Repression and Induction of Arylsulphatase Synthesis in *Aerobacter aerogenes*

By T. HARADA AND B. SPENCER

Institute of Scientific and Industrial Research, Osaka University, Japan, and Department of Biochemistry, Trinity College, Dublin, Eire

(Received 31 March 1964)

The arylsulphatase activity of fungi varies with the sulphur source used for growth (Harada & Spencer, 1962). In general, the use of sulphur compounds that are thought to be on the direct pathway of sulphate assimilation to cysteine, namely sulphate, sulphite, thiosulphate, cysteine-*S*-sulphonate and cysteine itself, led to low arylsulphatase activities. It has now been possible to show similar repression of arylsulphatase synthesis by sulphur compounds in a bacterium, *Aerobacter aerogenes*, ATCC 9621.

Many of our findings with *Aerobacter* have been independently reported by Rammler, Grado & Fowler (1964), who arrived at their conclusions by different techniques. The present study therefore confirms the work of these authors; but, in addition, the relationship between the sulphur sources and repression of arylsulphatase has been investigated further and the role of tyramine in the induction of the enzyme has been studied. Tyramine is a potent inducer of arylsulphatase synthesis in *A. aerogenes*, ATCC 9621, when the cells are grown on a chemically defined medium (Harada & Hattori, 1956). The enzyme could only be induced by tyramine of a number of similar compounds tested (Harada, 1957) and the induction was specific for arylsulphatase (Harada, 1963). It has now been shown that tyramine relieves the repression of arylsulphatase caused by various sulphur compounds.

EXPERIMENTAL

Materials. $\text{Na}_2^{35}\text{SO}_4$ (code SJS1, specific radioactivity greater than 100 mc/mg. of S) was obtained from The Radiochemical Centre, Amersham, Bucks. Choline sulphate was synthesized by the method of Schmidt & Wagner (1904). Phenyl sulphate and *p*-nitrophenyl sulphate were prepared by using chlorosulphonic acid in a modification of the method of Burkhardt & Lapworth (1926); cysteine-*S*-sulphonate was kindly supplied by Professor R. Sato; all other compounds were commercial samples.

Culture conditions. *Aerobacter aerogenes*, ATCC 9621, was grown at 34° in shaking culture in 100 ml. of media. Vessels were inoculated with 0.5 ml. of a cell suspension containing 10^7 – 10^8 cells/ml. in sterile water. The chemically defined medium contained (per l.): 10 g. of sodium citrate, 1.5 g. of $(\text{NH}_4)_2\text{HPO}_4$, 1 g. of KH_2PO_4 , 0.5 g. of MgCl_2 ,

6H₂O, 10 mg. of NaCl, 10 mg. of $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 10 mg. of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ and the appropriate amount of sulphur-containing compound. Tyramine (50 mg./100 ml.) was added in specific experiments. Media were adjusted to pH 7.0 before autoclaving. Sulphite, thiosulphate, cysteine-*S*-sulphonate, cysteinesulphinic acid, cysteine, methionine and sulphate esters (apart from choline sulphate, which is stable during sterilization by autoclaving) were Seitz-filtered after adjustment to pH 7.0 and then added to the autoclaved medium.

Preparation of acetone-dried cells. Cells were harvested by centrifugation and washed twice with water. A suspension of the washed cells was added dropwise with stirring to 10 vol. of acetone at 0° and the mixture left at 0° for 1 hr. The acetone was then removed by filtration under vacuum and the precipitate washed well with cold acetone. The cells were finally dried *in vacuo* over P_2O_5 .

