SUMMARY

1. Methods are described for the estimation of protein turnover in *Escherichia coli* by the use of strains with specific amino acid requirements.

2. In rapidly growing cells there was no detectable turnover of protein; in non-growing cells degradation and resynthesis of protein proceeded at the rate of 4–5 %/hr. for several hours.

3. Chloramphenicol, azide and ammonium salts inhibited protein breakdown after a latent period. The effect of 2:4-dinitrophenol was variable.

4. Protein turnover could not be attributed to lysis of cells.

5. The possible function of protein turnover in bacterial metabolism is discussed.

I wish to thank Dr J. Monod and Dr P. H. A. Sneath for leucine-requiring strains of *E. coli* and I am greatly indebted to Miss R. Coyle for invaluable technical assistance.

REFERENCES

Cohen, G. N. & Rickenberg, H. V. (1956). *Ann. Inst. Pasteur*, **91**, 693.
Gale, E. F. & Folkes, J. P. (1953). *Biochem. J.* **55**, 721.
Halvorson, H. O. (1958). *J. Bact.* (in the Press).
Hogness, D. S., Cohn, M. & Monod, J. (1955). *Biochim. biophys. Acta*, **16**, 99.
Koch, A. L. & Levy, H. R. (1955). *J. biol. Chem.* **217**, 947.
Mandelstam, J. (1954). *J. gen. Microbiol.* **11**, 426.
Mandelstam, J. (1956). *Int. Rev. Cytol.* **5**, 51.
Mandelstam, J. (1957). *Nature, Lond.*, **179**, 1179.
Mandelstam, J. (1958). *Biochem. J.* **69**, 103.
Melchior, J. B., Klioze, O. & Klotz, I. M. (1951). *J. biol. Chem.* **189**, 411.
Moore, S. & Stein, W. H. (1948). *J. biol. Chem.* **176**, 367.
Podolsky, R. (1953). *Arch. Biochem. Biophys.* **45**, 327.
Pollock, M. R. (1958). In *The Enzymes*. New York: Academic Press Inc.
Polson, A. (1948). *Biochim. biophys. Acta*, **2**, 575.
Rickenberg, H. V. & Lester, G. (1955). *J. gen. Microbiol.* **13**, 279.
Roberts, R. B., Abelson, P. H., Cowie, D. B., Bolton, E. T. & Britten, R. J. (1955). *Studies of Biosynthesis in* Escherichia coli. Washington, D.C.: Carnegie Institution Publication 607.
Rotman, B. & Spiegelman, S. (1954). *J. Bact.* **68**, 419.
Schneider, W. C. (1945). *J. biol. Chem.* **161**, 293.
Seidman, M. & Link, K. P. (1950). *J. Amer. chem. Soc.* **72**, 4324.
Simpson, M. V. (1953). *J. biol. Chem.* **201**, 143.
Steinberg, D., Vaughan, M. & Anfinsen, C. B. (1956). *Science*, **124**, 389.
Wainwright, S. D. & Pollock, M. R. (1949). *Brit. J. exp. Path.* **30**, 190.

Further Studies on the Inhibition of Acetate Metabolism by Propionate

By R. J. PENNINGTON and J. M. APPLETON
Rowett Research Institute, Bucksburn, Aberdeenshire

(*Received* 11 *December* 1957)

It has been shown previously (Pennington, 1957, and independently by Masoro, Felts, Panagos & Rapport, 1957) that acetate metabolism in rat-liver slices is strongly inhibited by propionate and, to a lesser extent, by other short-chain fatty acids. These investigations did not reveal the mechanism of the inhibition. The formation of $^{14}CO_2$ from [2-^{14}C]pyruvate was much less affected by propionate than was its formation from [*carboxy*-^{14}C]-acetate (Pennington, 1957), thus indicating that propionate (or a product of propionate metabolism) inhibits acetate metabolism by blocking the formation of acetyl-coenzyme A. It seems likely, however, that other interactions are also involved, since Masoro *et al.* (1957) found that the incorporation of isotope from labelled acetate into higher fatty acids was inhibited by propionate to a lesser extent than the incorporation of isotope into carbon dioxide. This was confirmed by the present authors (unpublished work).

As a step towards elucidating the mechanism of the inhibitory action of propionate, further experiments have been carried out with homogenates and soluble preparations from liver.

