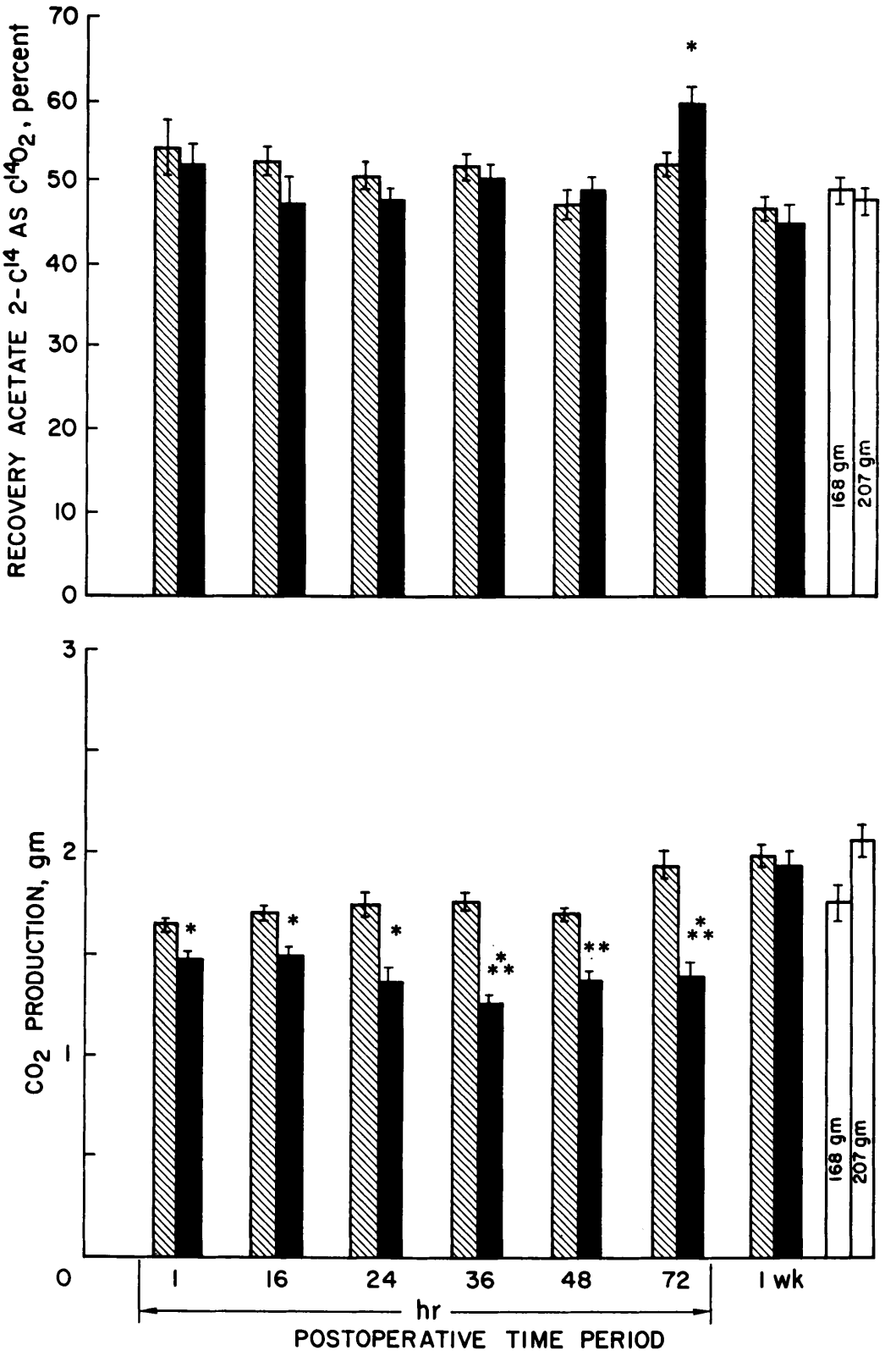
the end of 1 week as a result of the normal liver growth for these control rats during this period. A significant increase in the concentration of liver FA was noted as early as the 4-hr postoperative period. The liver FA concentration reached a significant ($p < .001$) maximum value of 9.02 gm/100 gm liver wet wt as compared to an SO control value of 3.17 gm/100 gm liver wet wt. The liver FA concentration returned to control values at the 75-hr postoperative period. The total liver FA content in the regenerating liver gradually increased from significantly less ($p < .05$) than SO control values at the early postoperative hours to a maximum of 307 mg at the 39-hr postoperative period. This maximum value at hour 39 was significantly greater ($p < .01$) than the SO control value of 246 mg. The total liver FA content was less than SO control values after hour 39 although this lowered value was not significantly different from controls.

