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Determination of ^{14}C-labelled Ketone Bodies by Liquid-Scintillation Counting

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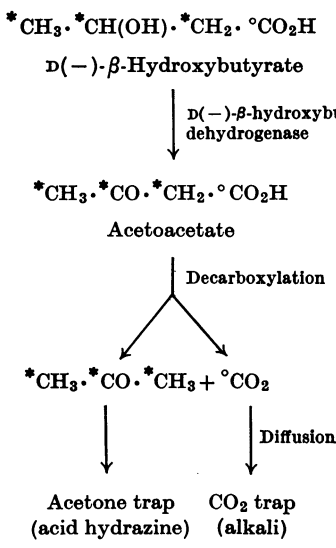
1. A method of assaying ^{14}C in ketone bodies present in blood by using liquid-scintillation counting is described. 2. D(−)-β-Hydroxy[^{14}C]butyrate is converted quantitatively into [^{14}C]acetoacetate by means of a coupled oxidoreduction reaction involving NAD^+, D(−)-β-hydroxybutyrate dehydrogenase and malic dehydrogenase in the presence of a high concentration of oxaloacetate. 3. [^{14}C]Acetoacetate is decarboxylated to acetone and carbon dioxide which are trapped separately in a double-well flask and counted subsequently. 4. The method permits the determination of ^{14}C activity in the individual ketone bodies and allows the activity in the carboxyl carbon atoms of acetoacetate or of D(−)-β-hydroxybutyrate to be assayed separately from the activity in the remainder of the molecule. 5. Recoveries of ^{14}C-labelled ketone bodies added to blood approach 100% with good reproducibility in replicate analyses.

There has been no method available for estimating ^{14}C in labelled ketone bodies which makes use of the rapidity and convenience of liquid-scintillation counting. Hitherto, ^{14}C-labelled ketone bodies have been assayed after their decarboxylation followed by precipitation of the acetone with Deniges' reagent as an acetone–mercuric sulphate complex. This complex was burnt to $^{14}CO_2$ and counted as barium [^{14}C]carbonate with a Geiger counter (Chaikoff, Goldman, Brown, Dauben & Gee, 1951; Shreeve & Tocci, 1961). Activity in the acetone moiety of acetoacetate has also been determined either in the 2,4-dinitrophenylhydrazine derivative (Hird & Symons, 1959; Connolly, Head & Williams, 1964) or in the formazan derivative (Sauer, 1961). The ^{14}C present in the carboxyl carbon atoms of acetoacetate has been removed by decarboxylation and counted as barium [^{14}C]carbonate (Chaikoff *et al.* 1951).

The method described here permits the estimation of ^{14}C in the individual ketone bodies by means of liquid-scintillation counting. The estimation of radioactivity present in total ketone bodies involves the enzymic conversion of D(−)-β-hydroxy[^{14}C]butyrate into [^{14}C]acetoacetate by using D(−)-β-hydroxybutyrate dehydrogenase (EC 1.1.1.30). The resulting [^{14}C]acetoacetate plus any preformed [^{14}C]acetoacetate is decarboxylated to [^{14}C]acetone and $^{14}CO_2$ by heating with acid in a sealed ampoule. The contents of the ampoule, including any preformed [^{14}C]acetone plus $^{14}CO_2$, undergo differential diffusion in a sealed flask fitted with two wells, one containing acid hydrazine to trap [^{14}C]acetone and the other containing sodium hydroxide to trap $^{14}CO_2$. The contents of each well are counted by liquid-scintillation techniques. The



Scheme 1.

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various steps involved and the fate of the label in each carbon atom of the ketone bodies are as shown in Scheme 1.

Separate estimations of radioactivity may be made in acetoacetate alone or in acetoacetate plus acetone plus carbon dioxide. Hence, the total radioactivity in each ketone body fraction may be calculated. Also, the distribution of ^{14}C between the carboxyl carbon atom and the remainder of the molecule in either β-hydroxybutyrate or acetoacetate is obtained.

