citric acid added to deproteinized serum filtrate has been studied, and the results of a series of three consecutive determinations are given in Table 2.

SUMMARY

A modified colorimetric procedure is described for the estimation of 10–80 μ g. of citrate in biological fluids. The final colour is developed in a 4% thiourea–2% borax solution containing 0.2% sodium sulphide.

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Studies in Carotenogenesis

15. THE ROLE OF CARBOXYLIC ACIDS IN THE BIOSYNTHESIS OF β -CAROTENE BY *PHYCOMYCES BLAKESLEEANUS**

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Schopfer & Grob (1950) found that a medium containing lactate as the sole carbon source supported only meagre growth of *Phycomyces blakesleeanus* which synthesized no β -carotene. Addition of acetate to this medium stimulated growth and initiated carotenogenesis; pyruvate, butyrate, palmitate, tartrate and citrate behaved similarly to acetate whilst glycerol, acetone and an unspecified ester of β -methylcrotonic acid were ineffective. Later Schopfer & Grob (1952) demonstrated that lactate was not necessary and that growth and carotenogenesis occurred when acetate was the only carbon source.

Grob, Poretti, von Muralt & Schopfer (1951) and Grob, Schopfer & Poretti (1952), using labelled acetate, found that the methyl and carboxyl carbon atoms were incorporated equally into the β -carotene molecule and, recently, Grob & Bütler (1954*a, b*) found that the lateral methyls of β -carotene arise from the methyl of acetate and the carbon atoms adjoining these methyl groups from the carboxyl of acetate. Glover, Goodwin & Lijinsky (1951), using methyl-labelled acetate, could not demonstrate specific incorporation of acetate into β -carotene either in growing cells of *P. blakesleeanus* in the presence of glucose or in vegetative mats placed on a medium containing only acetate and phosphate buffer.

Because of the importance of acetate as the possible primary precursor of β -carotene, further investigations into the role of this and other carboxylic acids in carotenogenesis in *P. blakes-*

leeanus have been undertaken. Some of the results presented here have already been briefly recorded (Friend, Goodwin & Griffiths, 1954).

EXPERIMENTAL

Cultures. *Phycomyces blakesleeanus* (–strain), obtained from the Centraalbureau voor Schimmelcultures, Baarn, was used throughout. Cultures were grown either in 15 ml. of medium in 8 oz. medicine bottles (Garton, Goodwin & Lijinsky, 1951) or in 100 ml. of medium in penicillin flasks. The latter were used with media not containing glucose, because of the smaller growth obtained. Incubation was carried out at 25° in a glass incubator (Garton *et al.* 1951).

The glucose media were those described by Garton *et al.* (1951). Five non-glucose media were used: (A) containing per 100 ml.: ammonium lactate 0.2 g.; magnesium lactate, 3H₂O, 0.2 g.; KH₂PO₄, 0.15 g.; MgSO₄·7H₂O, 0.05 g.; MnSO₄, 0.01 g. and thiamine 2 μ g. (Grob *et al.* 1951); (B) medium A + 1.0% (w/v) sodium acetate anhyd.; (C) medium A with ammonium lactate and magnesium lactate replaced with equivalent (carbon) amounts of the corresponding acetates; (D) medium B with ammonium lactate replaced by 0.12% (w/v) NH₄NO₃ and (E) medium A + 1% (w/v) glucose.

Dry weight, lipid and carotenoid determinations. With mycelia grown on glucose media, these determinations were carried out by the methods of Garton *et al.* (1951). On non-glucose media the pH increases during growth of the mycelium from 6.5–7.0 to about 8.5. This results in a considerable amount of precipitated inorganic phosphate accompanying the mycelium when it is filtered from the medium. In order to free the mycelia from this precipitate they are washed with about 5–10 ml. of 2N-HCl. Table 1 shows that washing reduces the dry weight very considerably but this is not surprising from visual examination of the crucibles in which can be seen large crystals. Washing

* Part 14: Goodwin, Land & Osman (1955).

with 1 ml. of acid (which facilitates transfer of the last traces of medium) is insufficient to remove all the inorganic material (Table 1), but this is achieved with a further 3 ml. The fact that further washing does not alter the dry wt. indicated that little or no cellular material is removed. This cannot be confirmed by nitrogen determinations because the inorganic precipitate contains nitrogen, but it is confirmed by the fact that mycelia grown on glucose media (in which there is no precipitation) do not lose weight after being washed in a similar manner. Routinely 5–10 ml. 2N-HCl are therefore used for washing non-glucose mats. Similar results are obtained if acetic acid is used instead of HCl or if the pH of the medium is taken to 1–2 before filtration.

