Some Effects of Propionate upon Acetate Metabolism in Animal Tissue Slices

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A number of instances of inhibitory effects of propionic acid on metabolic reactions have been reported. Grafflin & Green (1948) found that the oxidation of acetate by washed rabbit-kidney particles ('cyclophorase') was inhibited by propionate, which itself was not oxidized. The oxygen uptake of sheep-rumen epithelial tissue was shown (Pennington, 1954) to be markedly depressed by propionate in a medium without bicarbonate, but was increased by propionate in a medium buffered with bicarbonate-carbon dioxide. It was suggested that, in the absence of carbon dioxide, the metabolism of propionyl-coenzyme A was blocked and that coenzyme A, which would normally be available for the metabolism of endogenous substances, was immobilized as propionyl-coenzyme A. Propionate suppresses ketone-body formation by rumen epithelium from pyruvate and decreases slightly the uptake of pyruvate (Pennington & Sutherland, 1956a). Lang & Bässler (1953) found that propionate inhibited the oxygen uptake of particle preparations from rabbit liver and kidney in the presence of low levels of succinate; the formation of acetoacetate from acetate by the liver preparations was depressed also. These effects were attributed to effective competition by propionate for coenzyme A.

It has been established that propionate inhibits the growth of many micro-organisms (see Heseltine, 1952). Hill (1952) showed that the suppression of growth of *Streptococcus faecalis* by propionate could be reversed by acetate. He suggested that, by

combining with coenzyme A, propionate interferes with the oxidative decarboxylation of pyruvate to acetate.

The ruminant liver normally metabolizes considerable quantities of propionic acid, formed in the rumen. Consequently, any inhibitory effects of propionate may be of considerable physiological importance to this class of animals. The present studies were concerned with possible effects of propionate on the metabolism of acetate, which is present in relatively high concentration in the blood of ruminants. Most of the experiments described were carried out, for convenience, with rat liver. It subsequently became evident, however, that there was a marked species difference in the magnitude of the effects of propionate.

EXPERIMENTAL

Labelled compounds. These were obtained from the Radiochemical Centre, Amersham. (The D- ^{14}C]glucose is prepared by tobacco-leaf photosynthesis and is expected to be uniformly labelled.)

Animals. The rats were Hooded Lister strain, 3-4 months old, and were given rat cubes *ad lib.*; males were used in every case except Expt. 3, Table 7. The sheep livers were obtained from Scottish Blackface ewes, which had been fed on adequate amounts of hay and concentrates.

Tissue slices. The slices were prepared either with the tissue-chopping machine of McIlwain & Buddle (1953) or by free-hand slicing with the technique of Deutsch (1936). Considerable differences, summarized in Table 1, were observed between the rates of respiration, of ketone-body formation and of $^{14}\text{CO}_2$ formation from [*carboxy- ^{14}C*]acetate

Table 1. *Effect of slicing technique on metabolism of rat-liver slices*

Liver slices (500 mg., wet wt.) were incubated for 3 hr. at 38° in 3 ml. of Ringer-phosphate solution containing [*carboxy- ^{14}C*]acetate (0.01 M). The gas phase was O_2 . Q_{O_2} = $\mu\text{l. of O}_2/\text{mg. dry wt./hr.}$

Expt. no.	Method of slicing	Q_{O_2}		Ketone bodies produced ($\mu\text{moles}/100 \text{ mg. dry wt.}$)	Acetate carboxyl-C in CO_2 ($\mu\text{atoms}/100 \text{ mg. dry wt. of tissue}$)
		Initial	After 3 hr.		
1	Hand	8.19	4.86	12.46	8.36
	Machine, 0.416 mm.*	7.27	2.54	4.64	2.95
2	Hand	7.06	5.21	—	10.28
	Machine, 0.416 mm.*	7.49	1.62	—	5.95
	Machine, 0.572 mm.*	7.02	1.71	—	4.46
	Machine, 0.728 mm.*	7.07	2.46	—	6.41

* The machine was set to cut slices of the stated thickness.

by rat-liver slices cut by the different procedures. It is evident that the respiratory activity of machine-cut slices declined more rapidly than that of slices cut by hand. The method of slicing used in each experiment is stated in the legends to the tables.