EXPERIMENTAL

Preparation and incubation of tissue slices. Tissue slices were cut by hand and incubated as described previously (Pennington, 1957). To facilitate approximate comparison with the activity of the homogenates (which usually corresponded to 100 mg. of wet tissue) the results obtained with slices were corrected to 20 mg. (dry wt.).

Preparation of homogenates. These were prepared by homogenizing the tissue in 9 vol. of 0·25 M-sucrose for 2 min. in a glass homogenizer. The clearance between the walls was about 0·1 mm. and the pestle was driven by a $\frac{1}{50}$ h.p. motor; the specified speed of the motor (without load) was 2000 rev./min. and it was set at about two-thirds of its maximum speed. The tube was immersed in ice–water during the process.

Preparation of mitochondria. Homogenates, prepared as above, were centrifuged at 600 *g* for 10 min. to remove larger particles. The supernatant was centrifuged at 12 000 *g* for 15 min. and the mitochondria were resuspended in 0·25M-sucrose (one-half of the volume of the original homogenate). The centrifuging and resuspending were repeated twice.

Incubation of homogenates or mitochondria. The vessels usually contained: potassium phosphate buffer (pH 7·2), 10 mM; $NaHCO_3$, 10 mM; $MgCl_2$, 4 mM; adenosine triphosphate (ATP), mM; cytochrome *c*, 0·013 mM; potassium fumarate, mM. The total volume was 3 ml., including 1 ml. of the tissue preparation. Sufficient sucrose was added to ensure that the medium, after the addition of the tissue preparation, was isotonic with 0·25M-sucrose. The gas phase was $O_2 + CO_2$ (95:5), and the vessels were shaken at 38° for 1 hr. Further additions to the medium and any variations from the above procedure are indicated in the tables.

Measurement of acetate or propionate metabolism. The collection of $^{14}CO_2$ and measurement of its radioactivity were carried out as described previously (Pennington, 1957). In some experiments with homogenates or mitochondria the quantity of ^{14}C trapped in metabolic pools was also measured, a procedure similar to that of Aisenberg & Potter (1955) being used, involving determination of the non-volatile radioactivity in the protein-free filtrate.

In a few experiments the radioactivity in acetoacetate $[C_{(3)-(4)}]$ was measured as previously described (Pennington, 1957).

Experiments with cell-free extracts. Acetone-dried powders were prepared, extracted and aged according to Kaplan & Lipmann (1948). The following medium was used for studying the acylation of sulphanilamide: 2-amino-2-hydroxymethylpropane-1:3-diol–HCl buffer (tris), 0·2M (a 1·5M solution of this buffer was pH 8 at 38°); $MgCl_2$, 10 mM; cysteine, 10 mM (a freshly prepared solution of cysteine hydrochloride, which was adjusted to approx. pH 8 with KOH, was used); ATP, 5 mM; sulphanilamide, 0·4 mM; NaF, 50 mM; coenzyme A, 0·05 mM. The appropriate enzyme preparation and fatty acid or acids were also present; the concentration of each fatty acid was 10 mM. The total volume was 1·5 ml. The mixtures were incubated for 2 hr. at 38° and the remaining free sulphanilamide was determined on a trichloroacetic acid filtrate by the method of Bratton & Marshall (1939).

Materials. ATP (cryst. disodium salt) and coenzyme A (approx. 75% pure) were products of Sigma Chemical Co., St Louis, Mo., U.S.A.

Each result given in the tables represents the average obtained from two vessels. The tissue used in each numbered experiment, except in Tables 5 and 6, was obtained from a single animal.

RESULTS

Table 1 shows that the conversion of the carboxyl group of acetate into CO_2 by rat-liver homogenates was inhibited by propionate. The inhibition was even greater than that previously observed in slices (Pennington, 1957) and was, in fact, almost complete when the concentration of propionate was only one-tenth of that of acetate. The incorporation of isotope into acetoacetate was inhibited to a similar degree. Butyrate also inhibited the production of $^{14}CO_2$ from acetate but less effectively than propionate; this was found also with slices (Pennington, 1957).

Aisenberg & Potter (1955) found that when [*carboxy*-^{14}C]acetate was metabolized by kidney homogenates in the presence of fumarate and pyruvate a considerable amount of ^{14}C was trapped in acid-soluble non-volatile compounds.

