7. None of the amylases studied could degrade potato-starch granules.

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The Metabolism of C_2-Compounds in Micro-organisms

9. ROLE OF THE GLYOXYLATE CYCLE IN PROTOZOAL GLYCONEOGENESIS*

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Although evidence has previously been presented (Hogg, 1959) for the presence of the key enzymes of the glyoxylate cycle (Kornberg & Krebs, 1957; Kornberg & Madsen, 1958) in cells of *Tetrahymena pyriformis*, strain E, which can convert fats into glycogen (Wagner, 1956; Hogg & Wagner, 1956), the presence of isocitrate lyase (EC 4.1.3.1) and malate synthase (EC 4.1.3.2) in cells lacking this ability has brought into question the intracellular role of these enzymes (Hogg, 1959; Reeves, Seaman, Papa & Ajl, 1961).

It is the main purpose of this paper to present results obtained from analysis of *Tetrahymena* cells grown either in synthetic or in proteose–peptone media. Such results show that the intracellular activities of isocitrate lyase and malate synthase are profoundly affected by the composition of the growth media. Moreover, in cells capable of glyconeogenesis from fats, these enzymes were found to be confined to one of the two 'large-granule' fractions obtained after disruption of the cells in a mannitol solution; in cells not capable of glyconeogenesis from fats, the enzymes (though present) were not concentrated at one intracellular locale. These findings indicate that the conversion of fats into glycogen by *Tetrahymena* necessitates both the presence of large amounts of glyoxylate-cycle enzymes and their incorporation into an organized intracellular structure. This work has been presented in part to The Biochemical Society (Hogg & Kornberg, 1961).

* Part 8: Kornberg & Sadler (1961).

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MATERIALS AND METHODS

Growth of cells. The routine procedures for maintenance, growth and harvesting of *T. pyriformis* have been described by Elliott & Hogg (1952) and Wu & Hogg (1952). However, the stock culture medium, which contains (g./l.) proteose–peptone (Difco) 10, sodium acetate 1·0, glucose 1·0 and K_2HPO_4 1·0, was varied by omitting acetate or glucose or both. The minimal synthetic medium (Elliott & Hayes, 1953) was altered by reducing the concentrations of the essential ketogenic amino acids phenylalanine, leucine, isoleucine and lysine to 20, 20, 15 and 25 mg./l. respectively. In each case the altered concentration corresponded to 2·5 times the concentration required to obtain half-

maximal growth in the synthetic medium (Wu & Hogg, 1952). In addition, either glucose or acetate was omitted from this medium for the present use. Growth in such synthetic media required 1–2 weeks and cell densities were not more than one-fifth of those attained in the peptone media.

Fractionation of cells. Freshly harvested cells of *Tetrahymena* were washed twice in dilute Ringer phosphate solution (Ryley, 1952) and then twice in mannitol solution, the concentration of which was 0·25 M for log-phase cells and 0·125 M for stationary-phase cells. Cell concentration was estimated by centrifuging at 1000*g* in a Constable protein tube; the cell suspensions were adjusted to 5% (v/v) for log-phase cells (1–2 days' growth) and 10% (v/v) for stationary-phase cells (4–7 days' growth).

The cell suspension was chilled in ice and passed slowly through a 11 cm. (×3) fritted-glass filter under light suction. Considerable care was taken to prevent foaming. If microscopic examination under phase contrast showed cells to be still present, the filtration step was repeated once to ensure complete breakage. When stationary-phase cells were used, the filtrate was mixed with an equal volume of cold 0·375 M-mannitol, to achieve a final concentration of 0·25 M-mannitol. The filtrate was then centrifuged for 10 min. at 2000*g* in a refrigerated centrifuge.