The slices were kept in ice-cold Ringer solution before use. The time from killing to the end of the equilibration period was 60–90 min. All dry weights given are final dry weights. The metabolic data presented are, with few exceptions, means obtained from two runs with separate lots of tissue. Pooled slices, usually from a single animal, were used in any one numbered experiment.

Medium. The basal medium was prepared by mixing 90 parts of Krebs–Ringer solution (Cohen, 1949) from which CaCl_2 was omitted, 10 parts of 0.1M-Na phosphate buffer (pH 7.2) and 7.5 parts of NaHCO_3 (0.154M). Fatty acids and pyruvic acid were added as sodium salts. The flasks were gassed with $\text{O}_2 + \text{CO}_2$ (95:5, v/v) and the final pH was 7.1. For use with sheep tissue the concentration of all the components of the Ringer solution was increased to give a total concentration of 0.167M. For the respiration measurements of Table 1, the bicarbonate was omitted and the Warburg flasks were gassed with O_2 .

General procedure and measurement of $^{14}\text{CO}_2$. The medium and slices were shaken (80–90 strokes/min.) at 38° , either in Warburg vessels or in specially constructed flasks. The latter were similar to those described by Pennington & Sutherland (1956b), with the addition of a centre well like that of Warburg flasks. At the end of the experiment 0.5 ml. of N-HCl was injected to kill the tissue and liberate bound CO_2 , then 1 ml. of 2N-NaOH (CO_2 -free) was injected through the rubber cap into the centre well and the flasks were shaken for 75–90 min. to allow absorption of the labelled CO_2 . The $^{14}\text{CO}_2$ was counted as $\text{Ba}^{14}\text{CO}_3$.

When smaller quantities of medium and tissue were required, Warburg flasks were adapted for use by replacing the stopper by a rubber cap. To allow gassing of the flasks a hypodermic needle was inserted temporarily through the cap. (If such a cap is not readily available it can be made easily by boring a wide hole in a rubber stopper to within a short distance of the base. The stopper is inserted and the top turned down over the outside of the glass.) At the end of the experiment HCl was injected into the side arm and tipped into the main compartment. NaOH was then injected into the side arm to absorb the $^{14}\text{CO}_2$.

Ketone bodies. The tissue was washed three times with warm water and the medium and washings were combined. After a portion of the liquid had been neutralized, protein was precipitated with solutions of ZnSO_4 and $\text{Ba}(\text{OH})_2$. The filtrate was used for determination of acetone by the method of Thin & Robertson (1952) and of total ketone bodies and β -hydroxybutyric acid by the method of Greenberg & Lester (1944) as modified previously (Pennington & Sutherland, 1956a). Acetoacetate was determined by difference.

For radioactivity measurements on ketone bodies, these were converted into acetone by refluxing the protein-free filtrates with chromic acid, as in the procedure of Greenberg & Lester (1944). The acetone was aerated into a saturated solution of 2:4-dinitrophenylhydrazine in 2N-HCl (5 ml.) and the acetone dinitrophenylhydrazone extracted with CCl_4 (2 ml.). After colorimetric measurement of the dinitrophenylhydrazone, the CCl_4 was removed by evaporation and the radioactivity of the dinitrophenylhydrazone was measured by direct counting.

In one experiment, after a portion of the filtrate had been refluxed with chromic acid, the acetone was distilled off and precipitated, as the Hg complex, with Denigé's reagent. The complex was decomposed with 10% HCl, the acetone distilled off and the distillate aerated as described above. The specific activity of the dinitrophenylhydrazone was 88% of the value obtained by the usual procedure.

The radioactivity in the carboxyl group of acetoacetic acid or β -hydroxybutyric acid was not measured. In two instances (Table 7, Expt. 1; Table 8, Expt. 1) only the acetone and acetoacetic acid were assayed for radioactivity; instead of the samples being refluxed with chromic acid they were treated with aniline citrate (Edson, 1935), followed by aeration etc.

Fatty acid uptake. The disappearance of labelled fatty acid was determined by steam-distilling 1 ml. of the medium (and washings) in a small Markham still, after precipitation of protein with metaphosphoric acid. The distillate was made slightly alkaline, evaporated to a small volume and portions were evaporated to dryness on nickel planchets and counted.