Table 1. *Effect of propionate and butyrate on oxidation of acetate by rat-liver homogenates*

Homogenates (representing 100 mg. wet wt. of liver) were incubated at 38° for 1 hr. in the medium described in the Experimental section, with the addition of [*carboxy*-^{14}C]acetate (0·01M except in the last two tests in Expt. 4, when the concentration was 0·05M). Propionate or butyrate was added (as salts of potassium) where indicated. In Expt. 1 the cytochrome *c*, $NaHCO_3$ and CO_2 were omitted and the centre well of the flask contained KOH.

		Labelled C (μg. atoms) in		
Expt. no.	Addition (M)	CO_2	Acetoacetate $[C_{(3)-(4)}]$	Non-volatile compounds*
1	None	2·97	0·43	—
	Propionate (0·01)	0·01	0·001	—
2	None	3·60	—	—
	Propionate (0·01)	0·03	—	—
3	None	4·17	—	2·30
	Butyrate (0·01)	0·37	—	0·39
4	None	3·83	—	0·47
	Propionate (0·01)	0·04	—	0
	Propionate (0·003)	0·03	—	0
	Propionate (0·001)	0·07	—	0·05
	Propionate (0·0003)	0·20	—	0·03
	Propionate (0·0001)	3·07	—	0·45
	None	4·02	—	0·46
	Propionate (0·003)	0·17	—	0·14

* Present in protein-free filtrate: see text.

Table 2. *Effect of propionate on oxidation of pyruvate by rat-liver homogenates*

Conditions were as in Table 1, except that [2-^{14}C]pyruvate (concn. as indicated) was present instead of labelled acetate.

Expt. no.	Concn. of [2-^{14}C]pyruvate (M)	Addition (M)	Labelled C (μg. atoms) in CO_2	Acetoacetate $[C_{(2)-(4)}]$
1	0·01	None	2·24	—
		Propionate (0·01)	0·88	—
2	0·01	None	4·48	—
	0·01	Propionate (0·001)	2·41	—
	0·01	Propionate (0·01)	1·65	—
	0·01	Propionate (0·04)	4·93	—
	0·0025	None	3·56	—
	0·0025	Propionate (0·01)	1·51	—
	0·0025	Propionate (0·04)	3·32	—
3	0·01	None	3·18	0·25
	0·01	Propionate (0·01)	0·77	0·01
	0·01	Propionate (0·04)	1·34	0·01
	0·01	Acetate (0·01)	2·91	0·25
	0·01	Acetate (0·04)	2·94	0·44

In the present experiments, the relative quantity of ^{14}C thus held varied rather widely between experiments, but in any one experiment, as shown in Table 1, it roughly paralleled the amount appearing in the CO_2.

Other results, not reported, showed that when fumarate was omitted from the medium, the oxidation of acetate was decreased to about one-fifth; again, there was negligible oxidation in the presence of propionate. In contrast with the stimulation of oxidation of acetate in kidney homogenates by pyruvate, reported by Aisenberg & Potter (1955), the addition of pyruvate (mM) did not increase oxidation of acetate, either in the presence or absence of fumarate. In Expt. 1 the uptake of O_2 was also measured. In the absence of propionate its rate was practically constant, but where propionate was present it progressively decreased and, at the end of the 60 min. period, was only about one-tenth of the original rate.

The incorporation of the $C_{(2)}$ of pyruvate into CO_2 was affected by propionate to a much smaller extent than the oxidation of acetate (Table 2), as was previously observed when slices were used (Pennington, 1957). Rather unexpectedly, 0·04 M-propionate had very much less effect than 0·01 M-propionate. On the other hand, acetoacetate formation from pyruvate was almost completely suppressed by propionate. Acetate had little effect on pyruvate metabolism, except for an increase in acetoacetate formation at the higher level of acetate.

It was previously observed that the propionate-induced inhibition of acetate oxidation was much less marked in sheep- than in rat-liver slices. An experiment with ox-liver slices (Table 3) also showed a relatively small effect. Subsequently, large variations have been found in the magnitude of this effect of propionate in different experiments with sheep-liver slices, but in only one instance has the degree of inhibition been as large as the smallest effect obtained with rat-liver slices. The relative inhibition caused by propionate was higher when sheep liver was homogenized (Table 3). Pigeon-liver slices and homogenates showed a relatively small effect of propionate (Table 3). The relative effect of propionate in rat-kidney homogenates (Table 3) was much less than that obtained with rat-liver homogenates (Table 1).