The supernatant liquid layer (S) was transferred carefully to a fresh centrifuge tube and the original filtrate volume was restored by the cautious addition of cold 0·25 M-mannitol to the sediment. Gentle swirling or stirring with a spatula allowed the removal of a bulky, jelly-like layer of sediment (P 1). The tightly packed pellet remaining (P_n) was resuspended in cold 0·25 M-mannitol by vigorous stirring. Each fraction was centrifuged again at 2000*g* for 10 min., with a resultant separation of a small amount of P 1 fraction from S, and of P_n fraction from P 1; additional centrifuging at 2000*g* gave no further separation. The S fraction, however, would yield a submicroscopic granule fraction when centrifuged at 20 000*g* for 30 min. or more. Ordinarily, each fraction was diluted to the volume of the original filtrate for convenience in comparison of amount of constituents.

Examination of the particulate fractions by phase contrast and, after osmium fixation, by electron microscopy (Plate 1) indicated that P 1 was a uniform suspension of granules in the size range 0·5–1·0 μ. The P_n fraction, however, contained membrane fragments and a variety of granules, the latter being similar in size to P 1 but more diverse in ultrastructure. Both granule fractions gave the reaction characteristic of mitochondria with Janus Green B and the electron photomicrographs showed that many of these granules had the structural details consistent with such identification (Elliott & Bak, 1962; Pitelka, 1961; Roth & Minick, 1961). The structural integrity of these granules was further indicated by the many-fold increase of various enzyme activities that resulted from ultrasonic treatment of the granules (Table 4) (Schneider, 1953).

Where a French pressure cell is available, identical cellular fractions may be obtained after disruption at a pressure of 100–150 lb./in.^{2} With this technique all cells can be disrupted in 0·25 M-mannitol solution.

Extraction of cells. Cell suspensions in Ringer phosphate solution were treated in one of two ways to obtain extracts for enzyme assays.

(*a*) The chilled suspension was exposed for 1 min. to the output of a 600 w Mullard magnetostrictor oscillator, operating at 3 A and 25 kcyc./sec. The ultrasonically treated suspension was centrifuged at 2000*g* in the cold to obtain a clear extract, which ordinarily contained the enzymes. Since, in certain cases, the sediment was found to contain citrate-synthase (EC 4.1.3.7) activity, the sediment was also assayed or else the uncentrifuged, ultrasonically treated suspension was used for assay.

(*b*) The suspension was freeze-dried and the dry powder was extracted by addition of cold water (1 ml./50 mg. of solids) and grinding in a Potter homogenizer. The clear extract obtained after centrifuging in the cold for 15 min. at 10 000*g* was used for enzyme assays. The latter procedure has the advantages that the freeze-dried cell powder preserves enzyme activity for years at low temperature and that all enzymes are solubilized. Both procedures extracted approximately the same fraction of the total cellular protein.

Enzyme assays. Citrate synthase, malate synthase and isocitrate lyase were assayed by the spectrophotometric procedures of Dixon & Kornberg (1959). Succinate dehydrogenase (EC 1.3.99.1) was assayed by the spectrophotometric procedure of Redfearn & Dixon (1961). Malate dehydrogenase (EC 1.1.1.37) and isocitrate dehydrogenase (EC 1.1.1.41) were assayed as described by Kornberg & Madsen (1958), D(−)-3-hydroxybutyrate dehydrogenase (EC 1.1.1.30) according to Williamson, Mellanby & Krebs (1962).

Materials. Acetyl-coenzyme A was prepared from coenzyme A (Boehringer reagent) by the procedure of Stadtman (1957). Oxaloacetic acid was a gift from Professor Sir Hans Krebs, F.R.S. Potassium dihydrogen L_s-isocitrate was generously supplied by Dr H. B. Vickery. Other chemicals used were of analytical-reagent grade.

Analytical methods. The protein content of the various extracts used was determined by the biuret procedure of Weichselbaum (1946). Glucose determinations were made with glucose oxidase (EC 1.1.3.4) according to the procedure of Huggett & Nixon (1957).