Lipid and carotenoid determinations on the non-glucose media were carried out in the usual way.

Transfer experiments. Vegetative mats were prepared and examined according to the method of Goodwin & Willmer (1952).

RESULTS

Table 2 gives the results of one experiment showing the effect of various non-glucose media on carotenogenesis in *P. blakesleeanus*. Many other

Table 1. *The effect of acid washing on the dry weight of P. blakesleeanus mats*

Dry wt. determinations according to Garton *et al.* (1951) before and after washing with 1, 3 and 3 ml. portions of 2N-HCl; amounts produced on 100 ml. medium *C* (acetate) (with or without the addition of 1.0% (w/v) sodium acetate anhyd.) or on 15 ml. standard glucose-asparagine medium (Garton *et al.* 1951).

Medium	Before washing	Total dry wt. (mg.)		
		After washing		
		1st	2nd	3rd
Acetate	192	—	44.0	44.3
	—	97.8	58.3	58.0
	—	85.9	43.9	43.0
	—	113.5	76.0	75.6
	—	110.2	71.9	72.1
	209.5	—	—	47.4*
Glucose	149.2	—	—	147.4*
	127.3	—	—	126.7*
	142.9	—	—	141.5*

* These mycelia were washed once with 5 ml. 2N-HCl.

experiments have been carried out using these media, and although growth and carotenogenesis vary from experiment to experiment somewhat more than on glucose media (Garton *et al.* 1951), the general picture has always been the same. (The relevant columns in Tables 3–7 give further indications of the degree of variability on these media.) The observations of Schopfer and his group that (i) lactate is a poor supporter of growth and carotenogenesis, (ii) addition of acetate to the lactate medium stimulates both growth and pigment production, and (iii) acetate is equally effective in the absence of lactate, have been confirmed. It has not been possible to confirm the observations (Schopfer & Grob, 1952) that substitution of NH_4NO_3 for ammonium lactate or ammonium acetate specifically stimulates carotenogenesis or that no β -carotene is formed on the medium containing lactate only. We found that β -carotene is always produced on this medium, but in relatively smaller amounts than on the acetate media; occasionally, in experiments not quoted, the concentration of β -carotene on the lactate medium approached that on the acetate medium. Table 2 also shows that although acetate is superior to lactate as a carbon source for growth, it is much inferior to glucose. Pyruvate was found to support only the same degree of growth and carotenogenesis as did lactate.

The possibility that poor growth on the lactate-only medium was due to the small amount of carbon present was ruled out when it was found that incorporation of additional sodium lactate (1%, w/v) into this medium had no stimulatory effect on growth or carotenogenesis. The effect of increasing the acetate concentration of medium *C* was investigated in detail. Increasing the acetate level above 0.325% (w/v) did not stimulate growth. Concentrations above 1.12% (w/v), corresponding to the addition of 1% (w/v) sodium acetate anhyd., were inhibitory (Table 3). β -Carotene synthesis, however, increases up to an acetate concentration of 1.12% (w/v), but thereafter declines.

Table 2. *Dry weight lipid and β -carotene production by Phycomyces blakesleeanus cultured on various media*

7-day cultures; amounts produced in penicillin flasks containing 100 ml. medium; temp. 25°; mats from two flasks bulked for each analysis; for details concerning the composition of media see text.

Medium	Total dry wt. (mg.)	Lipid (mg.)	% lipid	β -Carotene amount ($\mu\text{g.}$)	Concn. (p.p.m.)
Lactate only (<i>A</i>)	10.5	1.2*	11.4	1.4	121
Lactate + acetate (<i>B</i>)	67.5	17.6	26.1	56.2	833
Acetate only (<i>C</i>)	50.3	15.5	30.8	37.0	736
Acetate + NH_4NO_3 (<i>D</i>)	55.1	16.2	29.4	34.5	626
Lactate + 1% (w/v) glucose (<i>E</i>)	584.0	—	—	217.5	370

* Value too small to be reliable.

Table 3. *The effect of varying the acetate concentration on growth, lipogenesis and carotenogenesis in Phycomyces blakesleeanus*

Basal medium C (0.325%, w/v, acetate) + varying amounts of sodium acetate anhyd.; amounts produced on 100 ml. of medium in penicillin flasks; 7-day cultures; temp. 25°; mats from two flasks bulked for each analysis.