The upper part of Fig. 1 shows that plasma FFA rose rapidly upon partial hepatectomy and reached 1590 μEq/liter at the 39-hr postoperative period, which was significantly different ($p < .001$) from SO control value of 388 μEq/liter. This was followed by a decreasing but still significantly elevated plasma FFA level at the 51- and 75-hr postoperative period. Finally, plasma FFA levels returned to control values at the end of 1 week.

Plasma TG (Fig. 1, lower) were significantly increased ($p < .001$) in the PH rat at the 39-hr postoperative period. This time corresponds to the time when plasma FFA and liver FA are at their maximum. No other changes in plasma TG were noted.

Plasma phospholipids (not shown) as measured by inorganic P showed no significant

FIG. 3. Percentage of recovery of acetate-2-^{14}C as $^{14}CO_2$ (upper) and the total CO_2 production (lower) during 3-hr period in two-thirds hepatectomized rats. Each value represents the mean ±SE of 6 rats; $*p < .05$, $**p < .01$, $***p < .001$. Hepatectomized, solid block; sham-operated, shaded block; nonoperated, open block.


RECOVERY ACETATE 2-C14 AS C14O2, percent
70
60
50
40
30
20
10
0
168 gm
207 gm
CO2 PRODUCTION, gm
3
2
1
0
1
16
24
36
48
72
1 wk
hr
POSTOPERATIVE TIME PERIOD

change during the post-operative period studied. The average values for P ranged from 8.2 to 12.4 mg/100 ml, the largest difference between any given PH group and its corresponding SO group being 0.4 mg/100 ml.

Figure 2 shows the $^{14}CO_2$ excretion patterns for rats injected with acetate-2-^{14}C. No changes in the excretion pattern are noted except during the 72–75-hr postoperative period, when the excretion of $^{14}CO_2$ by the PH rat increased as shown by the increased magnitude and the early occurrence of the time of maximum expiration of $^{14}CO_2$.