Glycogen was separated by treating cold 0·5 N-perchloric acid extracts of fresh cells with 1·1 vol. of 95% ethanol (Stadie, Haugaard & Marsh, 1951). In preparation for counting ^{14}C, the glycogen was reprecipitated once from 0·01 N-NaOH, washed with ethanol and finally redissolved in a volume of 0·1 N-NaOH equal to that of the original cell suspension. A protein fraction was obtained by treating the cold perchloric acid residue successively with hot 95% ethanol, alcohol–ether (3:1), hot 0·5 N-perchloric acid (15 min. at 90°; Volkin & Cohn, 1954) and 95% ethanol. The protein residue was dissolved in a volume of 0·1 N-NaOH equal to that of the original cell suspension. Radioactivity was measured by spreading duplicate samples (0·100 ml.) on 1 in. diam. glass planchets, drying and recording 2560 counts in an automatic Nuclear–Chicago Geiger counter with end window. A sample (0·100 ml.) of the original tracer solution (dil. 1 : 100) was plated and counted under the identical conditions as an internal reference. All counts were corrected to infinite thinness.

RESULTS

Effect of growth medium on glyoxylate-cycle enzymes. In earlier work (Wagner, 1956) the cells of *T. pyriformis*, strain E, which had been grown

Table 1. *Formation of glyoxylate-cycle enzymes in various peptone media*

All cultures were harvested 44 hr. after inoculation with 1% of their volume of a stock culture. Washed cells were ultrasonically disrupted and centrifuged at 2000*g*: the supernatant fluid was used for assays (see the Materials and Methods section) unless otherwise indicated. Assay results, however, are expressed in terms of the total protein content of the uncentrifuged ultrasonically treated preparation. In all glucose media, more than 10% of the original glucose concentration added (1 g./l.) remained at harvest. Only the cells from the medium containing proteose–peptone plus acetate were found to be capable of converting acetate into glycogen, the rate being 0·5 μmole of glucose formed/mg. of soluble protein/hr.

Composition of medium	Sp. activity of enzymes (μmoles of substrate transformed/mg. of soluble protein/hr.) Isocitrate lyase	Malate synthase	Citrate synthase
Proteose–peptone plus glucose and acetate	0·60	0·90	5·0
Proteose–peptone plus acetate	0·85	3·00	23·0
Proteose–peptone plus glucose	0·04	<0·01	12·0*
Proteose–peptone	0·055	3·5	26·0

* The major fraction of the total activity was found in the sediment.

in a medium containing proteose–peptone, glucose and sodium acetate as carbon sources, were found to develop the ability to convert fats into glycogen when their growth rate had declined and when glucose had disappeared from the culture medium. Younger cells were unable to effect glyconeogenesis from lipids: however, such cells were found to contain malate synthase and isocitrate lyase (Hogg, 1959). Since both the growth phase at times of harvesting the cells, and the composition of the media used, had varied in the earlier experiments, a series of simpler peptone media were prepared and cells were grown in them under exactly comparable conditions. All of the cultures grew well in these simpler media and it was concluded that cells harvested near the end of the log phase yielded enzyme patterns (Table 1) that were comparable and were uncomplicated by differences in growth phase.

Both malate synthase and isocitrate lyase were formed in the simple proteose–peptone medium, but the isocitrate-lyase content of the cell was greatly increased when this medium also contained acetate. Malate-synthase formation was repressed completely by glucose but the further addition of acetate partially relieved the repression.

Since it was considered likely that the formation of these enzymes was associated also with the concentrations of ketogenic amino acids in the peptone media, cells were grown in a chemically defined medium that had a growth-restrictive content of such ketogenic amino acids. Analyses of these cells (Table 2) indicated that formation of malate synthase may be elicited in the presence either of fatty acids or ketogenic amino acids, whereas isocitrate lyase is formed in significantly large amounts only when fatty acids are supplied to this organism. It is therefore concluded that the formation of these enzymes in *Tetrahymena*, as in bacteria (Kornberg, 1959; Kornberg, Collins & Bigley, 1960; Kornberg & Elsden, 1961), is influenced by the composition of the nutrient medium. Since glyconeogenesis was observed only in cells containing high activities of both malate synthase and isocitrate lyase, it is further concluded that these enzymes participate in the conversion of fats into carbohydrate in this organism, as has been found with fatty seedlings (Kornberg & Beevers, 1957*a*, *b*).