Radioactivity in lipids. The washed tissue was extracted five times by refluxing with 10 ml. of ethanol-ether (3:1, v/v) for 5 min. The combined extracts were evaporated to dryness, 5 ml. of hexane and 2 ml. of water were added and the tube was shaken vigorously for 15 min. (Katz & Chaikoff, 1954). Portions of the hexane layer were plated and counted. There was no serious interference from radioactive acetate in this procedure.

RESULTS

The effect of propionate on the formation of $^{14}\text{CO}_2$ from [*carboxy*- ^{14}C]acetate by rat-liver slices is shown in Table 2, which also shows the effect of acetate upon the oxidation of labelled propionate. It will be seen that the oxidation of acetate, as measured by the appearance of isotope in the CO_2 , was almost completely suppressed by propionate. On the other hand, the oxidation of propionate was actually increased by acetate in each instance.

In another experiment, not recorded, there was no difference in the extent of inhibition of acetate oxidation produced by commercial sodium propionate which had been purified by recrystallizing three times from aqueous ethanol and then steam-distilling as propionic acid.

Table 3 shows the effect of lower concentrations of propionate upon acetate oxidation. An inhibitory effect is noticeable, even when the concentration of propionate was only one-twentieth that of the acetate. The 1 hr. incubation was carried out because of the likelihood that such small amounts of propionate would be completely used up within 3 hr. However, by comparison of the 1 and 3 hr. results, it is evident that there was very little oxidation of acetate by machine-cut slices after the first hour. This was confirmed by another experiment, in which the labelled acetate was added after the tissue had been incubated in the basal medium for varying periods. It appears, therefore, that the

Table 2. *Formation of labelled $^{14}\text{CO}_2$ from acetate and propionate by rat-liver slices*

The slices (1 g. and 500 mg. respectively) in Expts. 1 and 2 were cut by chopper (set at 0.416 mm.); 600 mg. of slices, cut by hand, were used in Expts. 3 and 4. All results are corrected to 100 mg. (final dry wt.). The flasks contained Ringer-phosphate-bicarbonate (10 ml. in Expt. 1, 3 ml. in Expts. 2-4) and the additions shown. They were incubated at 38° for 3 hr. (Expts. 1 and 2), 30 min. (Expt. 3) or 2 hr. (Expt. 4).

Expt. no.	Additions		Labelled C in CO_2 (μatoms)
	Labelled [<i>carboxy-^{14}C</i>]	Unlabelled	
1	Acetate (0.01 M)	None	4.60
	Acetate (0.01 M)	Propionate (0.01 M)	0.21
2	Acetate (0.01 M)	None	4.45
	Acetate (0.01 M)	Propionate (0.01 M)	0.08
	Propionate (0.01 M)	None	1.32
	Propionate (0.01 M)	Acetate (0.01 M)	2.22
3	Acetate (0.01 M)	None	2.49
	Acetate (0.01 M)	Propionate (0.01 M)	0.06
4	Propionate (0.01 M)	None	6.96
	Propionate (0.01 M)	Acetate (0.01 M)	8.77
	Propionate (0.005 M)	None	6.94
	Propionate (0.005 M)	Acetate (0.01 M)	8.01
	Propionate (0.0025 M)	None	4.55
	Propionate (0.0025 M)	Acetate (0.01 M)	6.00

Table 3. *Effect of various concentrations of propionate on acetate oxidation by rat-liver slices*

Slices (250 mg., wet wt.) cut by the chopper set at 0.416 mm. were used in 3 ml. of medium. [*carboxy- ^{14}C*]-Acetate (0.01 M) was present in all the flasks, which were incubated at 38°. The figures are calculated to 100 mg. (dry wt.) of tissue.

Expt. no.	Concn. of propionate (M)	Incubation period (hr.)	Acetate carboxyl-C in CO_2 (μatoms)
1	0	3	4.16
	0.01	3	0.11
	0.005	3	0.15
	0.0025	3	0.20
2	0	3	4.32
	0.01	3	0.11
	0.00125	3	1.64
	0.0005	3	3.12
	0	1	4.12
	0.0005	1	2.00

Table 4. *Oxidation of acetate and propionate by slices of various animal tissues*

Slices cut by the chopper set at 0.416 mm. were incubated in 3 ml. of medium at 38° for 3 hr. The figures are calculated to 100 mg. (dry wt.) for the rat tissues, and 300 mg. (wet wt.) of rumen epithelium was used.

