FATTY ACID SYNTHESIS IN HUMAN LEUCOCYTES*

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It has been reported by several investigators (Buchanan, 1960, Marks et al., 1960, Rowe et al., 1960) that human leucocytes in vitro are able to synthesize long-chain fatty acids from acetate-1-C^{14}. It has also been found that the incorporation of the newly synthesized fatty acids into the various lipid fractions is affected by the physiological condition of the cell (Malamos et al., 1962) and lipemia of the host (Miras, 1963). In the present communication is described the composition of the fatty acids formed in the presence of acetate-1-C^{14} by intact leucocytes. This precursor was found to be incorporated into fatty acid mainly by chain-lengthening of preexisting fatty acids.

METHODS

Blood (4 volumes) from healthy men, fasted for 12 hours, was withdrawn into (1 volume) A.C.D. solution (1.32% sodium citrate, 0.48% citric acid hydrate, 1.4% dextrose). The separation of leucocytes took place as described previously with polyvinylpyrrolidone (Malamos et al., 1962). By improvement of this technique, it was possible to obtain a preparation contaminated with only 2 to 5 red cells and 10 to 20 platelets per 100 leucocytes. The leucocytes were washed with 0.85% sodium chloride solution and suspended in plasma free of platelets. Suspensions of about 1 x 10^8 cells per ml. of plasma were in-

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cubated in air with 10 μc per ml. acetate-1-C^{14} (specific activity 489 μc/mg.). Incubation took place at 37° in a shaking water bath for three hours. During this period the pH of the incubation mixture was found to remain between 7 and 7.2. Following the incubation, the leucocytes were extracted and the lipids washed according to the method of Folch et al. (1957), as described previously (Malamos et al., 1963). The total lipid extract was hydrolyzed with methanol-concentrated hydrochloric acid 5:1 v/v for six hours at 70° under nitrogen, and the fatty acids transformed into their methyl esters. The methyl esters were fractionated by gas chromatography* (Miras et al., 1964). The individual fatty acids and the areas between the peaks were trapped and the radioactivity of a portion of each was counted in a Nuclear Chicago Flow Counter. To check the coincidence of mass and radioactivity in the preparative gas chromatograph, a radioactive standard was injected and the radioactivity of several serial fractions counted during the elution of the peak. Coincidence was found to be very close to ideal. Calculation of the mass for the specific radioactivity data was obtained by calibrating the detector response with standards.

Individual fatty acid esters were isolated by separation first into saturated and unsaturated fractions (Kishimoto and Radin, 1959) and then by preparative gas chromatography. After their purity was established, the esters were saponified and the acids decarboxylated** by the Schmidt reaction, as modified by Brady et al. (1960).

RESULTS AND DISCUSSION

The results shown in Table I indicate that acetate-1-C^{14} is incorporated into a great variety of fatty acids with a preponderance in stearic acid and

*Preparative gas chromatography was performed on a Pye Argon Chromatograph with a 1 x 100 cm. column of 10% ethyleneglycol adipate on Celite. The identity and purity of all fatty acids was determined on an analytical unit of the same chromatograph. The columns used were 0.4 x 110 cm. ethyleneglycol adipate and 0.4 x 110 Apiezon L. Standard fatty acids and relative retention volumes were used for identification of the peaks obtained on both columns. Further identification of the unsaturated acids was performed by rechromatographing their hydrogenated products.

**$C^{14}O_2$ was collected in Hyamine and counted in a liquid scintillation counter (EKCO Electronic Ltd.).

in the C_{18}, C_{20}, C_{22} and C_{24} unsaturated acids. It is noteworthy that only a small percentage of radioactivity was found in the C_{16} acids, while the C_{20} mono- and polyunsaturated acids, as well as the $C_{24:1}$ acids possess a high specific activity. The results of decarboxylation indicate that even palmitic acid, which in other tissues is the primary product of the de novo system, in leucocytes is formed mainly through the elongation system since most of the radioactivity is in the carboxyl group. In the same way it is apparent that all the other labelled fatty acids were formed by the addition of one or more acetyl's to preexisting fatty acyl residues of differing degrees of saturation and chain lengths, presumably in the mitochondria, according to the elongation mechanism described by Wakil (1961). This finding does not exclude the possibility that both the elongation and the desaturation systems contribute to the in vitro biosynthesis of the polyunsaturated fatty acids in a manner similar to that described by Mead (1961).