Determination of arylsulphatase activity. To a suspension of 10 mg. of acetone-dried cells in 2 ml. of water was added 1 ml. of 0.2M-sodium acetate, pH 7.2, containing NH_4Cl (0.3M). The NH_4^+ ion is known to activate the arylsulphatase of *Aerobacter* (Harada & Kono, 1954). The mixtures were preincubated at 34° for 5 min. before the addition of 1 ml. of 0.01M-potassium *p*-nitrophenyl sulphate. After incubation with shaking at 34° for a period of between 10 min. and 1 hr., according to the activity present, 1 ml. of 0.5N-NaOH was added, the mixture centrifuged and the liberated *p*-nitrophenol in the supernatant measured colorimetrically at 430 m μ with the Hitachi grating spectrophotometer. A standard curve prepared with 0–120 μg . of *p*-nitrophenol/ml. of sample was linear. Control determinations were carried out in which the cell suspension and substrate were incubated separately and combined immediately before the addition of NaOH. A unit of arylsulphatase activity is defined as that amount of enzyme which liberates 1 μg . of *p*-nitrophenol/hr. under the conditions of assay.

Competitive metabolism. The chemically defined medium was supplemented with $\text{Na}_2^{35}\text{SO}_4$ and unlabelled K_2SO_4 to give a final sulphate concentration of 0.25 mM and radioactivities of 3820 counts/min./0.1 ml. of medium when tyramine was absent from the medium and 3750 counts/min./0.1 ml. of medium when tyramine was included. Other sulphur compounds were added at a concentration of 0.25 mM.

The bacteria were grown in 100 ml. of medium at 34° with shaking. At the commencement of the experiment and after 3 days' growth, 5 ml. of the medium was removed aseptically, centrifuged to remove cells and 1 ml. of the supernatant was diluted to 5 ml. with water. A portion (1 ml.) of the diluted solution was dried on a stainless-

steel planchet (5 cm.²) and the radioactivity measured with an end-window gas-flow apparatus (Aloca DC1 type). Corrections were made for dead-time of the instrument, background, self-absorption and decay. After 3 days' incubation the cells were harvested, washed well with water and dried *in vacuo* over P₂O₅. Radioactive cysteine was isolated and its specific radioactivity determined as described by Spencer & Harada (1960).

RESULTS

Arylsulphatase activity of acetone-dried cells. *A. aerogenes* was grown in the presence and absence of tyramine on a chemically defined medium to which were added different sulphur sources at a concentration of 1 mM. The arylsulphatase activities of acetone-dried cells produced on the various culture media are shown in Table 1. In the absence of tyramine, arylsulphatase activity could not be detected in cells grown on sulphate, sulphite, thiosulphate, cysteine-*S*-sulphonate, cysteinesulphinate and cysteine, although all of these sulphur sources gave good growth. However, in the presence of tyramine, appreciable arylsulphatase activity could be detected in cells grown on these media. Methionine, taurine, cystate, choline sulphate and *p*-carboxyphenyl sulphate produced high concentrations of arylsulphatase in the absence of tyramine, but these concentrations were increased when tyramine was present. These results suggest that tyramine reverses the repression of arylsulphatase synthesis caused by certain sulphur-containing compounds.

When the aryl sulphates (phenyl sulphate and *p*-nitrophenyl sulphate) were the sulphur sources

no growth was obtained in the absence of tyramine, but about one-third of the growth occurring on other sulphur compounds was obtained when tyramine was included. A small amount of arylsulphatase accompanied this growth.

No growth occurred in the presence or absence of tyramine when chondroitin sulphate was the sulphur source, and it must be assumed that *A. aerogenes* cannot liberate the sulphur of chondroitin sulphate in a form suitable for assimilation.

By harvesting cells after different periods of growth on media containing sulphate or taurine, each at two different concentrations (1.0 and 0.25 mM), and in the presence and absence of tyramine, it was seen that maximum arylsulphatase activity, expressed on the basis of cellular dry weight, occurred after 3 days. Growth almost stopped after 2–3 days. The growth rate in the presence of tyramine was 17–30 % greater than in the absence of tyramine. The arylsulphatase remained intracellular up to 3 days' growth but thereafter a small part of the activity appeared in the culture medium. No arylsulphatase activity could be detected in the cells or the culture medium when sulphate was used as the sulphur source in the absence of tyramine even after 6 days' growth.