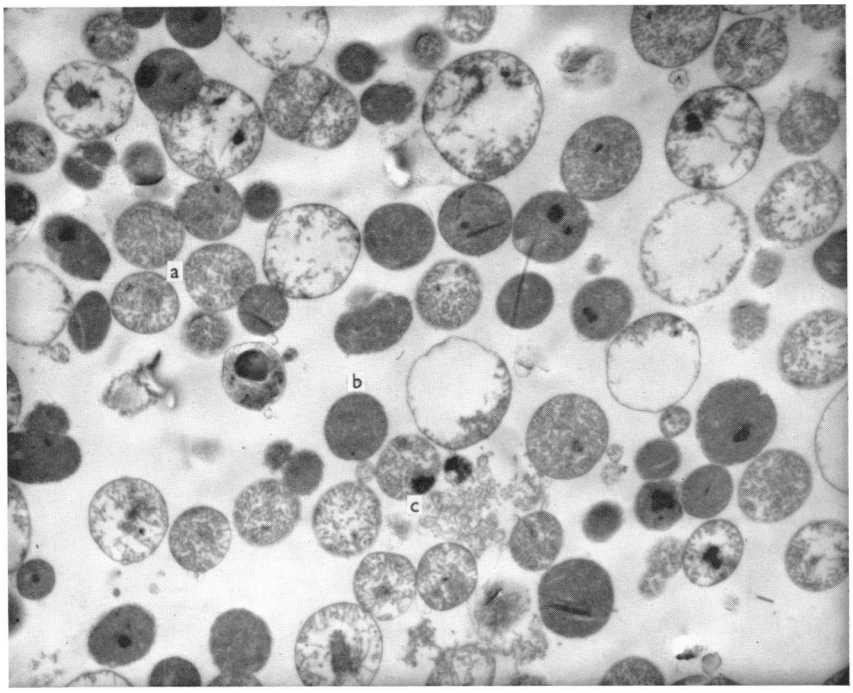
Table 2. *Formation of glyoxylate-cycle enzymes in chemically defined media*

Tetrahymena pyriformis grew in the minimal medium of Elliott & Hayes (1953), in which the concentrations of phenylalanine, leucine, isoleucine and lysine had been reduced to 20, 20, 15 and 25 mg./l. respectively, and to which either glucose (1 g./l.) or acetate (1 g./l.) had been added. Cultures were harvested after 1 week from the medium containing glucose and after 2 weeks from that containing acetate; only cells grown in the latter medium were found to be capable of glyconeogenesis from acetate. No growth occurred in the absence of either glucose or acetate. Cell extracts were prepared as described in Table 1.

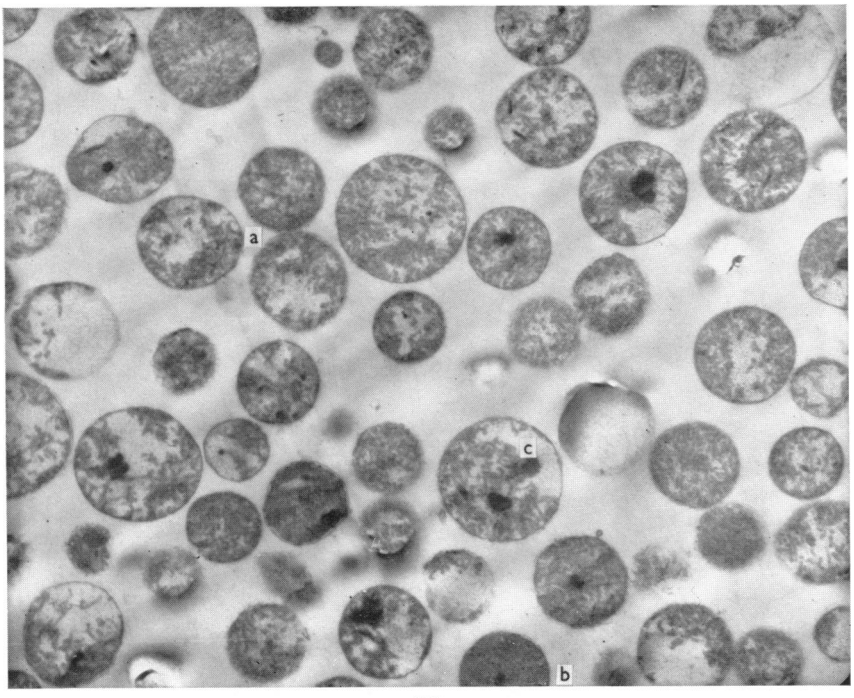
Composition of medium	Sp. activity of enzymes (μmoles of substrate transformed/mg. of soluble protein/hr.) Isocitrate lyase	Malate synthase	Citrate synthase
Minimal plus glucose	<0·01	<0·01	2·0*
Minimal plus acetate	0·75	5·0	2·0