MATERIALS AND METHODS

Assay for ^{14}C-labelled ketone bodies in blood

Reagents. These are as follows: hydrazine lactate, pH 5·0 (approx. 5 ml. of 88% lactic acid plus 2 ml. of hydrazine hydrate); ethanol (absolute); 1 M-Hyamine hydroxide in methanol (Packard Instrument Co. Ltd.); 0·1 M-tris buffer, pH 8·5; 10 mM-NAD^+ in tris buffer (pH 8·5); 0·2 M-sodium oxaloacetate (oxaloacetic acid adjusted to pH 8·5 with 1 N-NaOH: contamination of oxaloacetic acid with enzyme inhibitors is avoided by a preliminary extraction of an aqueous solution of oxaloacetic acid into diethyl ether); mixed enzyme solution containing D(−)-β-hydroxybutyrate dehydrogenase (100 units/ml.) and malate dehydrogenase (EC 1.1.1.37; 100 units/ml.), units of enzyme activity as defined by Williamson, Mellanby & Krebs (1962); scintillator solution, 2,5-diphenyloxazole (0·3%) and 1,4-bis-(5-phenyloxazol-2-yl)benzene (0·02%) in toluene. All reagents are of A.R. quality where possible. The enzyme fraction containing the two enzymes mentioned above was prepared from *Rhodopseudomonas spheroides* by a modification of the method of Williamson *et al.* (1962) developed by J. A. Mayerle, J. M. Felts & P. A. Mayes (unpublished work). Briefly, this modification involved omitting fractionation with $(NH_4)_2SO_4$ as described by Williamson *et al.* (1962), and substituting chromatography with a column of DEAE-cellulose. Alternatively, the mixed enzyme preparation may be obtained by mixing D(−)-β-hydroxybutyrate dehydrogenase with malate dehydrogenase, both of which may be obtained commercially (Boehringer Corp., London, Ltd.).

Apparatus. The only non-standard items used are double-well and single-well 50 ml. Erlenmeyer flasks and glass ampoules. The double-well flasks are screw-capped (no. 4985; Pyrex, U.S.A.), in which the caps are replaced with liquid-scintillation vial caps fitted with metal liners (Packard Instrument Co. Ltd.), or standard flasks may be used having a ground-glass socket (B 19) with a suitable stopper of dense polythene. Each double-well flask is fitted with wells of different size, having dimensions as shown in Fig. 1. The single-well flask has been described by Baruch & Chaikoff (1954). Glass ampoules are constructed from disposable Pasteur pipettes (Scientific Products, Evanston, Ill., U.S.A.) as shown in Fig. 1.

Deproteinization. All manipulations are carried out on ice or in the cold to minimize both decarboxylation of [^{14}C]-acetoacetate and volatilization of [^{14}C]acetone and $^{14}CO_2$. Perchloric acid (30%, w/v; 2·0 ml.) is added to 2·0 ml. of blood (defibrinated or heparinized), mixed and centrifuged. The supernatant (2·5 ml.) is neutralized with 20% (w/v) KOH solution and the pH adjusted to 8·0 with 1 N-KOH. The volume is made up to 5·0 ml. with water and the mixture allowed to stand for 30 min. before centrifuging. Portions of the supernatant are used for the assay of ^{14}C-labelled ketone bodies and may be used also for the enzymic determination of acetoacetate and D(−)-β-hydroxybutyrate by the method of Williamson *et al.* (1962).

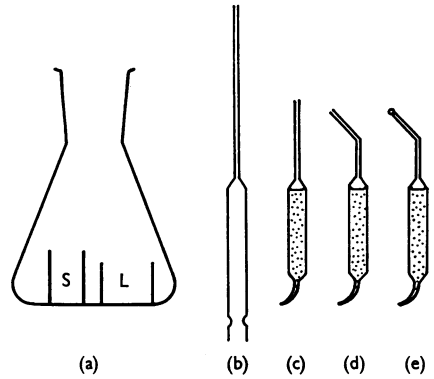

Fig. 1. (a) Double-well flask constructed from 50 ml. Erlenmeyer flask fitted with a standard B 19 socket. Well S measures 18 mm. × 11 mm. and well L measures 14 mm. × 17 mm. (b) Pasteur pipette used in making glass ampoule. (c), (d), (e) Stages in filling and sealing glass ampoule. Each ampoule contains 1·1 ml. of liquid and measures 8–9 cm. × 7 mm. overall.

