RADIOACTIVE CARBON AS A TRACER IN THE SYNTHESIS OF PROPIONIC ACID FROM CO_2 BY THE PROPIONIC ACID BACTERIA

RECENT investigations[1,2] using radioactive carbon C^{11} and the stable isotope C^{13} on CO_2 uptake by the propionic acid bacteria during the dissimilation of glycerol have demonstrated that the carbon from CO_2 is distributed between the two major products, namely, succinic and propionic acids. The equimolar ratio between CO_2 uptake and succinic acid formation has led to the hypothesis that the succinic acid is formed through the addition of CO_2 to a 3-carbon intermediate, thus yielding the 4-carbon compound.[1,2,3] Accordingly, succinic acid should contain labelled carbon (C*), which was found to be the case;[1] this was confirmed and extended to show that the labelled carbon was present only in the carboxyl groups of the succinic acid.[2] From this evidence it did not appear unreasonable that labelled propionic acid was derived from the succinic acid via decarboxylation.

In a continuation of our earlier studies we have investigated this hypothesis from two aspects, using radioactive C^{11} (half-life of 21 minutes). If propionic acid originates via succinic acid, then labelled succinic acid supplied to the bacteria in the presence of glycerol and inactive bicarbonate should yield labelled propionic acid through loss of one of the carboxyl groups. Furthermore, according to this hypothesis the propionic acid should contain labelled carbon only in the carboxyl group. We have tested this by preparing and separating radioactive propionic and succinic acids using propionic acid bacteria; these labelled acids were re-introduced separately into fresh bacterial suspensions in the presence of inactive CO_2 and glycerol. The fermentation in the presence of the labelled acids was allowed to proceed for 60 to 90 minutes. No convincing evidence for the reaction succinic acid $\rightleftarrows$ propionic acid was found under these conditions.

Decarboxylation of the radioactive propionate to oxalate and carbonate was effected by alkaline $KMnO_4$ oxidation at 100° C. for 30 minutes. The extent of the decarboxylation was followed by two methods, *viz.*, radioactive and gravimetric, with excellent agreement between the two. From 70 to 75 per cent. of the radioactivity was found in the oxalate fraction and 25 to 30 per cent. in the carbonate. The presence of C* in both the oxalate and carbonate fractions from the oxidation of the propionate suggests the presence of C* not only in the carboxyl group of the propionic acid but also in the α and/or β positions of the aliphatic chain. It is likely, however, that in the $KMnO_4$ oxidation of the propionate the carbonate liberated is not always the original carboxyl. Thus radioactive oxalate may be obtained from propionate containing C* only in the carboxyl group. In order to obtain more conclusive evidence for the uniform distribution of the C* within the propionic acid a dry distillation (at 350° C.) of labelled barium propionate was carried out which yielded labelled barium carbonate and diethyl ketone. If C* was present only in the carboxyl group then 50 per cent. of the radioactivity would be found in each of the products; if C* was uniformly distributed then 83.4 per cent. should be in the ketone and 16.6 per cent. in barium carbonate. Experimentally 88 per cent. was found in the ketone and 12 per cent. in the barium carbonate. In addition radioactive CHI_3 was obtained from the $(C_2H_5)_2CO$ by oxidation with I_2 in alkaline solution. In view of these results it seems reasonable to conclude that all the carbons in the propionic acid are labelled, although by the latter method it is not possible to distinguish between the α and β carbons. However, since two thirds of the C* is found in the oxalate fraction ($KMnO_4$ oxidation), it would appear that all the carbon atoms of propionic acid may originate from CO_2. While it does not necessarily follow that any one molecule of propionic acid is synthesized completely from CO_2, it appears that the net reaction:

$$\begin{matrix} CH_2OH & CH_3 \\ CHOH & \rightarrow CH_2 + H_2O \\ CH_2OH & COOH \end{matrix}$$

proceeds in part through a reduction of carbon dioxide to propionic acid by glycerol. A comparison of the amount of labelled propionic acid with total propionic acid formed by the bacteria during the experiment, as determined by chemical analysis, shows that 5 to 10 per cent. of the total carbon of propionic acid formed originated from C^*O_2. From these experiments a rather unexpected conclusion is reached, namely, the two main products of the glycerol fermentation, propionic and succinic acids are not formed via each other. It seems that an appreciable fraction of the propionic acid has been synthesized from C^*O_2 rather than from a simple transformation of glycerol without degradation of the carbon skeleton. In other words, CO_2 is reduced to propionic acid with an organic compound (in this case glycerol) acting as the ultimate reducing agent. The reduction of CO_2 by all living cells (photosynthetic and non-photosynthetic) for synthesis of cellular constituents or excretory products[4,5,6] is strongly supported by the present findings. This reduction of CO_2 may proceed to a greater or

[1] S. F. Carson and S. Ruben, *Proc. Nat. Acad. Sci.*, 26: 422, 1940.

[2] H. G. Wood and C. H. Werkman, *Jour. Biol. Chem.*, 135: 789, 1940.

[3] H. G. Wood and C. H. Werkman, *Biochem. Jour.*, 34: 129, 1940.

[4] S. Ruben and M. D. Kamen, *Proc. Nat. Acad. Sci.*, 26: 418, 1940.

[5] H. A. Barker, S. Ruben and J. V. Beck, *ibid.*, 26: 477, 1940.