* The major fraction of the total activity was found in the sediment.

Intracellular distribution of glyoxylate-cycle enzymes. The presence, in apparently significant quantities, of both malate synthase and isocitrate lyase in cells lacking the ability to convert acetate into glycogen (Table 1) suggested that not only the



(A)



(B)

Plate 1. Electron photomicrographs of isolated cellular fractions: (A) P_n fraction ($\times 6000$); (B) P 1 fraction ($\times 8000$). The fractions were treated by the procedure of Elliott & Bak (1962). a, 'Normal' mitochondrion; b, 'osmiophilic' mitochondrion; c, mitochondrion with intramitochondrial mass.

Table 3. *Intracellular distribution of glyoxylate-cycle enzymes*

Cells described under (A) were harvested from stationary-phase cultures from which free glucose had disappeared, those under (B) from log-phase cultures. Proteose–peptone medium containing glucose and acetate was used throughout. For the fractionation procedure and enzyme assays used see the Materials and Methods section.

Cell fraction	Average sp. activity of enzymes (μmoles of substrate transformed/mg. of soluble protein/hr.) P_n	P1	S
(A) Cells capable of glyconeogenesis from fats			
Isocitrate lyase	<0·01	3·7	0·4
Malate synthase	<0·01	4·8	<0·01
Citrate synthase	14·7	8·3	<0·01
Succinate dehydrogenase	2·7	0·87	<0·01
Isocitrate dehydrogenase (NADP)	3·9	4·3	6·8
Malate dehydrogenase (NAD)	730	440	40
D(−)-3-Hydroxybutyrate dehydrogenase	2·9	1·3	<0·01
(B) Cells not capable of glyconeogenesis from fats			
Isocitrate lyase	0·69	0·86	0·20
Malate synthase	<0·01	0·48	0·80
Citrate synthase	14·6	4·5	0·66

presence but also the intracellular location of these enzymes might affect the functioning of the glyoxylate cycle in *Tetrahymena*.

Fractionation procedures were therefore developed for determination of the intracellular distribution of these enzymes. Application of these procedures (see the Materials and Methods section) to cells capable of glyconeogenesis allowed the isolation of a large-granule fraction (P1), which contained essentially all of the isocitrate-lyase and malate-synthase activity of the cell (Table 3A), and which also contained other enzymes of the tricarboxylic acid cycle. The other large-granule fraction, P_n, contained no detectable isocitrate-lyase or malate-synthase activities, but was rich in citrate synthase, succinate dehydrogenase, isocitrate dehydrogenase, malate dehydrogenase and D(−)-3-hydroxybutyrate dehydrogenase.

Cells lacking glyconeogenic ability, fractionated by the same procedures, were found to contain the key enzymes of the glyoxylate cycle but differently distributed (Table 3B). Although malate synthase and isocitrate lyase were again present in the P1 granules, isocitrate lyase was also found in the P_n and S fractions, and the major portion of the malate-synthase activity was found in the S fraction. The pattern of distribution of citrate synthase, however, was similar to that observed with cells capable of glyconeogenesis.