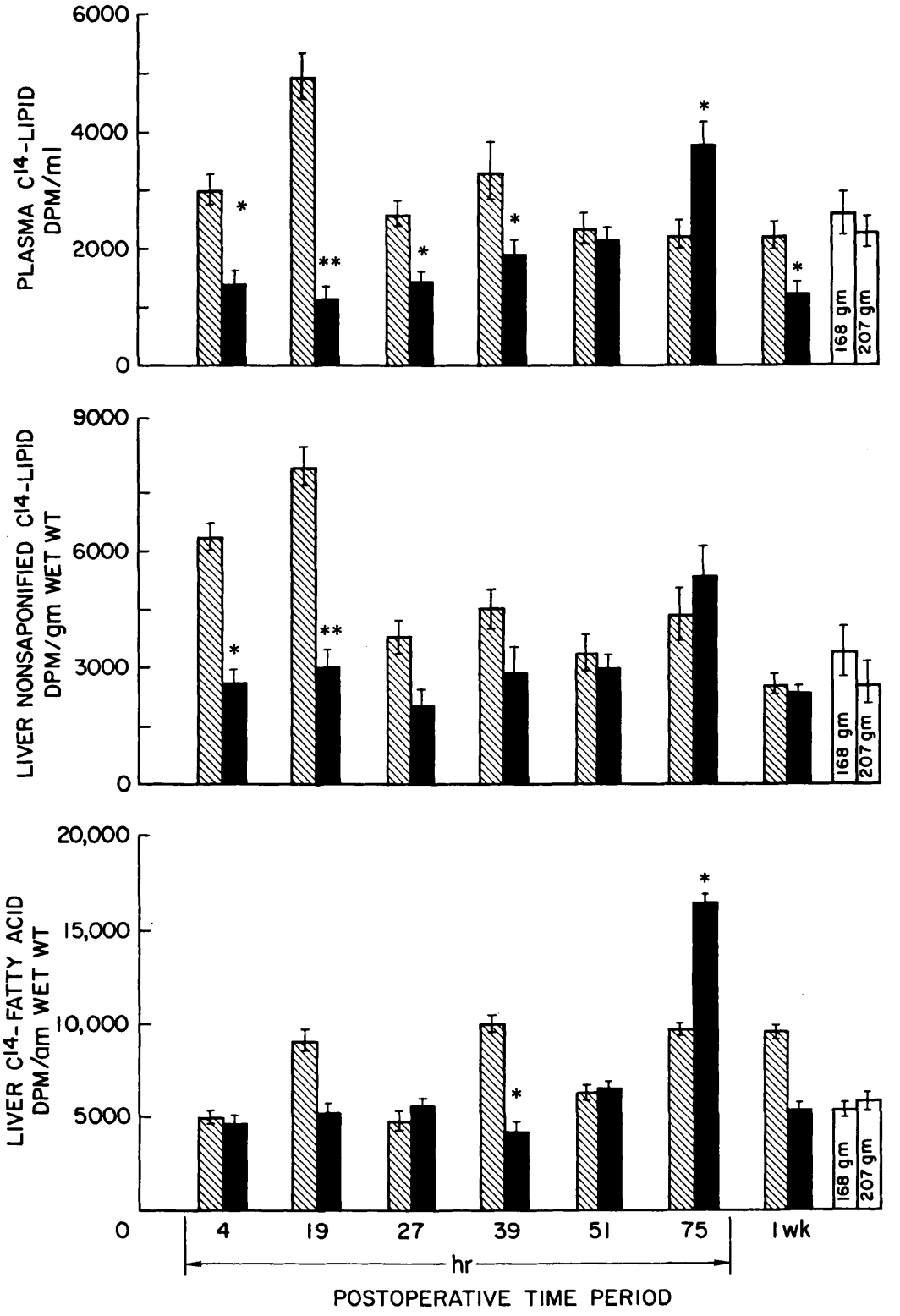
Figure 3 (upper) shows the total recovery of acetate-2-^{14}C as $^{14}CO_2$ during the 3-hr radiorespirometry period. The only significant change occurred at the 75-hr postoperative period when the PH rats showed an increased recovery. The total CO_2 production by the PH rats during the 3-hr radiorespirometry period was significantly less ($p < .05$) than for SO rats for all postoperative periods with the exception of the 1-week period (Fig. 3, lower).

Figure 4 shows the incorporation of acetate-2-^{14}C into plasma and liver lipids. The plasma ^{14}C-lipids (upper) of the PH rats were significantly less ($p < .01$) than those of the SO rats through the 39-hr postoperative period. The conversion of acetate-2-^{14}C to plasma lipids by the PH rats rose from 1325 dpm/ml at the 4-hr postoperative period to 3790 dpm/ml at the 75-hr postoperative period. At the 75-hr period the PH rats showed a significantly greater ($p < .01$) incorporation into plasma ^{14}C-lipids than did the SO rats. The conversion of acetate-2-^{14}C into nonsaponifiable lipids (middle) by the PH rats showed a slight but nonsignificant increase over SO rats only at the 75-hr postoperative period. The SO rats incorporated significantly more ($p < .01$) acetate-2-^{14}C into nonsaponifiable liver lipid at the 4- and 19-hr postoperative periods than did the PH rats. This change occurred at the same time periods that an increased plasma ^{14}C-lipid was noted in SO rats. The incorporation of acetate-2-^{14}C into liver FA (lower) by the PH rat was significantly greater ($p < .01$) than by the SO rats at the 75-hr postoperative period only.

