TABLE I. Summary of Typical Results Obtained by a Class of Students Using the Simple Basal Metabolism Apparatus. Oxygen consumption values are means for 32 rats corrected to N.T.P.

Treatment	Oxygen consumption, liters/m²/hr	Change from control	t	P
Control	8.6 ± .3			
Pentobarbital sodium (40 mg/kg)	5.6 ± .2	3.0	8.6	<.001
Tapazole (5 mg/kg/day)	7.8 ± .3	.8	2.0	.05

Griffith and Farris(6). After the oxygen consumption of each control animal was determined, the rat was injected intraperitoneally with 40 mg pentobarbital sodium‡ per kg and further determinations made when the animal became comatose. As expected(7), the oxygen consumption of the rat under pentobarbital anesthesia was significantly lower which was determined statistically by using the test for the significance of the difference between means given by Goulden(8).

Another group of 32 rats had been given 5 mg Tapazole§ per kg per day in their drinking water for 3 weeks. The oxygen consumption of these animals was determined by the same students in the same exercise. It is seen in Table I that administration of the antithyroid drug reduced the mean oxygen consumption by a small but statistically significant degree. Thus when used by an average group of students the simple basal metabolism apparatus herein described permits the measurement of even relatively small effects of drug administration.

‡ The pentobarbital sodium was generously provided by Abbott Laboratories.

§ The Tapazole (1-methyl-2-mercaptoimidazole) was generously provided by Eli Lilly and Co.

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Demonstration of β-Oxidation and Acetoacetate Formation in a Mouse Hepatoma.* (20720)

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The ability of normal tissues or their enzyme preparations to oxidize fatty acids via C_2 fragments (acetyl-S-CoA) has been demonstrated convincingly by many workers. Until recently, however, fatty acid oxidation by neoplastic tissues received little attention. Weinhouse and coworkers, in a series of papers on the metabolism of neoplastic tissues(1-3), have provided evidence for the occurrence of β-oxidation of fatty acids and the existence of the tricarboxylic acid cycle in such tissues. An interesting aspect of their studies was the search for isotopic acetoacetate which might have accumulated during the oxidation of C^{14}-labeled fatty acids. The amounts of C^{14} recovered in the acetoacetate fraction apparently approached the lower limits of detection. In-

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deed, as pointed out by Weinhouse *et al.*(3), their data did not exclude the possibility that there was *no ketogenesis at all* in tumor cells.

The formation and accumulation of acetoacetate by a tissue have obvious implications as to the manner by which that tissue oxidizes fatty acids. For this reason we sought to provide clear-cut evidence on acetoacetate formation in the case of tumors. It is shown here that during the oxidation of octanoate-1-C^{14} by slices of a hepatoma, isotopic acetoacetate not only is formed but also accumulates.

Methods. Labeled substrate. Sodium octanoate-1-C^{14} was obtained by carboxylation of the appropriate Grignard compound. *Non-isotopic acetoacetate.* The ethyl ester of acetoacetic acid was obtained commercially (Eastman Kodak), redistilled (b.p. 182-184°, uncorrected), and converted to sodium acetoacetate by hydrolysis with NaOH according to the procedure of Ljunggren(4). Ethyl alcohol, formed during hydrolysis, was not removed as it does not affect the use of the solution of sodium acetoacetate as carrier in the experiments described below. *In vitro procedures.* The tumor used in this study was an hepatocarcinoma, C_{954}, originally obtained from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine. It is maintained in this laboratory by serial transplantation in mice of the C_{57}-leaden strain. We are indebted to Mr. Carl Emanuel for the tumor tissue used in this study. Tumor slices, prepared free-hand, were placed in cold isotonic saline solution, blotted gently on filter paper, weighed, and placed in incubation flasks containing 5.0 ml calcium-free phosphate buffer(5) and 5.0 μ moles of octanoate labeled with octanoate-1-C^{14}. Pertinent information concerning incubation conditions is given in Table I. At the end of the incubation period, 300 μM of the non-isotopic acetoacetate were added, and the reaction was stopped with H_2SO_4. *Analytical procedures.* The deproteinized ($Ca(OH)_2$ plus $CuSO_4$) buffer was subjected to the thermal decarboxylation procedure described elsewhere (6). Mercury-acetone precipitates(7) were filtered, while hot, at the purification stage(6) to minimize contamination by sparingly-soluble isotopic substances. Weighed samples of the mercury-acetone complex were combusted with Van Slyke-Folch reagent(8) (iodate-free), and the resulting CO_2 was collected in 0.5 N NaOH (CO_2-free). Saturated $BaCl_2$ solution was added to aliquots of carbonate solutions representing the acetone and carboxyl carbons of acetoacetate and the respiratory CO_2. The resulting $BaCO_3$ precipitates were mounted on filter paper and counted with a gas flow counter (helium saturated with ethyl alcohol at 0°) of about 20 per cent efficiency. Na_2CO_3 was added as carrier to solutions containing the respiratory CO_2 in order to obtain sufficient mass of $BaCO_3$ for counting. All counting data have been corrected for background and are corrected to a standard mass of 40.0 mg $BaCO_3$. The distribution of isotope in acetoacetate is represented by the C*O: C*OOH ratio. The ratio is expressed in two ways: (c.p.m. in acetone carbon)/(c.p.m. in carboxyl carbon) and 3(s.a. acetone carbon)/(s.a. carboxyl carbon), where s.a. (specific activity) represents c.p.m./mg $BaCO_3$.

