Reçu le 2 juillet 1979.

# EFFECT OF EVISCERATION ON THE DISPOSAL OF [$^{14}$C] PALMITATE IN THE RAT

BY

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(1 figure)

The utilization *in vivo* of [1-$^{14}$C] palmitate was studied in hepatectomized-nephrectomized rats and their sham-operated controls. After i.v. injection of the tracer, the [$^{14}$C] lipids in plasma disappeared more slowly in eviscerated animals than in their controls. More label reappeared in plasma as esterified fatty acids in the latter group. At 30 min after the tracer, the amount of label found in the lipidic fraction of carcass and heart was much greater in eviscerated animals than in their controls although the percentile distribution of labelled lipidic fractions remained stable, a considerable proportion being present in the esterified fatty acid form. On the basis of these findings, the rapid increase in the plasma levels of FFA in eviscerated animals must be the result of augmented lipolytic activity more than reduced utilization of these metabolites.

## INTRODUCTION

Evisceration in the rat produces an increased concentration of free fatty acids (FFA) in the blood (PENHOS *et al.*, 1976) which may be related to the basal lipolysis augmented *in vivo* in adipose tissue described in animals with reduced hepatic function (PECTOR *et al.*, 1978). Reduced FFA utilization could also influence the enhanced FFA circulating levels in evisceration. This effect could be due either to diminished ability of different tissues to esterify FA, as a consequence of the decreased availability of glucose (RUSSEL, 1942; REINECKE & ROBERTS, 1944; CARMANIU, 1978), a main source of α-glycerophosphate, and/or to a reduced utilization of FFA by other pathways. This problem has been inves-

tigated by determining the utilization *in vivo* of [1-$^{14}$C] palmitate in hepatectomized-nephrectomized rats.

## Materials and Methods

Female Wistar rats weighing 160-180 g and maintained in a controlled environment (23 °C; 12 h light and dark cycles) were hepatectomized and nephrectomized according to the methods of Higgins & Anderson (1931) and Russell (1942) as described elsewhere (Carmaniu, 1978) and compared with sham-operated controls. Immediately after surgery, the animals were injected through the inferior cava vein with 1 μCi of [1-$^{14}$C] palmitate bound to 8% bovine albumin purified by the method of Chen (1967). Blood samples were collected from the inferior cava vein at 5, 10, and 30 min after hepatectomy. Organs and carcass (muscle and skeleton without skin and viscera) were rapidly dissected and placed in ice-cold saline. Animals were maintained under Nembutal anaesthesia throughout the procedures.

Aliquots of plasma samples were used for counting their radioactivity, for purification of lipids (Folch *et al.*, 1957) for further fractionation (Kerpel *et al.*, 1961), and for the analysis of FFA concentration (Falholt *et al.*, 1973). Lipid extraction (Folch *et al.*, 1957) and fractionation (Kerpel *et al.*, 1961) were also performed in weighed aliquots of organs and carcass.

Radioactive measurements were assessed in a PPO/POPOP based scintillation cocktail dissolved in xylene and Triton X-100 and the samples were counted in a Nuclear Chicago (Isocap 300) counter provided with an external standard device. All radioactive values were corrected considering $10^6$ des./min the administrated tracer.

## Results

Immediately after evisceration (hepatectomy and nephrectomy) in the rat (Fig. 1), there was a marked rise in the circulating levels of FFA which persisted for the 30-min experiment. After i.v. administration of [1-$^{14}$C] palmitate, there was a loss of radioactivity in plasma which was faster in the sham-operated controls than in the eviscerated rats (Table I). In the former group, after

TABLE I. *Radioactivity in plasma after i.v. injection of [1-$^{14}$C] palmitate in hepatectomized-nephrectomized (eviscerated) rats.*

| Group | Min after operation | Total radioactivity (des. min$^{-1}$ ml$^{-1}$) | [$^{14}$C] total lipids (des. min$^{-1}$ ml$^{-1}$) | Percentual distribution of radioactivity in lipidic fractions | |
|---|---|---|---|---|---|
| | | | | FFA | Esterified-FA |
| Eviscerated rats ..... | 5 | 8 314 ± 1 619 | 6 616 ± 1 284 | 91.11 ± 2.19 | 8.90 ± 2.21 |
| ($n$ = 5) ..... | 10 | 3 270 ± 642 | 1 970 ± 490 | 86.39 ± 3.36 | 13.60 ± 3.36 |
| | 30 | 1 573 ± 156 | 1 125 ± 174 | 68.21 ± 7.48 | 31.78 ± 7.48 |
| Sham-operated controls ... | 5 | 2 049 ± 166** | 1 115 ± 69** | 90.48 ± 1.02 | 9.68 ± 1.05 |
| ($n$ = 5) ..... | 10 | 1 413 ± 171* | 640 ± 114* | 88.24 ± 1.99 | 11.75 ± 1.99 |
| | 30 | 3 803 ± 426** | 3 280 ± 495** | 34.20 ± 2.07** | 67.79 ± 2.07** |

$P$ of eviscerated as controls at each time : * = <0.05
** = <0.01

$n$ = number of animal/group.