Procedure for assay of ^{14}C in ketone bodies. (*a*) In total ketone bodies. In this assay are included D(−)-β-hydroxy-[^{14}C]butyrate, acetoacetate, acetone and any free $^{14}CO_2$. Into a small test tube are placed tris buffer (0·25 ml.), NAD^+ solution (0·05 ml.), deproteinized supernatant (0·25 ml.), oxaloacetate solution (0·50 ml.) and mixed enzyme solution (0·20 ml.). After mixing, the reaction is allowed to proceed for 90 min. at room temperature. During this time 1·0 ml. of the mixture is transferred into a glass ampoule (Fig. 1c) by using an accurately calibrated syringe and needle. When the 90 min. period has expired, the ampoule is frozen in a bath of solid CO_2 in propan-2-ol or in a deep freeze (−20°); 0·1 ml. of 10 N-H_2SO_4 is introduced into the ampoule from a syringe and needle. A bend is made in the stem of the ampoule (Fig. 2d) and the ampoule is sealed with a micro-burner (Fig. 2e). When the contents have melted, the ampoule is placed in a boiling-water bath for 5 min. to decarboxylate acetoacetate, cooled to room temperature, and frozen again. Hydrazine lactate (0·5 ml.) is added to the larger well of a double-well flask and 0·05 ml. of 10 N-NaOH is placed on a piece of folded filter paper ($\frac{3}{4}$ in. × $\frac{3}{4}$ in.) in the smaller well. The frozen ampoule is inverted and pressed into the main compartment of the double-well flask until it breaks at the bend in the neck. The opposite end of the ampoule is broken off with forceps or thin-nosed pliers to allow subsequent drainage of the contents. Immediately, the flask is stoppered firmly. The contents of the ampoule melt and drain into the flask; [^{14}C]acetone diffuses into the well containing hydrazine lactate and $^{14}CO_2$ is trapped on the alkaline filter paper. After 24 hr. of diffusion, the hydrazine lactate is transferred quantitatively into a counting vial

with a syringe and needle, 6·0ml. of ethanol being used for rinsing. Scintillator solution (10·0ml.) is added, mixed and the vial counted in a liquid-scintillation counter. The counts which measure the total activity in free acetone and in the $C_{(2)}$–$C_{(4)}$ moiety of acetoacetate and D(−)-β-hydroxybutyrate, are corrected for quenching by adding to the vial 0·1ml. of [^{14}C]acetone dissolved in toluene and re-counting the sample. The filter paper from the other well is placed in the main compartment of a single-well flask containing 5ml. of water and 3ml. of methanol; Hyamine solution (0·5ml.) is added to the well. The flask is sealed with a rubber serum-bottle stopper or a Suba-Seal bung (Scientific Supplies Ltd., London) and approx. 5ml. of air withdrawn from the flask, with a syringe and needle, creating a partial vacuum which ensures that any leakage is into the flask. 5N-Sulphuric acid (0·5ml.) is injected into the main compartment of the flask, which is agitated for 90min. at room temperature. The contents of the well are transferred quantitatively to a counting vial, 6·0ml. of ethanol being used for rinsing. Scintillator solution (10ml.) is added, mixed and the vial counted. The counts, derived from $^{14}CO_2$ and from the carboxyl carbon atoms of acetoacetate and β-hydroxybutyrate, are corrected for quenching by adding an internal standard to the vial and recounting the sample. All counts are expressed in terms of 1·0ml. of blood.

(*b*) In acetoacetate, plus acetone, plus CO_2. To 1·0ml. of water in a small tube is added 0·25ml. of deproteinized supernatant; it is mixed and 1·0ml. of this solution is placed in a glass ampoule, which is processed as in the preceding paragraph.

(*c*) In acetoacetate. To 1·0ml. of water in a finely graduated centrifuge tube is added 0·25ml. of deproteinized supernatant; it is mixed and the volume noted. For 30min. a stream of air, saturated with water vapour, is directed to form a pocket in the surface of the solution to drive off preformed [^{14}C]acetone and $^{14}CO_2$. Any loss of volume is made up with water, the solution mixed and 1·0ml. placed in a glass ampoule, which is processed as in (*a*) above.