Results and discussion. The results are shown in Table I. The percentages of the incubated C^{14} recovered in CO_2 plus acetoacetate varied from 13 to 17%. It is of interest to note that from 40 to 45% of the total isotope recovered in these two fractions resided in the acetoacetate. Negligible amounts of isotope were found in the various fractions from flasks containing boiled slices or no slices. This finding is of special importance since it clearly indicates that the mercury-acetone precipitates were obtained almost free of contamination by the labeled substrate.

The C*O:C*OOH ratio varied from 0.93 to 1.02 (average value, 0.95). Within experimental error, the ratio was the same whether calculated on a total c.p.m. or s.a. basis. Both should provide a valid measure of the C*O: C*OOH ratio since negligible amounts of isotope were found in iodoform (representing the methyl and methylene carbons of acetoacetate) prepared from an acetone sample.

Our finding that the isotope from octanoate-1-C^{14} appears in acetoacetate in amounts approaching those found in $C^{14}O_2$ is in contrast to that observed by Weinhouse *et al.*(3). The latter reported that litle or no isotopic acetoacetate accumulated in slices of a hepatoma or other neoplastic tissues incubated with octano-

TABLE I. Oxidation of Octanoate-1-C^{14} by Mouse Hepatoma, C_{954}. (500 mg slices; 5 ml phosphate buffer, pH 7.4; oxygen in gas phase; incubation time 90 minutes; t = 37.5°; KOH in center well; octanoate-1-C^{14} 0.001M, 2.9×10^5 c.p.m. incubated per flask.)

Flask	C^{14}-c.p.m. recovered in: CO_2	Acetoacetate† Acetone	Acetoacetate† Carboxyl carbon	C*O : C*OOH Acetone COOH	C*O : C*OOH 3 s.a. acetone carbon‡ s.a. COOH
	A	B	C	B/C	
1	30200	9930 ± 40	10550 ± 150	.94	.93
2	22000	7385 ± 405	7960 ± 100	.93	.89
3	21200	8885 ± 585	8775 ± 125	1.01	1.02
Avg 1, 2, 3	24500	8730	9100	.96	.95
4 (boiled slices)	205	367	115		
5 (no slices)	96	214	117		

† Avg of 2 samples ± avg dev.
‡ s.a. = c.p.m. per mg $BaCO_3$.

ate-1-C^{14} as substrate. In order to demonstrate the formation of isotopic acetoacetate from butyrate-1-C^{14}, these workers added carrier acetoacetate at the start of the incubation so as to "trap" the ketone body. None of their studies, however, provided unequivocal evidence for the formation of ketone bodies. In experiments reported here, carrier acetoacetate was added at the *end* of the incubation period, and served merely to facilitate recovery and degradation of isotopic acetoacetate which accumulated.

In general, the metabolism of neoplastic tissues with respect to the end products of fatty acid oxidation resembles that of extrahepatic tissues(3). In our experiments, however, the tumor metabolized octanoate in a fashion reminiscent of the fasted liver(9). The tumor used here was taken from a mouse nearing the terminal state (3 weeks after implantation), and the degree of ketosis, perhaps reflecting the starved condition of the animal, may have served advantageously for the accumulation of large amounts of ketone bodies. Accumulation of sufficient amounts of isotopic acetoacetate for the determination of the isotope distribution in the ketone body enables us to draw the conclusion that fatty acid oxidation in the tumor proceeds by the generally accepted scheme of β-oxidation-cleavage-condensation. This conclusion is based on the following two findings: 1) accumulation of acetoacetate in which the isotope from octanoate-1-C^{14} appears in the expected carbons (carbonyl and carboxyl); and 2) observance of a C*O:C*OOH ratio which is not greater than unity(10).

Summary. Slices of a transplanted mouse hepatoma, C_{954}, oxidized octanoate-1-C^{14} to $C^{14}O_2$ and acetoacetate-1,3-C^{14}. Isotopic acetoacetate accumulated in quantities approaching that recovered in respiratory CO_2. The distribution of isotope in the ketone body is consistent with the view that the short-chain fatty acid was degraded by β-oxidation, the resulting C_2 fragments condensing to form acetoacetate as well as being oxidized to CO_2.

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