These results thus indicate that incorporation of the key enzymes of the glyoxylate cycle into an organized intracellular structure, with apparently mitochondrial properties, is an important factor in their physiological functioning.

That certain of the enzymes measured are indeed an integral part of the granules and not merely adsorbed on them is consistent with the fact that the apparent enzyme activity was increased markedly by ultrasonic treatment of the granules (Table 4). In addition, the incubation of P1 granules, low in malate-synthase activity, with a mannitol solution containing a high activity of malate synthase did not produce any detectable shift of enzyme activity from the soluble to the particulate phase (Table 5). Therefore surface adsorption on to the granules did not appear to be a factor in the intracellular distribution of the enzymes. The lack of malate synthase in P_n granules, especially where it was present in the S fraction (Table 3B), also agrees with this conclusion. Such data cannot be presented for isocitrate lyase and succinate dehydrogenase because the reagents used for their assay disrupt the granules. With isocitrate lyase, however, one-third of the enzyme activity of P1 granules, disrupted by ultrasonic treatment, was

Table 4. *Effect of ultrasonic treatment on apparent enzymic activities of protozoal granules*

P1 granules were prepared as described in the Materials and Methods section. 'Sonic. P1' granules were those granules, in mannitol, exposed to the output of a magnetostrictor oscillator for 30 sec.

Enzyme	Sp. activity of enzymes (μmoles of substrate transformed/mg. of soluble protein/hr.) P1	'Sonic. P1'
Malate synthase	4·8	11·2
Citrate synthase	4·0	10·0
Malate dehydrogenase (NAD)	33*	195*
D(−)-3-Hydroxybutyrate dehydrogenase (NAD)	1·20*	3·45*

* Measured on a separate preparation of granules.

found to be sedimented at 2000*g* and nine-tenths at 20 000*g*: thus this enzyme also appears to be an integral part of the granules. Similar results on malate synthase and isocitrate lyase have been reported for germinating fatty seedlings by Marcus & Velasco (1960).

That the glyoxylate cycle may be spatially separate from the sites responsible for oxidations and the production of carbon dioxide (i.e. the tricarboxylic acid cycle) in *Tetrahymena* was indicated in another manner by the pattern of utilization of L-[2-^{14}C]glutamine in protozoal cells actively converting acetate into glycogen (Table 6). Since the formation of $^{14}CO_2$ from L-[2-^{14}C]glutamine would be expected to necessitate the prior formation of labelled succinate, malate and oxaloacetate, which are also intermediates of the glyoxylate cycle, the differences in isotope distribution observed suggest that the C_4 acids produced from L-[2-^{14}C]glutamine do not equilibrate with the C_4 acids produced from [1-^{14}C]acetate, and hence that compartmentation occurs within the organism.

Table 5. *Lack of adsorption of malate synthase on to protozoal granules*

A protozoal extract containing malate synthase at high specific activity (11·0 μmoles/hr./mg. of protein) was made 0·25 M in mannitol and then mixed with an equal volume of a P1 fraction low in malate synthase (from cells not capable of glyconeogenesis from fats, cf. Table 3B). After 15 min. at ice-bath temperature, the suspension was centrifuged at 2000*g* for 10 min., the supernatant solution removed and the P1 granules, after one wash in 0·25 M-mannitol, were resuspended in mannitol solution to their original concentration. A separate portion of the P1 fraction was carried through the same process with the substitution of 0·25 M-mannitol for the protozoal extract.

Sample	Malate-synthase activity (μmoles/hr./ml.)
Protozoal extract in mannitol	67±7
P1 fraction, untreated, 5% (v/v) granules	11±4
Supernatant solution*	37±4
P1 fraction after treatment with protozoal extract	13±4

* A 1·95-fold dilution of the extract in mannitol.