Considerable quantities of acetate-1-C^{14} were incorporated into the $C_{24:1}$ acids which contained one third of their radioactivity in the carboxyl carbon; these findings indicate the presence in leucocytes of a system capable of forming the very long-chain fatty acids. These acids are presumably formed by an elongation mechanism similar to that described for the brain, in which Fulco and Mead (1961) and Kishimoto and Radin (1963) showed by in vivo studies that these acids are formed by the successive additions of three acetyl groups to oleic acid.

It has been shown by Ganguly (1960) and Numa et al. (1961), that the rate-limiting reaction in the de novo pathway of fatty synthesis by cell-free preparations is the carboxylation of acetyl-CoA. Although it is uncertain whether the same will hold for intact cells, malonate was used as a precursor of fatty acid synthesis by leucocytes, with the hope that further information could be obtained on the efficiency of the de novo system in these cells. The rate of malonate-2-C^{14} incorporation into leucocyte fatty acids showed a lag phase of 1 hr., was depressed by added acetate, and was about

TABLE I

Incorporation of Acetate-1-C^{14} into the Fatty Acids of Human Leucocytes

Fatty acid	Radioactivity* percent of total eluted	Specific activity Cpm/μg	Percent radio-activity in the carboxyl carbon**
C_{16}	0.8 ± 0.2		
$C_{16:0}$	3.4 ± 0.2	11.3	65
$C_{16:1}$	0.7 ± 0.1		
$C_{16:0}$-$C_{18:0}$	0.7 ± 0.2		
$C_{18:0}$	13.0 ± 0.5	22.0	95
$C_{18:1}$	9.8 ± 1.1	8.2	97
$C_{18:2}$	1.5 ± 0.1	2.2	
$C_{18:2}$-$C_{20:0}$	1.3 ± 0.2		
$C_{20:0}$	2.6 ± 0.5		85
$C_{20:1}$	13.8 ± 1.6	344.0	96
$C_{20:1}$-$C_{20:4}$	8.9 ± 0.9	288.0	
$C_{20:4}$	1.8 ± 0.2	5.7	
$C_{20:4}$-C_{22}	1.5 ± 0.1		
$C_{22:0}$	2.2 ± 0.3		62
$C_{22:1}$	7.3 ± 0.6		79
$C_{22:1}$-$C_{22:4}$	5.0 ± 0.7		
$C_{22:4}$	6.3 ± 2.0		
$C_{22:4}$-$C_{24:0}$	2.8 ± 0.9		
$C_{24:0}$	2.1 ± 0.3		34
$C_{24:1}$	11.6 ± 1.4	142.0	36
C_{24}	2.8 ± 0.3		

*Mean value (± standard error). Results from three experiments performed with leucocytes taken from three individuals.

**For de novo synthesis the carboxyl carbon would contain a proportion of the total radioactivity depending on the chain length. In contrast, the addition of one acetyl-1-C^{14}-CoA to preexisting acyl-CoA would produce a fatty acid with all of the radioactivity in the carboxyl carbon.

one sixth that of acetate-1-C^{14} at the same molar concentration*. Decarboxylation of the total fatty acids derived from malonate-1-C^{14} showed that 50% of the total radioactivity was in the carboxyl carbon. The above findings

* In these data the dilution of acetate-1-C^{14} by the plasma acetate was taken into consideration.

demonstrate clearly that a large part of the incorporated malonate is first decarboxylated to acetate which is then incorporated through the chain-elongating pathway. Therefore, only a small percentage of malonate is utilized by the cell for fatty acid synthesis through the de novo system.

In conclusion, it can be said that under the experimental conditions employed, the elongation system for fatty acid synthesis is of primary importance in human leucocytes. This system shows a specificity for the formation of C_{20} to C_{24} fatty acids and is more efficient than the de novo system.

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