Tissue	Additions (0.01 M)		Labelled C in CO_2 (μatoms)
	Labelled [<i>carboxy-^{14}C</i>]	Unlabelled	
Kidney (rat)	Acetate	None	85.9
	Acetate	Propionate	30.9
	Propionate	None	53.3
	Propionate	Acetate	43.5
Heart (rat)	Acetate	None	0.79
	Acetate	Propionate	0.37
Diaphragm (rat)	Acetate	None	2.91
	Acetate	Propionate	1.15
Rumen epithelium (sheep)	Acetate	None	1.20
	Acetate	Propionate	0.51

ability of such slices to oxidize acetate declines even more rapidly than the Q_{O_2} (cf. Table 1).

The effect of propionate upon acetate oxidation by some other tissues was examined (Table 4). Although acetate oxidation was depressed in each case, the relative effect of propionate was much less than was observed with rat liver. With kidney tissue, in contrast with liver, acetate slightly decreased the conversion of the propionate carboxyl-C into CO_2 .

Certain amino acids, namely methionine, isoleucine and valine, are believed to form propionic acid (or propionyl-coenzyme A) when metabolized by liver (Patwardhan, 1954; Coon, Abrahamsen & Greene, 1952; Kinnory & Greenberg, 1953). The effect of each of these on acetate oxidation was measured (Table 5). Butyrate and isovalerate, both of which may be expected to compete for available coenzyme A, were also examined. The latter, like propionate, inhibits the respiration of rumen

epithelium in a bicarbonate-free medium (Annison & Pennington, 1954). The effects of valine and butyrate were particularly marked, although none of the compounds tested were as effective as propionate in suppressing acetate oxidation.

Edson (1936) showed that sorbitol is an extremely strong inhibitor of endogenous ketone-body production by liver slices from fasted rats, being in this respect more effective than glucose. Table 6 shows the effect of sorbitol and of glucose on the metabolism of labelled acetate. The effects of acetate and propionate on the oxidation of labelled glucose are included in this table. It may be seen that neither sorbitol nor glucose has any marked effects on the conversion of acetate into CO_2 or ketone bodies. Both increased somewhat the incorporation of the isotope into the lipid. The effect of propionate on glucose oxidation was not comparable with its effect on the oxidation of acetate.

More extensive studies of the effects of propionate on acetate metabolism were undertaken in further experiments (Table 7), in which acetate uptake and ketone-body formation were also measured. The strong inhibition of acetate oxidation by propionate was paralleled by its effect on the total amount of acetate metabolized and on the incorporation of the isotope into ketone bodies (excluding the carboxyl groups of acetoacetate and β -hydroxybutyrate which were not assayed). A point of interest in Table 7 is the relatively large proportion of ketone bodies accounted for as acetone.

Table 8 shows the results of similar experiments carried out with sheep liver. Comparison with Table 7 reveals considerable quantitative differences in the effect of propionate on acetate metabolism. Both the overall disappearance of acetate and the formation of $^{14}CO_2$ were influenced far less by propionate than in rat liver. There are two other

Table 5. *Effect of various other compounds on acetate oxidation by rat liver*

Slices cut by the chopper set at 0.416 mm. were incubated in 3 ml. of medium for 3 hr. at 38°. All flasks contained [carboxy- ^{14}C]acetate (0.01M). The amino acids were neutralized. The figures are calculated to 100 mg. (dry wt.) of tissue.

Expt. no.	Addition	Acetate carboxyl-C in CO_2 (μ atoms)
1	None	3.36
	Propionate (0.01M)	0.11
	DL-Methionine (0.02M)	1.44
	DL-Isoleucine (0.02M)	2.12
	DL-Valine (0.02M)	0.63
2	None	2.85
	Propionate (0.01M)	0.09
	n-Butyrate (0.01M)	0.42
	isoValerate (0.01M)	1.06

Table 6. *Mutual interaction of sorbitol, glucose and fatty acids, metabolized by rat-liver slices*

Slices cut by the chopper set at 0.416 mm. were used. In Expt. 1, 600 mg. of slices was incubated in 3 ml. of medium for 2 hr. at 38°. In Expt. 2, 1 g. of slices was incubated in 10 ml. of medium for 3 hr. The concn. of each substrate was 0.01M. The quantities given are the totals for each experiment.

