in these animals than in rats injected with *E. coli* only. The organisms were gradually removed from the normal kidney and were no longer demonstrable by culture 30 days after injection. In contrast, at 30 days, significant numbers of *E. coli* were still present in the kidneys of some rats with aminonucleoside-induced nephrosis. It is suggested that functional vascular alterations or, possibly, changes in renal enzyme patterns may be the important factors in the pathogenesis of this type of pyelonephritis.

1. Guze, L. B., Beeson, P. B., *J. Exp. Med.*, 1956, v104, 803.

2. Lepper, E. H., *J. Path. Bact.*, 1921, v24, 192.

3. Braude, A. I., Shapiro, A. P., Siemienski, J., *J. Clin. Invest.*, 1955, v34, 1489.

4. De Navasguez, S., *J. Path. Bact.*, 1956, v71, 27.

5. Woods, J. W., Welt, L. G., Hollander, W., Jr., Newton, M., *J. Clin. Invest.*, 1960, 39, 28.

6. Woods, J. W., *ibid.*, 1958, v37, 1686.

7. Hopper, J., Jr., Yamauchi, H., personal observation.

8. Kleeman, C. R., Hewitt, W. L., Guze, L. B., *Medicine* (Baltimore) 1960, v39, 3.

9. Fiegelson, E. B., Drake, J. W., Recant, L., *J. Lab. Clin. Med.*, 1957, v50, 437.

10. Vernier, R., Papenmaster, B. W., Good, R. A., *J. Exp. Med.*, 1959, v109, 115.

11. Dubach, U. C., Recant, L., *J. Clin. Invest.*, 1960, v39, 1364.

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Oxidation of Palmitate-1-C^{14} by Red Blood Cells.* (26579)

JOSEPH P. HRACHOVEC, MICHÈLE LEBLANC† AND MORRIS ROCKSTEIN
New York University Medical Center, New York University School of Medicine

It is well established that many body tissues have the enzymatic equipment for oxidation of both glycerides and carbohydrates as a source of energy. Isotope tracer studies have shown that both these processes occur simultaneously in tissue cells, where either glucose or fatty acid oxidation may prevail to a different extent according to the experimental conditions. The non-esterified fatty acids (NEFA) of the blood plasma, present in water-soluble albumin complexes, have been shown to be highly active metabolites(1,2). They appear to be the prerequisite form for penetration of long-chain fatty acids into sites of oxidation(3,4) and the flux of calories carried by NEFA may reach more than twice the caloric value of glucose transport(5), although NEFA comprise only about 5% of the total fatty acids of the plasma.

It has long been known that red blood cells oxidize glucose as a source of energy(6). Although biosynthesis of fatty acids by red blood cells has been investigated by James, *et al.*(7), oxidation of fatty acids by red blood cells, to our knowledge, has not been examined.‡ However, oxidation of palmitate-1-C^{14} by skeletal muscle *in vitro* has been examined by Fritz, *et al.*(8), and its oxidation by hepatic and adipose tissues has been examined by Milstein and Briscol(9) and others. The aim of our investigation was to ascertain the existence of an enzymatic mechanism for fatty acid oxidation in red blood cells.

Methods. Normal, fed, adult, male rats, weighing between 200 and 400 g were killed by decapitation and rapid exsanguination. The blood was collected into about 15 to 20 times its own volume of modified Tyrode phosphate medium, (NaCl—0.137 M; KCl—0.004 M; Na_2HPO_4 buffered to pH 7.38—0.01 M). No anti-coagulants were used because this dilution of blood is sufficient to prevent formation of clots without their use.

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† Fulbright Research Scholar 1959; Present address: Lab. de Physiol. de la Faculté de Méd., Paris.

‡ A paper on "Incorporation of palmitate-1-C^{14} incorporation into blood cells" by Mines, C. J., Fillerup, D. L., Mead, J. F., *Nature* 1961, v190, 92, has just appeared as the present paper goes to press.

Red blood cells were washed and centrifuged several times in the modified Tyrode phosphate medium.