There was a possibility that arylsulphatase synthesis only took place at very low concentrations of sulphate, cysteine or other sulphur sources. The arylsulphatase activity of acetone-dried cells produced on media containing various sulphur sources at different concentrations was therefore tested (Table 2). All the sulphur compounds that led to the development of appreciable arylsulphatase activities showed maximum production of enzyme at concentrations that were clearly more than sufficient for growth (Table 2). Arylsulphatase synthesis would thus seem to be favoured by higher concentrations of the sulphur source than are required for growth purposes since the cell yield is almost independent of sulphur concentrations greater than 0.25 mM. The repressor sulphur sources, sulphate, sulphite, thiosulphate, cysteine-*S*-sulphonate, cysteinesulphinate and cysteine at concentrations (0.0625 mM) that limit growth to about 30 % of the maximum, still cause almost complete repression of arylsulphatase synthesis.

Attempts were made to ascertain the extent of the repression of arylsulphatase synthesis by sulphate, cysteine etc. *Aerobacter* was grown on 1 mM-*taurine* or 1 mM-methionine as the sulphur source with and without the addition of small amounts of potential repressors. As shown in Table 3, low concentrations (0.25 mM) of sulphate, sulphite, thiosulphate, cysteine-*S*-sulphonate, cysteinesulphinate and cysteine completely repressed arylsulphatase synthesis when either *taurine* or *methionine* was the sulphur source. At lower con-

Table 1. *Arylsulphatase activity of Aerobacter aerogenes*

Bacteria were grown in the presence and absence of tyramine (50 mg./100 ml. of medium) on culture media containing different sulphur sources. Experimental details are given in the text.

Sulphur source (1 mM)	Arylsulphatase activity (units/10 mg. of acetone-dried cells)	
	Without tyramine	With tyramine
Na ₂ SO ₄	0	230
Na ₂ SO ₃	0	110
Na ₂ S ₂ O ₃	0	150
Sodium L-cysteine- <i>S</i> -sulphonate	0	270
Sodium L-cysteinesulphinate	0	240
L-Cysteine hydrochloride	0	450
L-Methionine	590	1500
Taurine	930	2100
Sodium L-cystate	80	450
Choline sulphate	270	900
<i>p</i> -Carboxyphenyl sulphate	1700	2300
Phenyl sulphate	No growth	2
<i>p</i> -Nitrophenyl sulphate	No growth	15

centrations the repressors were not completely effective, but this may have been partially due to the utilization of the repressors before maximum growth had been obtained. Thiosulphate was the most effective repressor at the low concentrations. Repression of arylsulphatase synthesis was more complete in the methionine medium than in the taurine medium.

Competitive utilization of sulphur compounds. Bacteria were allowed to grow on a medium containing 0.25 mM- ^{35}S sulphate with and without tyramine and with the addition of various non-labelled sulphur compounds at a concentration of 0.25 mM. The rate of incorporation of ^{35}S sulphate into the cells and the specific radioactivities of the cysteine isolated from the cells were measured (Table 4).

Taurine, cysteate, choline sulphate or phenyl sulphate in the absence or presence of tyramine did not significantly inhibit the uptake of ^{35}S sul-

phate or decrease the specific radioactivity of the isolated cysteine. These sulphur compounds, which are not repressors of arylsulphatase synthesis, are thus hardly utilized for cysteine and methionine synthesis when sulphate is present. In the presence or absence of tyramine, however, sulphite, thiosulphate, cysteine-*S*-sulphonate, cysteinesulphinatate or cysteine, all of which are repressors of arylsulphatase synthesis, caused 50 % or more inhibition of sulphate uptake and decreased the specific radioactivity of the isolated cysteine by a similar amount. These results indicated that this second group of sulphur compounds can act competitively with sulphate in sulphur utilization. On a molar basis, thiosulphate was particularly effective, presumably owing to its ability to enter the sulphate metabolic pathway as its two degradation products sulphite and sulphide. The competitive power of sulphite was not as great as might be expected from its position in the sulphate assimilation pathway

Table 2. *Yields and arylsulphatase activities of Aerobacter aerogenes grown on media containing various sulphur sources at different concentrations*

Bacteria were harvested after 3 days' growth. Experimental details are given in the text.