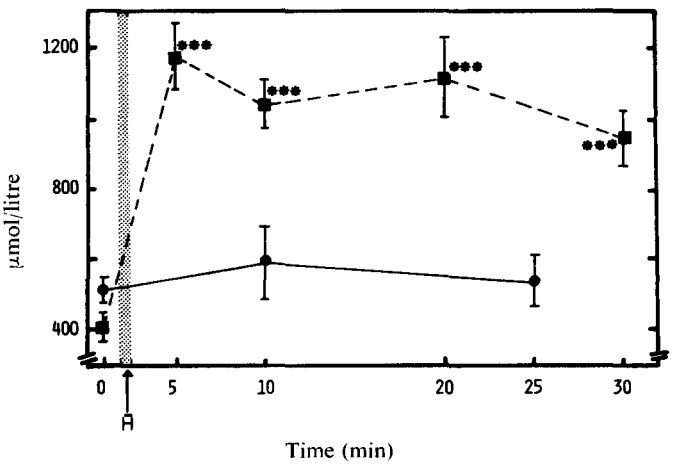


FIG. 1. *Plasma concentration of FFA at different times after hepatectomy-nephrectomy* ($\overline{H}$) *in the rat.*
Means ± SEM values of 5 animals in each group.
●——● : controls; ■ - - - - ■ : Hepatectomized.
*P* vs. 0 Time : *** $P < 0.001$.

TABLE II. *Radioactivity in tissues at 30 min after i.v. injection of* [*1-*$^{14}$*C*] *palmitate in hepatectomized-nephrectomized (eviscerated) rats.*

| | [$^{14}$C] total lipids (des./min whole tissue) | Percentual distribution of radioactivity of lipid fractions | |
|---|---|---|---|
| | | FFA | Esterified-FA |
| *Liver* | | | |
| Eviscerated | 97 ± 21 | — | — |
| Sham controls | 399 092 ± 19 597 | 49.72 ± 2.12 | 50.27 ± 2.12 |
| *P* | < 0.001 | | |
| *Lumbar Fat Pads* | | | |
| Eviscerated | 1 360 ± 394 | 18.43 ± 4.05 | 81.56 ± 4.05 |
| Sham controls | 2 076 ± 282 | 18.94 ± 2.23 | 81.06 ± 2.23 |
| *P* | NS | NS | NS |
| *Heart* | | | |
| Eviscerated | 31 480 ± 1 540 | 36.47 ± 2.66 | 63.52 ± 2.66 |
| Sham controls | 4 452 ± 300 | 36.55 ± 3.33 | 63.46 ± 3.31 |
| *P* | < 0.001 | NS | NS |
| *Carcass* | | | |
| Eviscerated | 395 094 ± 22 010 | 51.70 ± 2.24 | 48.22 ± 2.25 |
| Sham controls | 111 925 ± 9 583 | 51.91 ± 1.94 | 48.08 ± 1.95 |
| *P* | < 0.001 | NS | NS |

the period of lowest plasma radioactivity (10 min), there was an increase never observed in eviscerated animals. Most of the plasma radioactivity corresponded to lipids in both groups. Although initially all the label appeared in the form of [$^{14}$C] FFA, at 30 min there was a proportional decrease which contrasted with an increase in the [$^{14}$C] esterified fatty acids. At 30 min after the tracer, these changes were significantly smaller in the eviscerated animals than in their controls (Table I).

At 30 min after injection of the tracer, almost 40 % of the administered label appeared in the lipidic fraction of the liver in sham-operated controls while this percentage was found in the carcass of the eviscerated animals (Table II).

In addition to the enhancing effect of the quantity of label present in the lipidic fraction of the carcass, evisceration also produced a significant increase in the [$^{14}$C] lipids in the heart and a non-significant reduction in the label found in lipids of the lumbar fat pads. Evisceration, however, did not alter the percentile distribution of radioactivity in the lipidic fractions in each tissue (Table II). A greater proportion of lipids appeared in the lumbar fat pad esterified-FA fraction, followed by the heart, carcass, and liver.