The observation of Aisenberg & Potter (1955) that acetate metabolism by kidney homogenates is stimulated by pyruvate, even when furmarate is present, was confirmed. It was noted also that the rate of uptake of O_2 declined much more rapidly when pyruvate was omitted.

Cytochrome *c* was not included in the medium in the experiments of Table 3 except in Expt. 8. It was, however, found in separate tests that production of $^{14}CO_2$ from [*carboxy*-^{14}C]acetate by rat-kidney and sheep-liver homogenates was increased 20–30 % by the inclusion of cytochrome *c* (0·013 mM).

It was of interest to know whether propionate itself was rapidly oxidized by these preparations. Table 4 summarizes the results obtained. The oxidation of propionate by rat-liver homogenates was extremely slow; it appears that, unlike the oxidation of acetate, the rate of oxidation of propionate by rat liver is greatly decreased when the cells are broken down. Addition of coenzyme A (0·1 mM) did not increase the rate of oxidation of propionate by rat-liver homogenates. The oxidation of propionate by rat-kidney homogenates was also very slow, but the activity of sheep- and pigeon-liver homogenates in this respect much more nearly approached that of slices. Other results, not

Table 3. *Effect of propionate on oxidation of acetate by rat kidney and by livers of other animals*

Homogenates were incubated as in Table 1 except that: cytochrome *c* was omitted except in Expt. 8; CO_2 and $NaHCO_3$ were omitted (and KOH was present in the centre well) where indicated (*); pyruvate (mM) was included in the medium in Expts. 1 and 2. Homogenate from 100 mg. of tissue was used, except in Expt. 1 when 50 mg. was used. Slices were incubated as described previously (Pennington, 1957); 600 mg. (wet wt.) of slices was used, the total volume of medium was 3 ml. and the results are corrected to 20 mg. (dry wt.) of slices. Incubation was for 3 hr. in Expt. 6 and 1 hr. in all other experiments. The concn. of [*carboxy*-^{14}C]acetate was 0·01 M in all flasks.

Expt. no.	Tissue	Nature of preparation	Propionate (0·01 M, where present)	Labelled C in CO_2 (μg. atoms)	Acetate used (μmoles)
1	Kidney (rat)	Homogenate	−	1·27*	.
			+	0·21*	.
2	Kidney (rat)	Homogenate	−	1·69*	8·7
			+	0·74*	6·7
			−	1·92	.
			+	0·39	.
3	Liver (sheep)	Homogenate	−	0·262*	.
			+	0·007*	.
4	Liver (sheep)	Homogenate	−	0·69	.
			+	0·015	.
		Slice	−	0·65	.
			+	0·04	.
5	Liver (sheep)	Homogenate	−	0·62	.
			+	0·095	.
		Slice	−	0·58	.
			+	0·16	.
6	Liver (ox)	Slice	−	2·26	2·88
			+	0·85	1·86
7	Liver (pigeon)	Homogenate	−	0·47	.
			+	0·30	.
		Slice	−	0·88	1·44
			+	0·41	0·52
8	Liver (pigeon)	Homogenate	−	2·20	.
			+	0·85	.

Table 4. *Oxidation of propionate by tissue preparations*

Homogenates (100 mg. of tissue) were incubated as in Table 1, except that [*carboxy*-^{14}C]propionate was present instead of labelled acetate. Cytochrome *c* was omitted in Expts. 3–5 and CO_2 and $NaHCO_3$ were omitted in the first test in Expt. 3. (Expts. 3, 4, 5, 6, 7 and 8 were performed as part of Expts. 2, 4, 5, 6, 7 and 8 respectively, Table 3.) The results of the experiments with slices are corrected to 20 mg. (dry wt.).