Concn. of sulphur source (mM)	Growth (mg. of acetone-dried powder/100 ml. of medium)				Arylsulphatase activity (units/10 mg. of acetone-dried powder)			
	1.0	0.25	0.125	0.0625	1.0	0.25	0.125	0.0625
Sulphur source								
K_2SO_4	64	62	41	20	0	0	0	0
Na_2SO_3	60	59	38	20	0	0	0	1
$\text{Na}_2\text{S}_2\text{O}_3$	55	53	54	33	0	0	0	0
Sodium L-cysteine- <i>S</i> -sulphonate	60	55	20	8	0	0	1	12
Sodium L-cysteinesulphinatate	63	54	37	18	0	7	1	0
L-Cysteine hydrochloride	60	55	31	21	0	0	0	2
L-Methionine	58	53	32	14	590	270	110	—
Taurine	72	63	41	32	930	320	190	140
Sodium L-cysteate	24	22	21	17	120	92	81	24
Choline sulphate	56	53	26	14	330	310	6	0
<i>p</i> -Carboxyphenyl sulphate	61	60	—	—	1700	530	—	—

Table 3. *Inhibition of the synthesis of arylsulphatase in Aerobacter aerogenes by various sulphur compounds*

The bacteria were grown on either taurine or methionine (1 mM) as the main sulphur source and other sulphur compounds were added at the concentrations shown. Experimental details are given in the text. The values shown are percentage inhibitions calculated from the arylsulphatase activities of the cells grown on taurine or methionine in the absence of other sulphur compounds.

Concn. of sulphur source (mM) ...	Inhibition of arylsulphatase activity (%)							
	0.25		0.125		0.0625		0.031	
	Taurine medium	Methionine medium	Taurine medium	Methionine medium	Taurine medium	Methionine medium	Taurine medium	Methionine medium
Additional sulphur source								
Na_2SO_4	100	100	40	100	31	82	—	—
Na_2SO_3	100	100	43	100	35	78	—	—
$\text{Na}_2\text{S}_2\text{O}_3$	100	100	100	100	57	100	17	50
Sodium L-cysteine- <i>S</i> -sulphonate	100	100	43	100	10	48	—	—
Sodium L-cysteinesulphinatate	100	100	52	100	18	32	—	—
L-Cysteine hydrochloride	100	100	36	100	31	30	—	—

Table 4. *Competitive utilization of Na₂³⁵SO₄ and other sulphur compounds for cysteine synthesis by Aerobacter aerogenes*

Cells were grown in media containing 0.25 mM-Na₂SO₄ and small amounts of Na₂³⁵SO₄ so that the initial radioactivities of the media were 3820 and 3750 counts/min./0.1 ml. respectively in the presence and absence of added tyramine (50 mg./100 ml.). Unlabelled sulphur compounds (0.25 mM) were incorporated as shown. Experimental details are given in the text. Samples of the media were removed after 3 days' growth and the radioactivity remaining was measured after removal of the cells by centrifugation. In the absence of tyramine the radioactivity remaining in the supplemented medium was 560 counts/min./0.1 ml. and in the presence of tyramine the corresponding value was 570. The radioactivity of the cysteine isolated from the cells was measured as described in the text. The cysteine isolated from cells grown in the unsupplemented medium in the absence of tyramine was 1580 counts/min./mg. and the corresponding value in the presence of tyramine was 1490.