## Discussion

The rapid rise in plasma FFA after evisceration was partially compensated for by the smaller distribution space and reduced utilization rate of the injected [$^{14}$C] palmitate. Thus specific activity of the labelled FFA circulating soon after administration of the tracer was similar in eviscerated and sham-operated control animals, facilitating comparison between these groups. The subsequent appearance of labelled esterified fatty acids in plasma of sham-operated controls confirms other reports (Laurell, 1959; Jones & Havel, 1967; Clouet *et al.*, 1974) and may be caused by return to the blood of the [$^{14}$C] FFA being taken up and metabolized for triglyceride synthesis by the liver, as this phenomena did not appear in hepatectomized animals. This interpretation is also in agreement with our previous study in which a considerable proportion of [$^{14}$C] palmitate administered to the intact rat returned to blood in the form of esterified fatty acid in very low density lipoproteins (Carmaniu & Herrera, 1979). In addition, it is well

known that the liver is the main site of synthesis of these lipoproteins (SMITH *et al.*, 1978).

Evisceration produces an intense increase in the incorporation of [$^{14}$C] palmitate in carcass and heart lipids which demonstrates that both skeletal and heart muscles become the main consumer tissues of circulating FFA. Because the amount of label taken up by these tissues in the eviscerated animals is similar to that found in liver of the sham controls, the enhanced concentration of plasma FFA in the former group can be explained not by their reduced utilization but by their augmented release from adipose tissue through lipolysis. A significant proportion of the [$^{14}$C] lipids in these tissues appears in the esterified fatty-acid fraction, suggesting that esterification is an important pathway for the temporal retention of fatty acids before their consumption. This esterification in eviscerated animals must be supported by consumption of glycerol for the synthesis of $\alpha$-glycerophosphate more than glucose, as in these animals the circulating glucose becomes very low (RUSSELL, 1942; REINECKE & ROBERTS, 1944; CARMANIU, 1978), while glycerol levels are augmented (CARMANIU, 1978). This interpretation is in agreement with the previously reported increase in utilization of [$^{14}$C] glycerol but not of [$^{14}$C] glucose for glyceride glycerol formation by these specific tissues in eviscerated animals (CARMANIU, 1978).

Some of the observed findings must be ascribed to the altered circulatory function in eviscerated rats producing important changes in blood flow and oxygen availability in the different tissues. Other conditions that alter these parameters, such as exercise and hypoxia, are known to produce an enhancement in the turnover of FFA (JONES & HAVEL, 1967; ALBERGHINA *et al.*, 1977) and a specific increase in the incorporation of labelled palmitate into heart lipids (ALBERGHINA *et al.*, 1977).

## ACKNOWLEDGMENTS

Part of this study was carried out with a grant from the *Comisión Asesora de Investigación Cientifica y Técnica, Presidencia del Gobierno, Spain.*

The authors wish to express their gratitude to Caroline S. DELGADO for her editorial help.

## REFERENCES

ALBERGHINA, M., CAMBRIA, A., PETRONE, G. & MISTRETTA, A. (1977) *Ital. J. Biochem.* **26**, 331-341.
CARMANIU, S. (1978) *Doctoral Thesis*, Barcelona Univ.
CARMANIU, S. & HERRERA, E. (1979) *Rev. Esp. Fisiol.*, in press.
CHEN, R. F. (1967) *J. Biol. Chem.* **242**, 173-181.
CLOUET, E., PARIS, R. & CLÉMENT, J. (1974) *Biochimie* **56**, 145-152.
FALHOLT, K., LUND, B. & FALHOLT, W. (1973) *Clin. Chim. Acta* **46**, 105-111.
FOLCH, J. M., LEES, M. & SLOANE-STANLEY, G. H. (1957) *J. Biol. Chem.* **226**, 497-509.
HIGGINS, G. M. & ANDERSON, R. M. (1931) *Arch. Pathol.* **12**, 186-195.
JONES, N. L. & HAVEL, R. J. (1967) *Amer. J. Physiol.* **213**, 824-828.
KERPEL, S., SHAFRIR, E. & SHAPIRO, B. (1961) *Biochim. Biophys. Acta* **46**, 495-504.
LAURELL, S. (1959) *Acta Physiol. Scand.* **47**, 218-232.
PECTOR, J. Cl., WINAND, J., VERBEUSTEL, S., HEBBELINCK, M. & CHRISTOPHE, J. (1978) *Amer. J. Physiol.* **234**, E579-E583.
PENHOS, J. C., BUTLER, E., WOODBURY, C., BUCHLY, M. & RAMEY, E. (1976) *Endocrinology* **99**, 636-640.
REINECKE, R. M. & ROBERTS, S. (1944) *Amer. J. Physiol.* **141**, 476-479.
RUSSELL, J. A. (1942) *Amer. J. Physiol.* **136**, 95-104.
SMITH, L. C., POWNALL, H. J. & GOTTO, A. M., Jr. (1978) *Annu. Rev. Biochem.* **47**, 751-777.

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