(*d*) In acetone and CO_2. This is obtained by subtracting the [^{14}C] activity found in (*c*) from that found in (*b*) above. It is to be noted that the estimate for CO_2 does not give an accurate measure of [^{14}C]bicarbonate in blood since some loss is to be expected during the deproteinizing in $HClO_4$. If desired, this loss of $^{14}CO_2$ may be circumvented by omitting the deproteinization and carrying out the estimation directly in the ampoule on diluted whole blood (1:10 dilution). However, deproteinization should always be carried out whenever β-hydroxy[^{14}C]butyrate is converted into [^{14}C]acetoacetate.

(*e*) In D(−)-β-hydroxybutyrate. The radioactivity in D(−)-β-hydroxybutyrate may be calculated by subtracting the value found in (*b*) from that found in (*a*) above.

Procedures used in ascertaining the radiochemical purity of the labelled chemicals employed

[^{14}C]*Acetone*. An estimate of non-volatile radioactive impurity was made by aerating an aqueous sample as in (*c*) above. A further period of aeration showed that 30min. was sufficient time to drive off acetone under the conditions employed. Portions (0·1ml.) of the samples were counted before and after aeration to ascertain the percentage impurity due to non-volatile radioactive contaminants.

[3-^{14}C]*Acetoacetate*. Radioactive purity was determined by acidifying a sample with HCl and heating in a boiling-water bath for 5min. to decarboxylate the acetoacetate, followed by aeration for 30min. as described above. Carrier acetoacetate was added to some samples. Any radioactive residue left after this treatment was considered to be due to radioactive impurity.

β-Hydroxy[^{14}C]*butyrate*. Radioactive purity was determined by chromatography of a sample on Whatman no. 1 paper in the phenol system described by Katz & Chaikoff (1954). The paper was scanned for radioactivity and the R_F compared with that of pure β-hydroxybutyric acid (California Biochemical Corp., Los Angeles, Calif., U.S.A.).

Materials. The following radioactive compounds were subjected to the tests of purity outlined above. [1,3-$^{14}C_2$]-Acetone obtained from New England Nuclear Corp., Boston, Mass., U.S.A. contained 1% of non-volatile radioactive impurity. A solution of sodium [3-^{14}C]acetoacetate was prepared (Ljunggren, 1924) from ethyl [3-^{14}C]acetoacetate obtained from either New England Nuclear Corp. (containing 60% of radioactive impurity) or from Nuclear–Chicago Corp., Ill., U.S.A. (containing 10% of radioactive impurity). Only the latter product was used in recovery experiments to test the method. Potassium DL-β-hydroxy[3-^{14}C]butyrate (New England Nuclear Corp.) was chromatographically pure. However, sodium DL-β-hydroxy[1-^{14}C]butyrate (Volk Radiochemical Co., Chicago, Ill., U.S.A.) was found to be 60–70% impure. Only the rechromatographed compound was used. D(−)-β-Hydroxy[3-^{14}C]butyrate was prepared by causing [3-^{14}C]acetoacetate (Nuclear–Chicago Corp.) to react with D(−)-β-hydroxybutyrate dehydrogenase and NADH in a system similar to that described by Williamson *et al.* (1962). The mixture was deproteinized with $HClO_4$, neutralized with KOH and the supernatant acidified and extracted with diethyl ether in a liquid/liquid Soxhlet apparatus. After evaporation of the ether, the extract was chromatographed in the phenol system and the D(−)-β-hydroxy[3-^{14}C]butyric acid eluted from the chromatogram. [3-^{14}C]Acetoacetate was also prepared from D(−)-β-hydroxy[3-^{14}C]butyric acid by reaction with D(−)-β-hydroxybutyrate dehydrogenase, malate dehydrogenase, NAD^+ and excess of oxaloacetate in a system similar to that described for the estimation of ^{14}C in total ketone bodies (see *a* above). After deproteinization this product was contaminated with approx. 4% of D(−)-β-hydroxy[3-^{14}C]butyrate and with other non-radioactive products of the reaction. Sodium [^{14}C]bicarbonate (New England Nuclear Corp.) was used in certain recovery experiments.

The results reported have been corrected for any radioactive impurity present in the added material. All results were obtained with the ethanol–toluene counting medium described. The counting efficiency was of the order of 60% with a Packard liquid-scintillation spectrometer.