Expt. no.	Additions		^{14}C (μ atoms) in		
	Labelled	Unlabelled	CO_2^*	Total lipids	Ketone bodies (C_2 -4)
1	[carboxy- ^{14}C]Acetate	None	4.43	0.31	0.81
	[carboxy- ^{14}C]Acetate	Sorbitol	4.30	0.40	0.84
	[carboxy- ^{14}C]Acetate	D-Glucose	4.61	0.39	0.78
2	[carboxy- ^{14}C]Acetate	None	5.50	—	—
	[carboxy- ^{14}C]Acetate	Sorbitol	5.40	—	—
	[carboxy- ^{14}C]Acetate	D-Glucose	6.20	—	—
	D-[^{14}C]Glucose	None	3.71	—	—
	D-[^{14}C]Glucose	Acetate	2.64	—	—
	D-[^{14}C]Glucose	Propionate	3.33	—	—

* The glucose is assumed to be uniformly labelled.

marked differences between the results obtained with rat and sheep tissue. The total ketone-body production by sheep-liver slices was only a fraction of that observed with the rat tissue, and acetone formation by the sheep liver was negligible.

The fact that, in rat liver, acetate uptake and CO₂ and ketone-body production were all inhibited by propionate tends to suggest that the inhibition occurs at an early stage in the metabolism of acetate. In an attempt to define further the site of inhibition,

Table 7. *Effect of propionate on metabolism of acetate by rat-liver slices*

Slices (1 g., wet wt.) incubated at 38° for 3 hr. in 5 ml. of medium containing [carboxy-¹⁴C]acetate (0.01 M). Propionate was added where indicated. The ketone-body production in control flasks without substrate is shown in parentheses. The slices in Expt. 1 were cut by chopper set at 0.416 mm., those in Expts. 2 and 3 by hand. The quantities given are the totals for each experiment.

Expt. no.	Propionate (0.01 M, where present)	Acetate used (μmoles)	Acetate carboxyl-C (μatoms) in		Ketone bodies formed (μmoles)*			
			CO ₂	Ketone bodies (C ₂₋₄)	Total	Acetone	β-Hydroxy-butyrate	Acetoacetate
1	-	23.4	14.50	1.50†	7.05	2.52	1.55	2.98
	+	2.4	0.31	0.03†	3.40 (4.95)	1.05 (2.05)	0.45 (0.20)	1.90 (2.70)
2	-	31.7	17.37	1.70	12.30	4.72	5.25	2.33
	+	0.9	0.77	0.07	5.03 (11.17)	1.13 (5.14)	2.55 (4.57)	1.35 (1.46)
3	-	21.9	15.48	1.19	7.54	3.55	2.63	1.36
	+	0.7	0.93	0.05	2.04 (4.68)	0.67 (2.81)	1.03 (1.35)	0.34 (0.52)

* By chemical analysis.

† Acetoacetate + acetone, only.

Table 8. *Effect of propionate on metabolism of acetate by sheep-liver slices*

Conditions as for Table 7. The slices in Expt. 1 were cut by chopper set at 0.416 mm., those in Expts. 2 and 3 by hand. The quantities given are the totals for each experiment.

Expt. no.	Propionate (0.01 M, where present)	Acetate used (μmoles)	Acetate carboxyl-C (μatoms) in		Ketone bodies formed (μmoles)*			
			CO ₂	Ketone bodies (C ₂₋₄)	Total	Acetone	β-Hydroxy-butyrate	Acetoacetate
1	-	22.1	5.90	0.149†	1.76	0	1.11	0.65
	+	13.8	1.12	0.006†	0.67 (0.84)	0 (0)	0.21 (0.27)	0.46 (0.57)
2	-	24.5	13.02	0.72	2.21	0	1.84	0.37
	+	14.0	9.69	0.15	1.25 (2.07)	0 (0)	1.02 (1.66)	0.23 (0.41)
3	-	22.8	13.91	0.92	2.92	0.11	1.84	0.97
	+	13.8	9.10	0.05	1.30 (2.66)	0.20 (0.17)	0.83 (1.73)	0.27 (0.76)

* By chemical analysis.

† Acetoacetate + acetone, only.