Expt. no.	Tissue	Nature of preparation	Labelled C in CO_2 (μg. atoms)
1	Liver (rat)	Homogenate	0·18
2	Liver (rat)	Homogenate	0·08
3	Kidney (rat)	Homogenate	0·20
		Homogenate	0·29
4	Liver (sheep)	Homogenate	0·78
		Slice	1·22
5	Liver (sheep)	Homogenate	1·30
		Slice	1·57
6	Liver (ox)	Slice	4·60
7	Liver (pigeon)	Slice	1·70
8	Liver (pigeon)	Homogenate	1·06

included in the table, showed that the rate of production of $^{14}CO_2$ from [*carboxy*-^{14}C]propionate by ox or pigeon liver was not appreciably altered by the presence of an equimolar concentration of acetate.

Effect of propionate in cell-free extracts. The marked difference between the effects of propionate on acetate and pyruvate metabolism in rat-liver homogenates made it appear likely that propionate blocked the formation of acetyl-coenzyme A from acetate. To attempt to confirm this and investigate the nature of the inhibition, the effect of propionate on the acetylation of sulphanilamide by cell-free liver preparations was measured. Such acetylation is usually very feeble in rat liver, and therefore an aged extract of rat-liver acetone-dried powder, together with an acetone-precipitated fraction of pigeon-liver extract containing the amine-acetylating enzyme, but free from acetic thiokinase, was used. The amount of the pigeon-liver fraction used was sufficient to give the maximum acetylating activity obtainable with the quantity of the rat-liver extract present. The

Table 5. *Acylation of sulphanilamide by liver preparations*

Acylation was measured after incubation of the preparations for 2 hr. at 38° in the medium described in the Experimental section. The amounts used were: pigeon-liver extract, 0·25 ml.; rat-liver extract, 0·1 ml.; pigeon-liver fraction (50–60 % acetone fraction of pigeon-liver extract), 0·2 ml.

Expt. no.	Acylating system	Fatty acid added	Sulphanilamide acylated (μmole)
1	Pigeon-liver extract	None	0·102
		Acetate	0·345
		Propionate	0·186
		Butyrate	0·120
2	Rat-liver extract + pigeon-liver fraction	None	0·030
		Acetate	0·178
		Propionate	0·120
3	Rat-liver extract + pigeon-liver fraction	None	0·063
		Acetate	0·276
		Propionate	0·222
		Acetate + propionate	0·276

Table 6. *Effect of propionate on acetylation of sulphanilamide by acetate*

Conditions were as in Table 5. The rat-liver extract and pigeon-liver fraction were used. After incubation and removal of the unused labelled acetate, the acetylsulphanilamide was hydrolysed and the resulting steam-volatile radioactivity was measured.

Fatty acid added	^{14}C recovered (counts/min.)
[*carboxy*-^{14}C]Acetate	145
[*carboxy*-^{14}C]Acetate	27*
[*carboxy*-^{14}C]Acetate + propionate	97
[*carboxy*-^{14}C]Acetate + propionate	15*

* Sulphanilamide was omitted.

acetone fractionation was carried out according to Chou & Lipmann (1952) and the fraction which precipitated at between 50 and 60 % of acetone was used. This fraction, when used alone, did not acetylate sulphanilamide. Initially, some attempts were made to use chicken liver as a source of the amine-acetylating enzyme. A higher acetone concentration (60–75 %) than is effective with pigeon liver was necessary to precipitate the enzyme. However, although the fraction obtained thus was free from acetic thiokinase, the amount of the amine-acetylating enzyme present in chicken liver was only about one-quarter of that found in pigeon liver and the latter was used for the experiments reported. The amine-acetylating enzyme appears to be absent from sheep liver, since sheep-liver slices failed completely to acetylate sulphanilamide.

Table 5 shows that acylation of sulphanilamide by pigeon-liver extract or the combined preparations was obtained in the presence of propionate as well as of acetate. Hence, to determine the effect of propionate on acetylation, it was necessary to distinguish between acylation from acetate and propionate where both are present. To do this, [*carboxy*-^{14}C]acetate and unlabelled propionate were used in the incubation mixture. After incubation, the protein was precipitated with $Ba(OH)_2$ + $ZnSO_4$ and the filtrate evaporated to about 1 ml. Potassium hydrogen phthalate (1 ml., 0·2 M), $MgSO_4,7H_2O$ (about 2 g.), acetic acid (0·25 ml., 2 N) and bromophenol blue were added, the mixture adjusted to pH 3·8–3·9 and rapidly steam-distilled in a Markham still. When at least 95 % of the acetic acid had distilled over (about 75 ml. of distillate) a further 0·25 ml. of acetic acid was added and the distillation repeated. Separate experiments showed that acetylsulphanilamide was hydrolysed to only a slight extent by this procedure. The liquid was removed from the still and H_2SO_4 added to a concentration of approx. N. It was heated under reflux for 1 hr. in boiling water to hydrolyse the acetylsulphanilamide. The liquid was then steam-distilled and the distillate neutralized, evaporated to dryness and its radioactivity measured. The weight of the residue was not large enough to necessitate a correction for self-absorption. Table 6 shows the results obtained. The amount of radioactivity recovered was relatively small when sulphanilamide was omitted from the incubation mixture. It may be seen that the effect of propionate on acetylation, as measured by the recovery of radioactivity, was small compared with the effect of propionate on oxidation of acetate by rat-liver homogenates.