Procedure for recovery of ^{14}C*-labelled ketone bodies from blood*

Samples of labelled ketone bodies were dissolved in 0·9% NaCl and added to defibrinated or heparinized rat blood in the ratio 1:7 respectively. The labelled blood was used in experiments to test the recovery of ^{14}C-labelled ketone bodies by the procedures described. Portions of the

solutions of labelled ketone bodies (0·1 or 0·05ml.) were counted in the ethanol–toluene counting system to estimate the radioactivity added to the blood.

RESULTS AND DISCUSSION

Assay of ^{14}C in ketone bodies

In acetone and carbon dioxide. Acetone and carbon dioxide are the common end products of all the procedures described here for the assay of ^{14}C in ketone bodies. Because of differences in the ratio of labelling between C-1 and C-3 of acetoacetate formed in the course of β-oxidation of ^{14}C-labelled fatty acids (Crandall & Gurin, 1949), it is desirable to estimate the activity separately in these positions. It was considered that this could be achieved most effectively by decarboxylation followed by a double-diffusion procedure in which the acetone moiety was trapped separately from the carbon dioxide derived from the carboxyl carbon atoms of acetoacetate. It was necessary therefore, if the estimations of ^{14}C in the two parts of the molecule were to be carried out simultaneously, to use a medium for trapping [^{14}C]acetone which would be unaffected by the presence of $^{14}CO_2$ and which would dissolve in the solvents used for liquid scintillators. A mixture of hydrazine in lactic acid at pH 5·0 was found to be suitable and dissolved in ethanol–toluene mixture with only slight opalescence. Use of this counting system led to a loss of approximately 3–4% of the counts in [^{14}C]acetone due presumably to slight phase separation. The small loss could be corrected for completely by using an internal standard of [^{14}C]acetone when correcting for quenching.

Further experiments showed that if the organic base Hyamine were used in the double-well flask to trap $^{14}CO_2$ it would also take up some [^{14}C]acetone, due probably to acetone dissolving in the methanol used as a solvent for Hyamine. This cross-contamination was overcome by using 0·05ml. of 10N-sodium hydroxide on filter paper as a trap for $^{14}CO_2$. Subsequently, the paper was transferred to the outer compartment of a single-well Erlenmeyer flask (50ml.) and the [^{14}C]carbonate displaced with acid and trapped by Hyamine contained in the well.

To test the efficiency of recovery and the extent of cross-contamination between the assay for [^{14}C]-acetone and $^{14}CO_2$, experiments were carried out with [1,3-$^{14}C_2$]acetone, potassium DL-β-hydroxy-[1-^{14}C]butyrate or sodium [^{14}C]bicarbonate added to water. These solutions were assayed for radioactivity by the procedure outlined previously, but deproteinization was omitted. The results (Table 1) demonstrate that [^{14}C]acetone is recovered in the acetone trap of the double-well flask to the extent of 97·0% with a standard deviation of ±1·7% and that contamination of the carbon dioxide trap is negligible (0·2%). Recovery of added [^{14}C]bicarbonate in the carbon dioxide trap was virtually complete (98·9% ± 1·7) with no contamination of the acetone trap. A recovery of 49·4% of added ^{14}C in DL-β-hydroxy[1-^{14}C]butyrate was obtained. A maximum recovery of only 50% of the label would be expected in this assay since the method is specific for the D(−)-isomer.

Results from experiments to ascertain the capacity of the reagent used to trap acetone in the double-well flask showed that the recovery of acetone was undiminished up to at least 7μmoles, which is in excess of the acetone expected in the estimation for ^{14}C in total ketone bodies from the most severe cases of ketosis. Likewise, the capacity of the carbon dioxide trap is greatly in excess of the requirement to trap carbon dioxide from the decarboxylation of ketone bodies and is some five times greater than the capacity required for trapping carbon dioxide derived from the decarboxylation of the large quantities of oxaloacetate used as a reagent in the assay of ^{14}C in total ketone bodies.

A diffusion time of 16hr. (i.e. overnight) at room temperature was adequate for maximal recovery of [^{14}C]acetone but in practice a standard time of 24hr. has been adopted. The time allowed for

Table 1. *Recovery of* [^{14}C]*acetone, β-hydroxy*[^{14}C]*butyrate and* [^{14}C]*bicarbonate from water*

The standard procedures were used for estimating ^{14}C in CO_2 and ketone bodies as described in the text but omitting deproteinization. The percentage recoveries are expressed as the means ± S.D. The numbers of tests are shown in parentheses.