Unlabelled sulphur compound	Percentage inhibition of incorporation of [³⁵ S]sulphate into cells		Percentage incorporation of ³⁵ S into cysteine	
	Without tyramine	With tyramine	Without tyramine	With tyramine
None	—	—	100	100
Na ₂ SO ₃	39	36	52	60
Na ₂ S ₂ O ₃	94	94	19	18
Sodium L-cysteine-S-sulphonate	66	41	36	58
Sodium L-cysteinesulphinat	72	59	49	48
L-Cysteine hydrochloride	78	63	38	45
L-Methionine	53	53	74	86
Taurine	3	1	92	100
Sodium L-cysteate	1	1	88	99
Choline sulphate	0	1	85	97
p-Phenyl sulphate	0	0	98	100

(see Scheme 1), but aeration during shaking culture would considerably decrease sulphite concentrations by oxidation to sulphate (Postgate, 1963). Methionine, which is a non-repressor, inhibited sulphate uptake and decreased the specific radioactivity of the isolated cysteine to an extent midway between that effected by the two groups of sulphur compounds. This suggested that exogenous methionine supplied the methionine requirements of the organism but that cysteine was more readily formed from sulphate than from methionine when both were present in the medium. Tyramine slightly increased the uptake of [³⁵S]sulphate with some sulphur sources, but hardly affected the specific radioactivity of the isolated cysteine.

DISCUSSION

The products of arylsulphatase action are inorganic sulphate and phenols, so that the physiological role of arylsulphatase in micro-organisms may be to provide both a sulphur and a carbon source. *Aerobacter*, for example, can catabolize some phenols such as tyramine and *p*-hydroxybenzoic acid (Harada, 1959), and inorganic sulphate is assimilated to organic sulphur compounds. Arylsulphatase synthesis may therefore be regulated through mechanisms concerned with sulphate assimilation or phenol dissimilation. Previous work with fungi (Harada & Spencer, 1962) sup-

ported the view that there was a connexion between arylsulphatase synthesis and sulphur metabolism, since growth on sulphur compounds such as taurine and cysteate, which are not direct intermediates in the assimilation of sulphate, allowed large amounts of the enzyme to develop in the fungi, whereas sulphate, sulphite, thiosulphate and cysteine, which are thought to be direct intermediates, gave only weak activity or none at all.

The present experiments on the synthesis of arylsulphatase in *A. aerogenes* gave a similar clear division of sulphur sources into repressors and non-repressors. The results, obtained from the 3-day growth experiments at 34°, accord very closely with those derived from the 18 hr. growth experiments at 28° of Rammler *et al.* (1964). Minor deviations between the two studies can probably be attributed to the use of different media and growth conditions. In addition to the sulphur compounds (sulphate and cysteine) tested by Rammler *et al.* (1964), the use of sulphite, thiosulphate, cysteine-S-sulphonate and cysteinesulphinat as the sole sulphur sources in the medium caused complete repression of arylsulphatase synthesis. The non-repressor sulphur compounds were methionine and taurine (which were included in the list of Rammler *et al.* 1964), cysteate, choline sulphate and *p*-carboxyphenyl sulphate. An alternative interpretation of these facts, i.e. that the 'non-repressors'

were actually inducers, can be discounted, since in both studies enzyme synthesis by cultures growing on methionine was also completely repressed by repressor sulphur sources.

Differences between the present work and that of Rammler *et al.* (1964) were seen in the respective attempts to show arylsulphatase synthesis in *Aerobacter* when cultured on growth-limiting concentrations of repressor sulphur source. Lowering the concentration of repressor sulphur source to 0.0625 mM decreased the growth of *A. aerogenes* over 3 days to only 30 % of the normal amount, but arylsulphatase activity was only just detectable (Table 2). In the study of Rammler *et al.* (1964) a technique involving short growth periods (4.5 hr.) allowed the use of lower sulphate concentrations, and reasonably high arylsulphatase concentrations were obtained at growth-limiting sulphate concentrations of 0.015 mM and less.