Table 9. *Effect of propionate on metabolism of butyrate and pyruvate by rat-liver slices*

Slices (1 g., wet wt.) cut by hand were incubated at 38° for 2 hr. in 5 ml. of medium containing the additions shown. The ketone-body production in control flasks without substrate is shown in parentheses. The quantities given are the totals for each experiment.

Expt. no.	Additions	Pyruvate used (μmoles)	¹⁴ C (μatoms) in		Ketone bodies formed (μmoles)			
			CO ₂	Ketone bodies (C ₂₋₄)	Total	Acetone	β-Hydroxy-butyrate	Acetoacetate
1	[carboxy- ¹⁴ C]Butyrate (0.006 M)	—	8.78	0.97	15.00	6.08	5.55	3.37
	[carboxy- ¹⁴ C]Butyrate (0.006 M) + propionate (0.006 M)	—	9.55	—	18.80 (6.32)	3.04 (2.14)	7.05 (2.18)	8.71 (2.00)
2	[carboxy- ¹⁴ C]Butyrate (0.01 M)	—	7.48	1.30	11.51	5.18	3.38	2.95
	[carboxy- ¹⁴ C]Butyrate (0.01 M) + propionate (0.01 M)	—	9.02	1.38	13.35 (4.35)	3.10 (2.37)	7.73 (1.98)	2.52 (0)
3	[2- ¹⁴ C]Pyruvate (0.006 M)	24.8	8.93	0.42	5.14	1.49	2.85	0.80
	[2- ¹⁴ C]Pyruvate (0.006 M) + propionate (0.006 M)	16.2	4.83	0.01	2.95 (3.91)	1.07 (1.36)	1.34 (1.77)	0.54 (0.78)

the effect of propionate on the metabolism of labelled butyrate and pyruvate was studied (Table 9). The production of $^{14}\text{CO}_2$ and the uptake of pyruvate were depressed by propionate, although the effects were much less than the effects upon acetate metabolism; on the other hand, ketone-body production from pyruvate was virtually abolished by propionate. Propionate had little or no effect on the metabolism of butyrate.

DISCUSSION

The main finding in the work described above was that propionate strongly suppressed the metabolism of acetate by rat-liver slices. Sufficient is known of the pathways of acetate metabolism in liver to make it possible to consider possible sites of inhibition. Available evidence indicates that, although the carbon of acetate has several alternative fates in liver, the initial step common to all is the formation of acetyl-coenzyme A. Since propionate inhibited both the uptake of acetate and its conversion into carbon dioxide and ketone bodies, it seems likely that it blocks the combination of acetate with coenzyme A. This hypothesis is supported by the observations (Table 9) that the oxidation of pyruvate was decreased much less, and that of butyrate not at all, by propionate. Both these compounds, as far as is known, can be oxidized by liver only after the formation from them of acetyl-coenzyme A. If the site of inhibition by propionate is subsequent to acetyl-coenzyme A formation, it would be expected that the oxidation of pyruvate and butyrate would be likewise prevented.

A more detailed investigation of the nature of the inhibition will necessitate the purification of the enzymes involved. Such data of this type as are available at present, however, throw little light on the problem. Hele (1954) purified from ox-heart muscle what appears to be a single enzyme which reversibly converts acetate and propionate into their coenzyme A derivatives. The Michaelis-Menten constant is greater with propionate (5.00×10^{-3}) than with acetate (1.42×10^{-3}). Straightforward competition between acetate and propionate for an enzyme with these characteristics obviously would not explain the effectiveness of propionate in inhibiting acetate metabolism. No enzyme which has been shown to form coenzyme A derivatives from both acetate and propionate has been purified from liver. The fatty acid-activating enzyme purified from ox liver (Mahler, Wakil & Bock, 1953) formed coenzyme A derivatives from fatty acids with 4–12 carbon atoms; it also had slight activity towards propionate but none towards acetate. The acetate-activating fraction from pigeon liver (Chou & Lipmann, 1952) was apparently not tested with propionate. Drysdale & Lardy (1953)

found that extracts of an acetone-dried powder from rat-liver mitochondria activated butyrate and higher acids but failed to activate acetate.