Total 'acetate' present (%, w/v)	Total dry wt. (mg.)	Lipid (mg.)	% lipid	β -Carotene	
				Amount (μ g.)	Concn. (p.p.m.)
0.325 (basal medium C)	43.5	10.7	24.6	15.9	411
0.722	46.6	13.2	28.4	29.4	632
1.120	46.5	14.5	31.2	28.6	621
1.915	25.6	5.3	20.7	13.7	536
2.710	18.2	2.1	11.6	5.4	297

Table 4. *Effect of yeast on growth, lipogenesis and carotenogenesis in Phycomyces blakesleeanus*

With non-glucose media amounts produced in penicillin flasks containing 100 ml. of medium. With glucose media, amounts produced in 8 oz. medicine bottles containing 15 ml. medium; temp. 25°; 7-day cultures. Mycelia from two flasks or three bottles bulked for each analysis. In experiments with yeast extract Oxoid yeast extract was used in all cases except *b*, where Difco yeast extract was used.

Medium	Total dry wt. (mg.)	Lipid		β -Carotene	
		Amount (mg.)	Concn. (%)	Amount (μ g.)	Concn. (p.p.m.)
		Without yeast extract			
Lactate + acetate (<i>B</i>)	72.6	19.8	27.3	45.6	628
Basal acetate (<i>C</i>)	56.9	15.8	27.8	23.5	413
(<i>C</i>) + 1% (w/v) Na acetate anhyd.	49.9	15.6	31.3	31.2	625
Acetate + NH ₄ NO ₃ (<i>D</i>)	55.1	16.2	29.4	34.4	626
Glucose 1.0% (w/v) + asparagine, 0.2% (w/v) (Garton <i>et al.</i> 1951)	55.5	11.2	20.2	15.6	285
With yeast extract (0.1%, w/v)					
Lactate + acetate (<i>B</i>)	145.0	22.9	15.8	64.1	442
Basal acetate (<i>C</i>)	67.4	17.2	25.5	52.5	780
(<i>C</i>) + 1% (w/v) Na acetate anhyd.	144.3	43.0	29.8	121.4	841
Acetate + NH ₄ NO ₃ (<i>D</i>)	81.2	25.9	31.9	68.5	844
Glucose 1.0% (w/v) + asparagine, 0.2% (w/v) (Garton <i>et al.</i> 1951)	(a) 54.7 (b) 55.8	8.6 6.6	15.7 11.8	11.7 12.2	213 219

Effect of yeast extract on growth and carotenogenesis

As relatively poor growth on acetate media might have been due to the absence of a factor necessary for optimum acetate utilization, the effect of the addition of yeast extract (Oxoid, Difco and Marmite) was examined. The results of an experiment recorded in Table 4 show that yeast extract stimulates growth and β -carotene synthesis on all non-glucose media examined, but has no effect on media containing glucose. Except on the lactate-acetate medium, carotenogenesis is always stimulated to a greater extent than growth. On the acetate-lactate media, β -carotene stimulation is relatively slight and less than growth stimulation. The reason for this anomaly, which is always reproducible, is not apparent. Although addition of yeast extract does not give growth comparable with that on a similar volume of glucose medium, the

concentration of β -carotene obtained on 'yeast mycelia' approaches the maximal values recorded for glucose mycelia, viz. 800–1000 p.p.m. dry wt.

Lipogenesis was stimulated by yeast extract to the same degree as growth. The maximal effect on both growth and carotenogenesis is obtained with 0.05–0.10% (w/v) yeast extract (Table 5); stimulation occurs to the same extent throughout the growing period (Fig. 1) and is also apparent at levels of acetate ranging from 0.325 to 2.17% (w/v) (Table 6) (for comparable values in the absence of yeast extract see Table 3).