The major finding in this and a previous study (Harada & Spencer, 1962) was that all the tested intermediates in the sulphur assimilation pathway (Scheme 1) repressed the synthesis of the initial enzyme, i.e. arylsulphatase. The phenomenon was previously explained (Harada & Spencer, 1962) by assuming that all the repressor sulphur sources are metabolized to a common repressor, which could be sulphate. An alternative explanation, for which there is some evidence (Rammler *et al.* 1964), is that the common repressor is cysteine (cf. Ellis & Pasternak, 1962). Cysteine represses the arylsulphatase of fungi (Harada & Spencer, 1962) and *A. aerogenes*. In *Escherichia coli*, cysteine represses sulphite reductase (Mager, 1960) and the synthesis of adenosine 3'-phosphate 5'-sulphatophosphate (Pasternak, 1962). In *Salmonella typhimurium*, cysteine suppresses the enzyme systems that reduce adenosine 3'-phosphate 5'-sulphatophosphate, sulphite and thiosulphate (Dreyfuss & Monty, 1963). All the major reactions (except the last) in the pathway outlined in Scheme 1 are thus known to be repressed by cysteine. Since the loci of the structural genes governing this pathway are not co-ordinated, at least in *S. typhimurium* (Dreyfuss & Monty, 1963) and in fungi, the repression in the pathway would seem to be an example of 'parallel repression' similar to that found with the enzymes of arginine biosynthesis (Gorini, Gunderson & Burger, 1961) rather than the 'sequential co-ordinate repression' suggested by Ames & Garry (1959).

The repression of arylsulphatase by various sulphur sources can thus be seen as a reflection of the ease with which a particular compound can be converted into cysteine. When the rate of cysteine production is greater than its rate of incorporation into protein, cysteine would accumulate and repress the enzymes of the sulphur assimilation pathway (Scheme 1). Where the rate of cysteine production from a particular sulphur source is lower than the potential growth rate then conditions of de-repression will occur. This explanation is the same as that presented by Ellis & Pasternak (1962) for the repression of adenosine 3'-phosphate 5'-sulphatophosphate synthesis in *E. coli* by sulphite, sulphide and thiosulphate.

In support of this explanation in the case of arylsulphatase Rammler *et al.* (1964) note that when the growth rate of *A. aerogenes* is retarded because of sulphur deprivation, compared with its growth rate on sulphate, then arylsulphatase synthesis occurs. The growth rates on non-repressor sulphur sources were lower than those on sulphate. This point was not brought out in the present study since only final amounts of bacteria were considered and over a 3-day growth period similar quantities were produced on both repressor and non-repressor sources. However, the competitive utilization experiments clearly showed that repressor sulphur sources all competed strongly with sulphate in cysteine synthesis (Table 4), suggesting that cysteine is formed from these sources at a faster rate than from sulphate. The rate of cysteine production from the non-repressor sources, which do not compete strongly with sulphate (Table 4), is thus less than that from sulphate, and, as Rammler *et al.* (1964) suggest, probably growth rate-limiting. The competitive-metabolism results with methionine show that this sulphur source is partially successful in competing against sulphate in cysteine synthesis yet it is a non-repressor of arylsulphatase synthesis. If methionine metabolism in *A. aerogenes* is similar to that in *E. coli*, there is no direct conversion of methionine into cysteine (Roberts, Cowie, Abelson, Bolton & Britten, 1957) and the ability of methionine to compete with the [³⁵S]sulphate in cysteine production must be due to unlabelled sulphate formed from the methionine by oxidation processes.

Previous work (Harada & Hattori, 1956; Harada, 1957) had shown that in *Aerobacter* arylsulphatase was formed only when tyramine was added to the

Scheme 1. Sulphur assimilation pathway.

culture medium. It is now realized that arylsulphatase can be produced in the absence of tyramine if a non-repressor sulphur compound is used as the sulphur source, and that the enzyme can also be formed with repressor sulphate sources if tyramine is included. Sulphur sources intermediate in the assimilation of sulphate to cysteine thus repress arylsulphatase synthesis whereas tyramine relieves the repression. The experiments on competitive metabolism with different sulphur sources in the presence and absence of tyramine suggest that the tyramine is an actual inducer and that it does not act by changing the sulphate assimilation pathway. Synthesis of arylsulphatase is produced by adding tyramine to cell suspensions and this synthesis is inhibited by chloramphenicol and puromycin without any lag (T. Harada, unpublished work). Tyramine, which is widely distributed in Nature, is a possible product of arylsulphatase action and can be catabolized by micro-organisms. It is not clear why the induction effect is specific to tyramine since other aryl compounds, e.g. *p*-hydroxybenzoic acid, can be products of arylsulphatase action and can be dissimilated but do not act as inducers.