Table 6. *Utilization of* L-[2-^{14}C]*glutamine during glyconeogenesis*

Portions (2 ml.) of a 2% (v/v) cell suspension in dilute Ringer phosphate solution (Ryley, 1952) containing 1 mg. of sodium acetate/ml. and the specified ^{14}C-labelled compound (2×10^5 counts/min.) were shaken in closed 50 ml. Cavett flasks for 2 hr. at 30°. The incubation was stopped by the addition of 1·0 ml. of 10% perchloric acid. Carbon dioxide was absorbed by 0·5 ml. of 0·1 N-NaOH in the centre cup. A total of 2560 counts was recorded for duplicate plates prepared from samples from each fraction (for details, see the Materials and Methods section).

	Fraction of ^{14}C recovered (%)		
Compound added	In glycogen	In CO_2	In protein
L-[2-^{14}C]Glutamine	0·4	5·7	0·56
Sodium [1-^{14}C]acetate	18·0	46·0	2·8

DISCUSSION

Previous work with bacteria and fungi has shown that the key enzymes of the glyoxylate cycle are formed constitutively in only small amounts, and that the elaboration by the growing cells of larger quantities of isocitrate lyase and malate synthase is associated with the utilization of acetyl-coenzyme A for the net synthesis of cell constituents (for review, see Kornberg & Elsden, 1961). The results here presented show that in *Tetrahymena* also, the intracellular activities of these key enzymes vary widely under different conditions of nutritional environment. Thus malate synthase was formed in high activity by *Tetrahymena* growing in proteose–peptone medium and the rate of formation of this enzyme was not enhanced by the further addition of acetate. Addition of glucose, however, strongly repressed malate-synthase formation: this repression was but partially relieved by the further provision of acetate. Similarly, although cells growing in the minimal medium to which acetate had been added were found to contain malate synthase in high activity, cells growing in the minimal medium to which glucose had been added were virtually devoid of this enzyme.

Unlike malate synthase, isocitrate lyase was formed in only trace amounts by cells growing in proteose–peptone medium. Cells grown in this medium to which glucose had also been added were likewise low in isocitrate-lyase content, but the addition of acetate either in the absence or in the presence of glucose increased the specific activity of this enzyme 15–20-fold. This apparent induction of isocitrate lyase by acetate, even in the presence of a compound known to act as a repressor of the formation of the enzyme, has been previously observed with a mutant of *E. coli* K 12 by Umbarger (1961), with *Achromobacter* sp. by Rosenberger (1962) and with glutamate-grown *Escherichia coli* w by Kornberg (in preparation); the formation of significant quantities of isocitrate lyase by bacteria grown on glucose but in the absence of exogenous acetate has been reported by Umbarger (1961), working with a serine–glycine auxotroph of *E. coli* K 12, and by Shiio, Otsuka & Tsunoda (1959) and Shiio (1960), working with a strain of *Brevibacterium flavum* that released large quantities of glutamate and α-oxoglutarate. In each of these instances it is

likely that the intracellular concentration of a repressor molecule (such as oxaloacetate) normally derived from glucose was lowered either by the addition of acetate or as a consequence of a metabolic lesion that would drain intermediates from the tricarboxylic acid cycle.

Although the mechanism of control of the biosynthesis of glyoxylate-cycle enzymes is as yet unknown, the results here presented show that, in *T. pyriformis*, as in other micro-organisms, isocitrate lyase and malate synthase are inducible. They further indicate that the factors governing their formation operate largely independently of one another and that these two enzymes, though operationally linked in the glyoxylate cycle, are not co-ordinately repressible.

The observation that cells capable of glyconeogenesis contain high activities of both isocitrate lyase and malate synthase indicates that the glyoxylate cycle plays a necessary role in the conversion of fat into carbohydrate by *Tetrahymena* as it does in fatty seedlings (for review, see Stumpf & Bradbeer, 1959; Beevers, 1961). Lower activities of isocitrate lyase and malate synthase were also found in cells that were not capable of glyconeogenesis but, whereas these enzymes were found in only one of the two large-granule fractions when the cells were capable of glyconeogenesis, they were distributed among several fractions in cells not capable of glyconeogenesis. In contrast, citrate synthase showed essentially the same intracellular distribution in each kind of cell, which indicates that the observed alterations in the pattern of intracellular enzyme location were not due to the use of a fractionation procedure inappropriate to one kind of cell.