Discussion. The data presented emphasize the role of plasma FFA in contributing to the energy requirements of the PH rat. Partial hepatectomy effects a rise in plasma FFA which reaches a maximum at the 39-hr postoperative period. Concomitantly, the concentration of liver FA rises and total CO_2 production decreases as a function of the plasma FFA levels. Since the amount of energy derived from fat is greater than that from protein or glucose for the same amount of CO_2 liberated, the lower CO_2 production strongly suggests that the PH rat selectively derives a greater proportion of its energy from the oxidation of fat than does the SO control. Johnson and Lederkremer (13) have shown that the utilization rate of palmitate by liver mitochondria obtained from PH rats is increased. However, it is not known whether these results can be extended to muscle and other tissue. Other studies (6) have shown that the utilization of plasma FFA by muscle is proportional to the plasma FFA concentration. The decrease in the total liver FA after the 39-hr postoperative period shows that the regenerating liver is utilizing a larger amount of fat as energy or releasing increased amounts of triglycerides into the plasma. This is the time when plasma triglycerides are elevated and the total liver FA content decreases even though the liver is increasing in size and the plasma FFA levels remain elevated. Simek et *al.* (14) have shown that the respiratory quotient of liver slices from PH rats decreases with increased liver lipid content. The decreased respiratory quotient was attributed mainly to an increased O_2 consumption, thus showing that regenerating liver has increased its utilization of lipid as an energy source.

Although the endogenous acetate pool size

FIG. 4. Recovery of acetate-2-^{14}C as plasma ^{14}C-lipids (upper), liver C^{14}-nonsaponifiable lipids (middle), and liver ^{14}C-fatty acids (lower) in two-thirds hepatectomized rats. Each value represents the mean ±SE of 6 rats; $*p < .01$, $**p < .001$. Hepatectomized, solid block; sham-operated, shaded block; nonoperated, open block.


PLASMA C14-LIPID DPM/ml
6000
4000
2000
0
LIVER NONSAPONIFIED C14-LIPID DPM/gm WET WT
9000
6000
3000
0
LIVER C14-FATTY ACID DPM/gm WET WT
20,000
15,000
10,000
5000
0
168 gm
207 gm
4
19
27
39
51
75
1wk
hr
POSTOPERATIVE TIME PERIOD

cannot be ascertained, it appears that any dilution of acetate resulting from plasma FFA breakdown is compensated for by an increased turnover of acetate. This is shown by the equal $^{14}CO_2$ excretion patterns for all the time periods studied except the increased $^{14}CO_2$ excretion noted at the 72–75-hr postoperative period. If a dilution of the injected acetate occurred without an increased turnover rate, the recovery of $^{14}CO_2$ for PH rats would be less than SO control values. On the other hand, if no dilution occurred, the $^{14}CO_2$ excretion patterns suggest that the increased levels of plasma FFA are not contributing to the energy requirements of the PH rat, which is contrary to the data presented here. The dilution of the injected acetate by an increased endogenous acetate pool size is probably proportional to the plasma FFA levels (15).