Material added	Percentage of ^{14}C recovered	
	In acetone trap	In CO_2 trap
[1,3-$^{14}C_2$]Acetone (1·4μmoles; 7800counts/min.)	97·0 ± 1·7 (12)	0·2 ± 0·1 (6)
Sodium DL-β-hydroxy[1-^{14}C]butyrate (0·08μmole; 500counts/min.)	0·0 ± 0·0 (8)	49·4 ± 1·1 (8)
Sodium [^{14}C]bicarbonate (0·007μmole; 35800counts/min.)	0·0 ± 0·0 (6)	98·9 ± 1·7 (6)

diffusion of acetone is adequate also for carbon dioxide, which was found to diffuse under present conditions at a much faster rate than acetone.

In acetoacetate. Widmark's (1920–21) finding that acetoacetate is decomposed rapidly and completely to acetone and carbon dioxide when heated in an acid solution is made use of in the estimation of acetoacetate by many methods. It was adopted in the present method for estimating ^{14}C in labelled acetoacetate. To eliminate completely the possibility of loss of acetone or carbon dioxide, the decarboxylation was carried out in sealed glass ampoules. Subsequently, the contents were transferred to double-well flasks and [^{14}C]acetone and carbon dioxide caused to diffuse and were trapped according to the principles discussed above.

In D(−)-β-*hydroxybutyrate.* D(−)-β-Hydroxy-[^{14}C]butyrate must first be converted into [^{14}C]-acetoacetate. Chemical methods for estimating ketone bodies have all used the oxidizing action of an acid dichromate solution (Shaffer, 1908–09) for this purpose but this is a relatively non-specific reaction which can lead to the formation of volatile aldehydes and ketones from other substances. Greater specificity would be conferred by using the enzyme D(−)-β-hydroxybutyrate dehydrogenase, which catalyses the conversion:

$$\text{D(}-\text{)-}\beta\text{-Hydroxy[}^{14}\text{C]butyrate} + \text{NAD}^+ \rightleftharpoons \text{[}^{14}\text{C]acetoacetate} + \text{NADH} + \text{H}^+ \quad (1)$$

However, the equilibrium constant of this reaction is $1{\cdot}42 \times 10^{-9}$ at 25°, which necessitates the use of a ketone-trapping agent if the reaction is to go to completion from left to right (Williamson *et al.* 1962). The presence of a ketone-trapping agent would prevent the effective diffusion of acetone into the acetone trap of the double-well flask. Some success was obtained by using a non-volatile ketone competitor such as levulinic acid (Keeney, 1957) to displace acetone from a ketone-trapping agent such as hydrazine. However, the more satisfactory method was to make use of the contamination of the preparation of D(−)-β-hydroxybutyrate dehydrogenase by malate dehydrogenase. This latter enzyme catalyses the reaction:

$$\text{Oxaloacetate} + \text{NADH} + \text{H}^+ \rightleftharpoons \text{L-malate} + \text{NAD}^+ \quad (2)$$

By combining reactions (1) and (2), D(−)-β-hydroxy[^{14}C]butyrate could be converted into [^{14}C]acetoacetate in the presence of a high concentration of oxaloacetate without the use of a ketone-trapping agent. The overall reaction is:

$$\text{D(}-\text{)-}\beta\text{-Hydroxy[}^{14}\text{C]butyrate} + \text{oxaloacetate} \rightarrow \text{[}^{14}\text{C]acetoacetate} + \text{L-malate} \quad (3)$$

Recovery of ^{14}C-labelled ketone bodies from whole blood

[1,3-$^{14}C_2$]Acetone,sodium[3-^{14}C]acetoacetate and sodium D(−)-β-hydroxy[3-^{14}C]butyrate were added to whole blood either separately or mixed and the blood was analysed completely for ^{14}C-labelled ketone bodies by the methods described. The results (Table 2) show recoveries approaching 100% of the radioactivity added. As would be expected from the location of the label in each compound, all the activity was trapped as acetone with negligible contamination of the carbon dioxide trap. When the labelled ketone bodies were mixed and added to blood they were completely separated upon

Table 2. *Recovery of ^{14}C-labelled ketone bodies from blood*

The standard procedures were used for estimating ^{14}C in ketone bodies as described in the text. The percentage recoveries are expressed as the means ± S.D. The numbers of tests are shown in parentheses.