The finding of two particulate fractions that differ in enzymic composition suggests that *T. pyriformis* contains two types of granule, one being a 'glyoxylate-cycle granule'. The size and fine structure of these granules (Plate 1), their reaction with Janus Green B and their content of citrate synthase and succinate dehydrogenase (Schneider, 1953) further suggests that these large granules may indeed be described as mitochondria: it is not yet established whether the different granules are different types of mitochondria or whether they are different forms of mitochondrion. (The granules cannot be further characterized on the basis of cytochrome-oxidase content as this enzyme appears to be absent from *T. pyriformis*; Ryley, 1952; Eichel, 1956).

These findings are in accord with previous studies of *T. pyriformis* by electron microscopy. Elliott & Bak (1962) described three types of mitochondria in this organism, one being a 'normal' mitochondrion comparable in appearance with the mitochondrion in other organisms, one being more osmiophilic than this particle and one containing an intramitochondrial mass (cf. Roth & Minick, 1961). The photomicrograph of the P_n fraction (Plate 1) shows these various mitochondrial varieties, which are characteristic of ciliates in the stationary phase. The P1 fraction, however, appears to contain only one variety of mitochondrion: both the more osmiophilic variety and that containing the intramitochondrial mass are virtually absent. Although much has yet to be learned of the nature of these granules, the existence of both structural and enzymic differences between the P_n and P_1 fractions appears to be indisputable.

These observations also provide an attractive physical basis for the finding (Table 6) that, in *T. pyriformis* actively converting acetate into glycogen, the glyoxylate cycle is spatially separate from the sites responsible for oxidations and the production of carbon dioxide.

SUMMARY

1. *Tetrahymena pyriformis*, strain E, cells grown in proteose–peptone media contained malate synthase in high activity but were low in isocitrate lyase.

2. The formation of malate synthase was repressed by glucose; this repression was partially relieved by acetate. The formation of isocitrate lyase was stimulated by acetate in the presence or in the absence of glucose.

3. Cells grown in chemically defined media containing acetate contained both isocitrate lyase and malate synthase in high activity; cells grown in such media containing glucose were virtually devoid of both enzymes.

4. These findings show that isocitrate lyase and malate synthase, though operationally linked in the glyoxylate cycle, are not co-ordinately repressible.

5. *Tetrahymena* capable of converting fats into glycogen contained both enzymes in higher activity than those incapable of glyconeogenesis. In the former type of organism, the enzymes were found in only one of the two mitochondrial fractions obtained after disruption of the cells; in the latter type, the enzymes were not localized in any single cellular component.

6. These results indicate that the conversion of fat into glycogen by *Tetrahymena* necessitates both the presence of high activities of glyoxylate-cycle enzymes and their incorporation into an organized intracellular structure.

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A Study of the Composition and Structure of the Cell-Wall Mucopeptide of *Micrococcus lysodeikticus*

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Previous work (Salton, 1953; Perkins & Rogers, 1959) has shown that acid hydrolysates of wall preparations from *Micrococcus lysodeikticus* that have been made by the method of Cummins & Harris (1956) contain glutamic acid, lysine, alanine, glycine, glucosamine, muramic acid (3-*O*-carboxyethylglucosamine) and glucose. These substances make up some 80% of the dry weight of the preparations, or 90% if it is assumed that the amino sugars are *N*-acetylated. It has been claimed in other work that the amino sugars in wall preparations are relatively easily broken down during acid hydrolysis with the liberation of ammonia. Thus uncertainty exists about the true amounts of amino sugars present and about the origin of the ammonia found in hydrolysates of wall preparations from both staphylococci (Strominger, Park & Thompson, 1959) and lactobacilli (Ikawa & Snell, 1960). This question obviously has an important bearing on ideas about the structure of mucopeptides, more particularly in relation to the liberation of ammonia from other amino sugar–peptide sub-

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