It seemed possible that the marked difference between the effects of propionate on acetate metabolism in homogenates and the soluble systems might be due to the addition of an adequate amount of coenzyme A to the latter. Consequently, the effect of coenzyme A on the oxidation of acetate by rat-liver homogenates in the presence of propionate was studied (Table 7).

It is evident that coenzyme A consistently increased the production of $^{14}CO_2$ from the labelled acetate in the presence of propionate. The effect,

Table 7. *Effect of coenzyme A on inhibited acetate oxidation*

Conditions were as in Table 1, except in Expt. 2, when homogenate from only 50 mg. of tissue was used and the total vol. was 1·5 ml. The concn. of [*carboxy*-^{14}C]acetate was 0·01 M in all flasks.

Expt. no.	Inhibitor (M)	Coenzyme A (mM)	Labelled C (µg. atoms) in CO$_2$	Non-volatile compounds
1	None	0	4·17	2·30
	Propionate (0·01)	0	0·04	0·04
	None	0·05	4·25	2·99
	Propionate (0·01)	0·05	0·29	0·38
	None	0·10	3·91	3·38
	Propionate (0·01)	0·10	0·45	0·64
2	None	0	1·54	.
	Propionate (0·01)	0	0·02	.
	None	0·10	.	.
	Propionate (0·01)	0·10	0·15	.
	None	0·10*	1·20	.
	Propionate (0·01)	0·10*	0·09	.
	Butyrate (0·01)	0	0·36	.
	Butyrate (0·01)	0·10	0·49	.
3	None	0	2·58	0·35
	Propionate (0·01)	0	0·03	0
	None	0·10	2·77	0·56
	Propionate (0·01)	0·10	0·20	0·15
	None	0·20	2·96	0·65
	Propionate (0·01)	0·20	0·15	0·10
	None	0·30	2·36	0·79
	Propionate (0·01)	0·30	0·11	0
4	None	0	4·87	2·17
	Propionate (0·001)	0	0·12	0·15
	Propionate (0·001)	0·10	0·62	0·94

* Additional coenzyme A (0·15 µmole each time) was added after 15, 30 and 45 min.

Table 8. *Effect of propionate on oxidation of acetate by washed mitochondria*

Conditions were as in Table 1. Mitochondria from 400 mg. of rat liver were used in each flask; the concn. of labelled acetate was 0·01 M.

Expt. no.	Addition	Labelled C (µg. atoms) in CO$_2$	Non-volatile compounds
1	None	7·68	3·55
	Propionate (mM)	0·30	0·10
	Propionate (mM) + coenzyme A (0·1 mM)	0·63	1·52
2	None	9·31	3·68
	Propionate (10 mM)	0·07	0·15

however, was small compared with the inhibition produced by propionate and decreased when concentrations of coenzyme A greater than 0·1 mM were used. Coenzyme A had a similar effect on the amount of ^{14}C trapped in the tissue metabolites. In the absence of propionate, coenzyme A had no consistent effect on the production of $^{14}CO_2$ from acetate, although it invariably increased the amount of ^{14}C trapped. [Coenzyme A (0·1 mM) also increased the gas uptake by about 50 %, although this is difficult to interpret since the uptake represents the excess of O_2 consumption over CO_2 production.]

Table 8 shows that the inhibition of acetate metabolism by propionate could be observed also when washed mitochondria were used, and that coenzyme A caused a small increase in the inhibited rate.