Material added	Percentage of ^{14}C recovered	
	In acetone trap	In CO_2 trap
[1,3-$^{14}C_2$]Acetone		
(0·001 μmole, 2000 counts/min.)	97·5 ± 1·7 (4)	—
(0·17 μmole, 2000 counts/min.)	99·2 ± 3·0 (5)	—
Sodium [3-^{14}C]acetoacetate		
(0·1 μmole, 3300 counts/min.)	98·8 ± 4·1 (7)	—
Sodium D(−)-β-hydroxy[3-^{14}C]butyrate		
(0·004 μmole, 2000 counts/min.)	101·4 ± 5·6 (7)	0·70 ± 0·1 (7)
(0·625 μmole, 2000 counts/min.)	101·2 ± 2·1 (7)	0·25 ± 0·1 (7)

Table 3. *Recovery of a mixture of ^{14}C-labelled ketone bodies from blood*

The standard procedures were used for estimating ^{14}C in ketone bodies as described in the text. The mixture of ^{14}C-labelled ketone bodies present in each sample consisted of [1,3-$^{14}C_2$]acetone (0·004 μmole, 870 counts/min.), sodium [3-^{14}C]acetoacetate (0·04 μmole, 1340 counts/min.), and sodium D(−)-β-hydroxy[3-^{14}C]butyrate (0·004 μmole, 2120 counts/min.). The percentage recoveries are expressed as the means ± S.D. The numbers of tests are shown in parentheses.

Assay procedure for ^{14}C in	Radioactivity expected in acetone trap (counts/min.)	Percentage of expected recovery found	
		In acetone trap	In CO_2 trap (contamination)
Total ketone bodies (*A*)	4330	96·8 ± 1·5 (7)	0·12 ± 0·03 (7)
Acetone plus acetoacetate (*B*)	2210	96·7 ± 4·2 (7)	0·12 ± 0·05 (7)
Acetoacetate (*C*)	1340	97·2 ± 3·2 (7)	0·25 ± 0·05 (7)
D(−)-β-Hydroxybutyrate (counts in *A* minus counts in *B*)	2120	96·6	—
Acetone (counts in *B* minus counts in *C*)	870	96·2	—

analysis (Table 3), with recoveries similar to the experiments in which each ketone body was added separately. The replication as shown by the standard deviation varied between ±4·2% for the estimation of ^{14}C in acetone plus acetoacetate and ±1·5% for the estimation of ^{14}C in total ketone bodies.

In the presence of labelled long-chain fatty acids, the normal precursors of ketone bodies, there is no interference with the method. Interference from compounds such as volatile aldehydes or ketones would not be expected in normal physiological experiments. High specific activity in oxaloacetate in blood would cause interference as it would be decarboxylated and give a falsely high value for the activity in the carboxyl carbon atoms of acetoacetate.

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REFERENCES

Baruch, H. & Chaikoff, I. L. (1954). *Proc. Soc. exp. Biol., N.Y.*, **86**, 97.
Chaikoff, I. L., Goldman, D. S., Brown, G. W., Dauben, W. G. & Gee, M. (1951). *J. biol. Chem.* **190**, 229.
Connolly, J. D., Head, H. H. & Williams, W. F. (1964). *J. Dairy Sci.* **47**, 386.
Crandall, D. I. & Gurin, S. (1949). *J. biol. Chem.* **181**, 829.
Hird, F. J. R. & Symons, R. H. (1959). *Biochim. biophys. Acta*, **35**, 422.
Keeney, M. (1957). *Analyt. Chem.* **29**, 1489.
Katz, J. & Chaikoff, I. L. (1954). *J. biol. Chem.* **206**, 887.
Ljunggren, G. (1924). *Biochem. Z.* **145**, 422.
Sauer, F. (1961). *Canad. J. Biochem. Physiol.* **39**, 1635.
Shaffer, P. A. (1908–09). *J. biol. Chem.* **5**, 211.
Shreeve, W. W. & Tocci, P. M. (1961). *Metabolism*, **10**, 522.
Widmark, E. M. P. (1920–21). *Acta med. scand.* **53**, 393.
Williamson, D. H., Mellanby, J. & Krebs, H. A. (1962). *Biochem. J.* **82**, 90.