SUMMARY

1. *Aerobacter aerogenes*, ATCC 9621, synthesized arylsulphatase when grown on media containing methionine, taurine, cysteate or choline sulphate as the sulphur source, but enzyme synthesis was repressed when sulphate, sulphite, thiosulphate, cysteine-*S*-sulphonate or cysteine was present either as the sole sulphur source or in addition to taurine or methionine.

2. The addition of tyramine to the medium relieved the repression of arylsulphatase synthesis.

3. The repressor sulphur sources were utilized for cysteine synthesis in preference to, or simultaneously with, sodium [³⁵S]sulphate supplied at the same time, whereas the non-repressor sulphur sources did not significantly affect the utilization of sulphate. The addition of tyramine to the media did not alter this competitive behaviour.

REFERENCES

- Ames, B. N. & Garry, B. (1959). *Proc. nat. Acad. Sci., Wash.*, **45**, 1453.
 Burkhardt, G. N. & Lapworth, A. (1926). *J. chem. Soc.* p. 437.
 Dreyfuss, I. & Monty, K. J. (1963). *J. biol. Chem.* **238**, 3781.
 Ellis, R. J. & Pasternak, C. A. (1962). *Biochem. J.* **84**, 97p.
 Gorini, L., Gundersen, W. & Burger, M. (1961). *Cold Spr. Harb. Symp. quant. Biol.* **25**, 173.
 Harada, T. (1957). *Bull. agric. chem. Soc., Japan*, **21**, 267.
 Harada, T. (1959). *Bull. agric. chem. Soc., Japan*, **23**, 222.
 Harada, T. (1963). *Mem. Inst. sci. Res. Osaka Univ.* **20**, 111.
 Harada, T. & Hattori, F. (1956). *Bull. agric. chem. Soc., Japan*, **20**, 110.
 Harada, T. & Kono, K. (1954). *J. agric. chem. Soc., Japan*, **28**, 608.
 Harada, T. & Spencer, B. (1962). *Biochem. J.* **82**, 148.
 Mager, J. (1960). *Biochim. biophys. Acta*, **41**, 553.
 Pasternak, C. A. (1962). *Biochem. J.* **85**, 44.
 Postgate, J. R. (1963). *J. gen. Microbiol.* **30**, 481.
 Rammler, D. H., Grado, C. & Fowler, L. R. (1964). *Biochemistry*, **3**, 224.
 Roberts, R. B., Cowie, D. B., Abelson, P. H., Bolton, E. T. & Britten, R. J. (1957). *Publ. Carnegie Instn no. 607: Studies of Biosynthesis in Escherichia coli*, pp. 318-405.
 Schmidt, E. & Wagner, W. (1904). *Ann. Chim.* **337**, 51.
 Spencer, B. & Harada, T. (1960). *Biochem. J.* **77**, 305.

Biochem. J. (1964), **93**, 378

Citrate and the Conversion of Carbohydrate into Fat

A COMPARISON OF CITRATE AND ACETATE INCORPORATION INTO FATTY ACIDS

BY ALICE SPENCER, L. CORMAN AND J. M. LOWENSTEIN

Graduate Department of Biochemistry, Brandeis University, Waltham 54, Mass., U.S.A.

(Received 3 December 1963)

Citrate is incorporated into fatty acids by high-speed supernatant preparations of lactating mammary gland and liver (Kallen & Lowenstein, 1962; Spencer & Lowenstein, 1962; Formica, 1962; Srere & Bhaduri, 1962; Bhaduri & Srere, 1963). In the present paper the rates of citrate and acetate

incorporation by cell-free preparations of liver and lactating mammary gland are compared. The ratio of these rates in liver preparations varies widely, depending on the nutritional state of the animal. Variations in the magnitude of citrate incorporation are much greater than those of acetate