DISCUSSION

The conclusion from the previous work (Pennington, 1957) that propionate inhibits the formation of acetyl-coenzyme A from acetate appears to be substantiated by the data in Tables 1 and 2; the quantitative difference between the effects on acetate and pyruvate metabolism were even larger than was found when liver slices were used.

Such an action of propionate is not supported by the relatively small inhibition of acetylation obtained with the soluble preparations. It is, however, not necessarily excluded by this result; two possibilities might be suggested. Inhibition may be caused, not by propionate, but by a metabolite of propionate such as propionyl-coenzyme A, which may occur in much higher concentration at the active enzyme site in the mitochondria than is attained in the soluble system. The relatively slow oxidation of propionate by rat-liver homogenates may result in the accumulation of an intermediate in greater concentration than would occur if the metabolism of propionate proceeded normally. A second possibility is suggested by the work of Aisenberg & Potter (1956), who found that activation of acetate by the enzyme in rat-kidney mitochondria was more sensitive to fluoride than was activation by the enzyme in the supernatant fraction of the cells. It is conceivable that a parallel situation occurs in rat liver, i.e. that the cell contains two acetic thiokinases and the one within the mitochondria is more susceptible to inhibition by propionate. The present results could then be explained if, of the two, only the mitochondrial enzyme were linked with the enzymes of the Krebs cycle. Both of these possibilities should be open to testing.

SUMMARY

1. The production of $^{14}CO_2$ from carboxyl-labelled acetate by rat-liver homogenates was almost completely abolished by propionate in only one-tenth of the concentration of the acetate. Butyrate was a less powerful inhibitor of oxidation of acetate.

2. The oxidation of [2-^{14}C]pyruvate was affected to a much smaller extent by propionate, although the incorporation of isotope into acetoacetate was largely suppressed.

3. Oxidation of acetate was strongly inhibited by propionate in sheep-liver homogenates; in pigeon-liver homogenates the inhibition was much less.

4. The oxidation of propionate by rat-liver homogenates was much slower than its oxidation by sheep- or pigeon-liver homogenates.

5. The acetylation of sulphanilamide by a rat-liver extract, together with an acetone fraction of pigeon liver containing the amine-acetylating enzyme, was inhibited to only a relatively small extent by propionate.

6. The very low rate of oxidation of acetate by rat-liver homogenates in the presence of propionate was increased by the addition of coenzyme A.

REFERENCES

Aisenberg, A. C. & Potter, V. R. (1955). *J. biol. Chem.* **215**, 737.

Aisenberg, A. C. & Potter, V. R. (1956). *J. biol. Chem.* **220**, 831.

Bratton, A. C. & Marshall, E. K., jun. (1939). *J. biol. Chem.* **128**, 537.

Chou, T. C. & Lipmann, F. (1952). *J. biol. Chem.* **196**, 89.

Kaplan, N. O. & Lipmann, F. (1948). *J. biol. Chem.* **174**, 37.

Masoro, E. J., Felts, J. M., Panagos, S. S. & Rapport, D. (1957). *Arch. Biochem. Biophys.* **68**, 270.

Pennington, R. J. (1957). *Biochem. J.* **65**, 534.

Connective Tissue Growth Stimulated by Carrageenin

3. THE NATURE AND AMOUNT OF POLYSACCHARIDE PRODUCED IN NORMAL AND SCORBUTIC GUINEA PIGS AND THE METABOLISM OF A CHONDROITIN SULPHURIC ACID FRACTION*

BY H. G. B. SLACK
Rheumatism Research Centre, University of Manchester

(*Received* 11 *October* 1957)

It is well known that new connective tissue formed during the repair of wounds in scorbutic animals is abnormal histologically and in other respects, e.g. the tensile strength of this repair tissue is much less than that formed when adequate amounts of ascorbic acid are available (Wolbach & Bessey, 1942). Evidence of impaired collagen formation in the scorbutic tissue has been presented, e.g. Robertson & Schwartz (1953), Kodicek & Loewi (1955), Dunphy & Udupa (1955). Abnormalities in the mucopolysaccharides in the ascorbic acid-deficient repair tissue have also been the subject of a number of reports during the past few years, but the exact nature of these changes, qualitative and quantitative, is incomplete and still the subject of some dispute. Persson (1953) found more hexosamine in scorbutic tissues than in normal tissues. Dunphy & Udupa (1955) also refer to increased

* Part 2: Slack (1957).