One of the most important and difficult problems in hepatectomy studies is determining whether the ^{14}C-FA in the regenerating liver arises from within or outside the liver. The recovery of ^{14}C-lipids in tissue for this purpose is complicated because of the lipid transport system in the intact animal. Camargo *et al.* (16) have shown that fatty livers do not occur in rats that have had 30% of the liver removed. The data presented here show the regenerating liver had obtained approximately 60% of its original mass when a decline from the peak level of plasma FFA was noted. Thus, the question arises: Are the increased plasma FFA levels in the two-thirds hepatectomized rat the result of increased FFA mobilization from adipose tissue or of the liver's inability to clear the normal flux of FFA from adipose tissue? Sham operation alone caused an increased plasma FFA level in the early postoperative period; however, this level had returned to presurgical values by hour 19. Glende and Morgan (17) determined the chain length of FA in adipose tissue and liver in the PH rat and concluded that the increased liver FA content originated from adipose tissue via mobilization and deposition. However, these results do not rule out the possibility that the regenerating liver synthesizes FA *in situ*.

Several aspects of the data indicate that the ^{14}C-FA content in the regenerating liver originates within the liver and that FA synthesis increases during the premitotic phase. The plasma ^{14}C-lipid is proportional to the liver mass, increasing as the liver mass increases. Thus, the plasma ^{14}C-lipids might be the result of synthesis in the liver rather than FA synthesis and release from adipose tissue. If the plasma ^{14}C-lipids originated from adipose tissue in the form of ^{14}C-FFA, then the liver ^{14}C-FA content in the PH rat would be much greater than SO or NO control values since the absorption and retention of plasma FFA in the regenerating liver is increased. This would be especially true during the 1–4-hr postoperative period when the regenerating liver is increasing in FA at the rate of approximately 4–5 mg/gm liver/hr, which compares to a rate of 0.20–0.25 mg/gm liver/hr during the 24–27-hr post-operative period. Simultaneously, the ^{14}C-FA/gm liver in the PH rat remained comparable to SO or NO control values during the premitotic phase even though the deposition of plasma FFA increased. Also, the ^{14}C-FA/gm liver in the PH rat remained comparable to SO or NO control values at the time a dilution of the injected acetate-2-^{14}C occurred with an enlarged endogenous acetate pool in the PH rat. Thus, the regenerating liver contributes to its increased liver FA content through synthesis during the premitotic phase as well as during the mitotic phase. Although the conclusions are that the fatty liver in the PH rat is the result of a combination of increased synthesis *in situ* and an increased deposition through transport, the relative importance of either mechanism cannot be assessed.

The dilution factor probably accounts for the plasma ^{14}C-lipids not increasing at the 39-hr postoperative period when plasma TG are significantly elevated. This is the time when plasma FFA levels and liver FA content are at their maximum. Also, the specific activity of the liver ^{14}C-FA is at its minimum. Therefore, large amounts of TG can be released by the liver without increasing the plasma ^{14}C-lipid level. This dilution effect is also shown by the slight decrease in the

recovery of liver ^{14}C-FA during this period.

Summary. Partially hepatectomized (67%) rats were injected with acetate-2-^{14}C at various times postoperative and the expired $^{14}CO_2$ and CO_2 measured for 3 hr. Liver ^{14}C-nonsaponifiable lipids, ^{14}C-fatty acids, and total fatty acid content were determined. Plasma triglycerides, phospholipids, free fatty acids, and ^{14}C-lipids were determined. The recovery of $^{14}CO_2$ showed no significant difference from sham-operated controls except during the 72–75-hr postoperative period when an increased excretion was noted. The specific activity of the expired CO_2 of the partially hepatectomized rat was increased as a result of a lower CO_2 production. The lower CO_2 production was attributed to an increased lipid oxidation by the partially hepatectomized rat. The appearance of ^{14}C as plasma lipids was proportional to the mass of the regenerating liver. Recovery of ^{14}C as liver fatty acids was comparable to sham-operated and nonoperated control values. The results suggest that, in addition to deposition via transport, the fatty acid content of the regenerating liver is also increased by synthesis *in situ* during the premitotic as well as the mitotic phase.

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Received Oct. 14, 1968. P.S.E.B.M., 1969, Vol. 130.