Palmitic-1-C^{14} acid (specific activity 1.99 millicuries/millimole) was purchased from Nuclear-Chicago Corp. and stored dissolved in benzene. For each experiment an aliquot of the benzene solution is pipetted into a small volumetric flask, evaporated in a mild stream of air, then neutralized with 0.01 M KOH solution in slight excess and dissolved at 75°C, and, finally, adjusted to the mark with modified Tyrode phosphate medium. Because non-enzymatic evolution of $C^{14}O_2$ from palmitate-1-C^{14} has been shown to be of substantial magnitude(9,10), freshly prepared substrate is used for each experiment.

The incubation of washed red blood cells with palmitate-1-C^{14} is done in Conway microdiffusion units. Into their outer well are added: (1) 0.5 to 2.0 ml of a 1.0 to 10% suspension of washed red blood cells; (2) 0.5 to 1.0 ml of a 0.05 mM solution of palmitate-1-C^{14} in modified Tyrode phosphate medium; (3) plus a sufficient volume of modified Tyrode phosphate medium to adjust the total volume to 4.0 ml.

Into the center well of the Conway microdiffusion unit is inserted a small removable stainless steel cup, containing one ml of 10% KOH solution, to capture the $C^{14}O_2$ evolved during the incubation. After an incubation period of exactly 2 hours at room temperature (about 24°C), the microdiffusion units are opened and the removable cup with the 10% KOH, containing the evolved $C^{14}O_2$, is replaced by another cup with 10% KOH, and the microdiffusion units are sealed again for a new period of incubation. The $C^{14}O_2$ evolved during each incubation period and captured in 10% KOH is precipitated with an excess of 1 M barium chloride and $BaC^{14}O_3$ is recovered and assayed for radioactivity. For each such experimental determination, controls were run in microdiffusion units, with the same amounts of palmitate-1-C^{14}, but with modified Tyrode phosphate medium alone, in substitution for the red blood cell suspension, with final volume of incubation adjusted again to 4.0 ml. Isotope measurements are carried out with the Automatic Scaler Unit, model 161A, from Nuclear-Chicago Corp. (The background is about 16 to 18 counts per minute.) Radioactivity is usually measured as the time for 6400 counts.

TABLE I. Oxidation at Room Temperature of Palmitate-1-C^{14} (0.0125 mM Concentration) by Varying Concentrations of Washed Red Blood Cells.

	Evolved $C^{14}O_2$*		
	Red blood cell concentration		
Time (hr)	1%	2%	4%
0-2	34.5	61.1	124.2
2-4	66.4	99.3	173.4
4-6	36.6	57.4	125.4
6-8	18.2	26.4	39.5

* Counts/min. for $BaC^{14}O_3$ precipitate, representing difference between experimental and blood cell-free control.

Results. After numerous trial experiments for establishment of suitable experimental conditions for oxidation of palmitate-1-C^{14} by red blood cells *in vitro,* many comprehensive experiments of the type shown in Tables I and II were carried out. Data of these tables are typical of those obtained from all these experiments, which have been performed with various amounts of red blood cells, incubated with various concentrations of palmitate-1-C^{14} during the various length of incubation time. At least 8-10 such experiments are represented by each value shown in the tables.

Table I shows that oxidation of palmitate-1-C^{14} is proportional to number of red blood cells, and that oxidation increases with length of incubation time, though not quite proportionally. The amount of the metabolic $C^{14}O_2$ evolved is lower in the first interval of incubation, consistently higher in the second in-

TABLE II. Oxidation at Room Temperature of Varying Concentrations of Palmitate-1-C^{14} by a 4% Suspension of Washed Red Blood Cells.

	Evolved $C^{14}O_2$*				
	Concentrations of palmitate-1-C^{14} in mM				
Time (hr)	.0008	.0016	.0032	.0063	.0125
0-2	18.1	26.4	48.3	77.4	124.2
2-4	23.2	43.6	64.1	114.4	173.4
4-6	10.3	28.5	48.4	69.2	125.4
6-8	6.8	13.7	17.6	23.4	39.5

* Counts/min. for $BaC^{14}O_3$ precipitate, representing difference between experimental and blood cell-free control.

terval, and decreases in the third and fourth interval of incubation.