Activation of acetate and propionate by a common enzyme having a much greater affinity for propionate is an obvious possibility to explain the observed inhibition. This seems unlikely, however, since individually acetate and propionate are metabolized at about the same rates by liver slices. On the other hand, separate acetic and propionic thiokinases [for nomenclature see *Biochem. J.* (1956), **64**, 782] may exist in liver and the acetic enzyme may be blocked by propionate. Any mechanism considered for the inhibition of acetate metabolism by propionate must take into account the fact that butyrate also markedly inhibited acetate oxidation (Table 5). In this connexion the hypothesis (Avigan, Quastel & Scholefield, 1955) that the reactions of acetyl-coenzyme A are inhibited by acyl-coenzyme A compounds is of interest.

The antiketogenic activity of propionate has often been looked on merely as a property possessed as a result of its glycogenic nature, thus posing no separate problem concerning the mechanism of the antiketogenic effect. However, Quastel & Wheatley (1933) suggested that propionate inhibits acetoacetate formation (in this case, from butyrate) by competition for enzymes. More recent work (Lang & Bässler, 1953; Pennington & Sutherland, 1956b) and the data in the present paper point to a similar conclusion. In this respect, however, Table 9 is of particular interest. The incorporation of the labelled carbon of the pyruvate into ketone bodies was depressed by propionate to a relatively much greater extent than was its conversion into carbon dioxide. Possibly propionate may inhibit ketone-body formation by another mechanism, in addition to blocking the formation of acetyl-coenzyme A.

The failure of sorbitol appreciably to influence the conversion of acetate into carbon dioxide or ketone bodies (Table 6) throws no light on its antiketogenic effect. The small increase in the isotope appearing in the lipids accords with the suggestion of Blakley (1952) that sorbitol may stimulate fat synthesis. However, glucose, which was a less effective antiketogenic agent than sorbitol (Edson, 1936), also increased the incorporation of ^{14}C into the lipids.

The marked difference in the effect of propionate on acetate metabolism by rat and sheep liver is being investigated further. It is pertinent, perhaps, to mention the differences between acetate metabolism in rat and sheep mammary tissue observed by Folley and his co-workers (e.g. Folley & French, 1950; Balmain, Folley & Glascock, 1952). The relatively large proportion of acetone in the ketone bodies produced by rat- compared with sheep-liver slices may be due to non-enzymic decarboxylation,

in the medium, of acetoacetate. This process is known to be catalysed by amino groups and it was found that during incubation considerably more protein escaped into the medium from rat-liver slices than from sheep-liver slices. On the other hand, this may represent another metabolic difference between rat and sheep liver. It should be noted that, since the incorporation of isotope into ketone bodies was always much less than into carbon dioxide, only a small proportion of the $^{14}\text{CO}_2$ produced during incubation of the rat-liver slices would be derived from the decarboxylation of acetoacetate.

SUMMARY

1. Propionate, in equimolar concentration, largely abolished the production of $^{14}\text{CO}_2$ from carboxyl-labelled acetate by rat-liver slices. Butyrate, isovalerate, valine, isoleucine and methionine inhibited acetate oxidation to varying degrees. The oxidation of propionate was not decreased by acetate.

2. Acetate oxidation by slices of other rat tissues was inhibited by propionate, but to a lesser degree than with rat-liver slices.

3. The uptake of acetate and the incorporation of its carboxyl-carbon into ketone bodies by rat-liver slices were also strongly inhibited by propionate.

4. The oxidation of carboxyl-labelled butyrate by rat liver was not inhibited by propionate; the oxidation of C_{13} -labelled pyruvate was affected to a much smaller extent than that of acetate.

5. The effects of propionate on acetate metabolism by sheep-liver slices were much smaller than those observed with rat liver.

6. The endogenous ketone-body production of sheep liver was less than that of rat liver.

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The Protein Composition and Nucleic Acid Content of the Rat Uterus in Different States

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This work arose out of our interest in the proteins of the contractile mechanism of the uterus. Csapo (1950a) has reported that more myosin and actomyosin were present in extracts of human, rabbit and rat uteri at the end of pregnancy than in extracts from the non-gravid uterus. In the rabbit some weeks after ovariectomy there was a marked fall in the amount of these proteins present per gram wet

weight of uterus, and injection of oestrone into castrated animals led to a gradual rise towards the normal value (Csapo, 1950b). Csapo used exclusively the viscosimetric method worked out by Balenovic & Straub (1942; see also Szent-Györgyi, 1945) for estimation of the purified proteins, and it is possible that in tissue extracts interfering substances might be present. The value for the myosin-actomyosin