[6] A. J. Kluyver, *Suomen Kemistilehti*, 12-A: 81, 1939.

lesser extent, depending on the biological system involved, and may require special conditions.

We have also carried out some experiments on the fermentation of pyruvic acid by the propionic acid bacteria. During this fermentation carried out in the presence of C^*O_2 a relatively large amount of the fixed C* was found in the form of a carbonyl compound, precipitable as a 2,4 dinitrophenylhydrazone along with pyruvic and oxalacetic acid present as carriers. The possibility of this compound being an α-keto acid with C* in the carboxyl group is strongly suggested by the enzymatic decarboxylation of the radioactive compound with yeast carboxylase, in which C^*O_2 was obtained. Radioactive propionic and succinic acids were also formed in this fermentation. Details of these and other experiments will be published elsewhere.

We are indebted to Professor C. B. Van Niel and Professor H. A. Barker for much valuable advice and discussion. We wish to thank Professor E. O. Lawrence and members of the Radiation Laboratory for their cooperation.

S. F. CARSON
J. W. FOSTER[7]
S. RUBEN
M. D. KAMEN

HOPKINS MARINE STATION OF
STANFORD UNIVERSITY,
DEPARTMENT OF CHEMISTRY AND
RADIATION LABORATORY OF THE UNIVERSITY
OF CALIFORNIA AT BERKELEY

MASSIVE "ACUTE" PRECIPITATION OF FREE SULFATHIAZOLE IN THE URINARY TRACT*

THE formation of concrements in the urinary tract after chronic administration of sulfanilamide derivatives has been reported repeatedly in the literature.[1, 2, 3] The uroliths were always found to contain a high percentage of the very insoluble *acetylated* form of the different compounds. After administration of sulfapyridine or sulfamethylthiazole the concrements were located mainly in the renal pelves, ureters and bladder, whereas, after sulfathiazole administration, considerable intrarenal precipitation (in the collecting tubules) was observed.[4]

In the course of an investigation on the acute intraperitoneal toxicity, in rats and mice, of the 3 derivatives[5] mentioned above, the peculiar observation was made that all animals dying from a single dose of sulfathiazole or its sodium salt, without exception, showed massive precipitation of the *free* drug in the urinary tract. Depending upon the dose, and consequently upon the time-interval between injection and death of the animal, the precipitate was found in different parts of the urinary tract. If death occurred only a few hours after the injection, the collecting tubules and the papillary ducts were filled with a whitish material distinctly visible macroscopically and extending into renal pelves, ureters and bladder. After a longer time-interval, the precipitate in the kidneys diminished in amount and finally disappeared (usually after 24 hours), while the bladder became completely filled and even distended with a white crystalline material, which in some cases reached back into the lower parts of the ureters. Within 10 to 20 hours this soft precipitate in the bladder was converted into hard aggregates weighing between 5 and 30 mg and composed almost entirely of free sulfathiazole. If the animals survived for at least several hours, anatomical signs of irritation were often found in the kidneys (marked enlargement with congestion and edema).

The picture as described in the different stages was seen in 54 rats and 20 mice injected with various doses of sulfathiazole or its sodium salt.

This phenomenon of acute precipitation was further investigated by sacrificing groups of 3 rats at different time-intervals after the intraperitoneal injection of a sublethal dose of sodium sulfathiazole (1.0 g/kg). The urinary tract was examined carefully and drug determinations in blood and various tissues were performed. Some of the results are summarized in Table I.

TABLE I

DETERMINATION OF SULFATHIAZOLE

Time after injection	Blood		Kidney				
	mg. per cent.		mg. per cent. per moist tissue		Total amount in both kidneys in mg.		Gross precipitation in the renal papilla
	Free	Acetyl.	Free	Acetyl.	Free	Acetyl.	
5 min.	103	0	145	0	2.12	0	0
15 min.	101	0	259	0	4.32	0	±
30 min.	115	0	396	0	6.06	0	++++ massive
60 min.	107	0	322	0	5.06	0	+++
3 hrs.	58	0	266	25	4.96	0.46	++
12 hrs.	44	7	67	27	1.76	0.70	+
24 hrs.	10	5	17	9	0.32	0.18	0

Intraperitoneal injection of sodium sulfathiazole 1.0 g/kg in rats. All figures are the mean of the values from 3 animals.

It can be seen from the table that precipitation (composed entirely of *free* sulfathiazole) reaches its maximum in the renal papilla 30 minutes after the

[7] National Research Council Fellow.

* Aided by a grant from the Sidney C. Keller Research Fund.

[1] W. Antopol and H. Robinson, *Proc. Soc. Exper. Biol. and Med.*, 40: 428, 1939; *Arch. Path.*, 29: 67, 1940.

[2] P. Gross, F. B. Cooper and M. Lewis, *Proc. Soc. Exper. Biol. and Med.*, 40: 448, 1939.

[3] H.Molitor and H. Robinson, *Proc. Soc. Exper. Biol. and Med.*, 41: 409, 1939; *Arch. internat. de pharmacodyn. et de therap.*, 62: 281, 1939.

[4] P. Gross, F. B. Cooper and R. E. Scott, *Urol. and Cutan. Review*, 44: 205, 1940.

[5] D. Lehr, W. Antopol, J. Churg and H. Sprinz, *Proc. Soc. Exper. Biol. and Med.* In press.