Table II shows that the amount of evolved $C^{14}O_2$ during oxidation of palmitate-1-C^{14} by a constant number of red blood cells increases with increasing concentration of palmitate-1-C^{14} in the solution. However, for the 16-fold increase in concentration of palmitate, release of the metabolic $C^{14}O_2$ is only about 9 to 12 times higher, which indicates an increasing "saturation" of red blood cells with palmitate as a source of energy, even at comparatively very low concentrations of palmitate. At higher concentrations of palmitate, this saturation becomes even more pronounced.

Discussion. The data clearly demonstrate that red blood cells oxidize palmitic acid to CO_2. However, it is likely that the *in vivo* rate of fatty acid oxidation by red blood cells is considerably reduced by the presence of naturally occurring substances having inhibitory effects. The factors which may influence rate of fatty acid oxidation by red blood cells *in vivo* are largely unknown and are being investigated. Preliminary experiments indicate that their influence increases with age of the animal.

Experiments reported here do not permit inference as to the fate of other carbons in the fatty acid molecule. The generally accepted scheme of fatty acid metabolism suggests that all straight chain fatty acids are activated and then oxidized to acetyl CoA(11). Once a fatty acid begins to be oxidized, it continues to be cleared of acetyl fragments until the whole molecule is degraded(12). The odd-numbered carbons on a long-chain fatty acid share nearly, but not exactly, the same metabolic fate(12).

Since the appearance of $C^{14}O_2$ is the result of a series of enzymatic reactions, it is not possible to give absolute conversion rates of added labeled palmitate to CO_2. Any unlabeled substrate that contributes to the final common acetyl CoA pool would dilute the collected $C^{14}O_2$. The data in Tables I and II demonstrate relative changes in rates of fatty acid oxidation which can be induced by changes in number of red blood cells, in fatty acid concentration, in duration of incubation, and by changes in other experimental conditions, some of which are currently being investigated.

Summary. Washed, red blood cells from normal, fed, adult rats were incubated *in vitro* in a modified Tyrode phosphate medium with palmitate-1-C^{14}, the oxidation of which was demonstrated and measured by following the incorporation of radioactivity in the respiratory CO_2. Rate of palmitate oxidation to CO_2 has been shown to increase with number of red blood cells in the incubating vessels and with concentration of palmitate, and also depends on duration of the incubation. The data are discussed in relation to the present concepts of fat metabolism *in vivo*.

1. Felts, J. M., *Fed. Proc.*, 1957, v16, 37.

2. McCalla, C., Gates, H. S., Jr., Gordon, R. S., Jr., *Arch. Biochem. Biophys.*, 1957, v71, 346.

3. Gordon, R. S., Jr., *J. Clin. Invest.*, 1957, v36, 810.

4. Gordon, R. S., Jr., Cherkes, A., *ibid.*, 1956, v35, 206.

5. Dole, V. C., *Arch. Int. Med.*, 1958, v101, 1005.

6. Barron, E. S. G., Harrop, G. A., Jr., *J. Biol. Chem.*, 1928, v79, 65.

7. James, A. T., Lovelock, J. E., Webb, J. P. W., in: *Essential Fatty Acids*, edited by H. M. Sinclair, Butterworths Scientific Publications, London, 1957, p72.

8. Fritz, I. B., Davis, D. G., Holtrop, R. H., Dundee, H., *Am. J. Physiol.*, 1958, v194, 379.

9. Milstein, S. W., Driscoll, L. H., *J. Biol. Chem.*, 1959, v234, 19.

10. Artom, C., *ibid.*, 1953, v205, 101.

11. Green, D. E., *Biol. Rev.*, 1954, v29, 330.

12. Chaikoff, I. L., Brown, G. B., in: *Chemical Pathways of Metabolism*, edited by D. M. Greenberg, Acad. Press, N. Y., 1954, vI, p277.

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