Although not a major aspect of this investigation, attempts were made to simulate the effect of yeast extract with pure vitamins; none of the following at the concentrations stated (μ g./100 ml.) was effective: riboflavin (50), nicotinamide (300), biotin (50), pyridoxal phosphate (50), inositol (50), choline chloride (440) Ca D-pantothenate (100), folic acid (50), lipoic acid (natural and synthetic,

25) and citrovorum factor (20); lipoic acid was the gift of J. Bibby and Son Ltd. and citrovorum factor of the Lederle Laboratories. Unfortunately coenzyme A, the most likely factor, was not available for examination. The possibility that small traces of glucose in the yeast extract might be the stimulating factor was ruled out by experiments in which it was demonstrated that, even added at a level of 0.1% (w/v), glucose had no effect on growth or carotenogenesis on medium C in the absence of yeast extract. A further possibility, that the presence of carotenogenic amino acids in traces could account for the stimulation of carotene synthesis, and possibly growth, by yeast extract was excluded by experiments (recorded in full in the next section) which showed that the yeast extract effect also occurs in the presence of excess of these amino acids. It was also possible that the normal limited growth on medium C in the absence of yeast extract was due to the presence of sufficient of the unknown factor in the spore suspension normally used for inoculation (Garton *et al.* 1951); the same degree of growth was, however, obtained when the acetate media (without yeast extract added) were inoculated with a loop drawn across the sporangia of a stock culture.

Fig. 1. Dry wt., lipid, and β -carotene production by *P. blakesleeana* in an acetate medium with and without addition of yeast extract; medium C+1% (w/v) Na acetate anhyd., corresponding to 1.12% (w/v) total acetate; yeast extract, 0.1% (w/v). Amounts produced in 100 ml. medium in penicillin flasks. Mats from two flasks analysed for each value; dry wt., without yeast $\bigcirc$ --- $\bigcirc$, with yeast $\bullet$ --- $\bullet$; lipid, without yeast $\bigcirc$ --- $\bigcirc$, with yeast $\bullet$ --- $\bullet$; β -carotene, without yeast $\bigcirc$ --- $\bigcirc$, with yeast $\bullet$ --- $\bullet$.

Table 5. *The effect of varying the concentration of yeast extract on growth, lipogenesis and carotenogenesis in Phycomyces blakesleeana*

Amounts produced on 100 ml. medium in penicillin flasks; 7-day cultures: temp. 25°; medium C+1% (w/v) Na acetate anhyd.; mats from two flasks combined for each analysis.

Amount of yeast extract added (%, w/v)	Total dry wt. (mg.)	Lipid		β -Carotene	
		Amount (mg.)	Concn. (%)	Amount (μ g.)	Concn. (p.p.m.)
0	60.1	14.6	24.3	28.9	481
0.01	71.2	15.5	21.8	34.8	489
0.02	92.0	21.2	23.1	45.8	498
0.05	105.0	24.0	22.9	86.0	820
0.10	144.3	43.0	29.8	121.4	841
0.20	122.4	20.1	16.4	80.0	719

Table 6. *The effect of yeast extract on growth, lipogenesis and carotenogenesis in Phycomyces blakesleeana on media containing various concentrations of acetate*

Amounts produced per 100 ml. medium C in penicillin flasks; temp. 25°; 7-day cultures: mats from two flasks bulked for analysis: yeast concn. 0.1% (w/v).

Amount of acetate present (%, w/v)	Total dry wt. (mg.)	Lipid		β -Carotene	
		Amount (mg.)	Concn. (%)	Amount (μ g.)	Concn. (p.p.m.)
0.325	67.4	17.2	25.5	52.5	780
0.722	107.4	31.9	29.7	100.0	931
1.120	126.0	33.6	26.7	99.0	786
1.915	101.1	15.4	15.2	67.6	663
2.710	62.7	7.5	12.0	43.8	700

*Effect of alteration of nitrogen source
in acetate media*

The effect of replacing ammonium acetate in medium *C* by asparagine [the source of nitrogen in the standard glucose medium (Garton *et al.* 1951), glycine or asparagine plus leucine has been investigated. On the glucose medium, glycine (slightly) and leucine and valine (considerably) are the only amino acids which stimulate carotenogenesis; all the others either have no effect or do not support growth (Garton *et al.* 1951; Goodwin & Lijinsky, 1952). Valine was not examined here because of the similarity of its effect to that of leucine; leucine plus asparagine was examined in preference to leucine alone because on leucine alone growth is very slow, whilst with a mixture of the two amino acids growth rate is normal, although the carotenogenic effect of leucine is still observed. Table 7 shows that none of the amino acids is as efficient as NH_4NO_3 from the point of view of carotene synthesis, although the superiority of leucine over asparagine is again apparent. Yeast extract was again stimulatory in all cases.

the presence of β -ionone was 124 $\mu\text{g.}$ compared with 40 $\mu\text{g.}$ produced in its absence.

Diphenylamine inhibited β -carotene synthesis and stimulated the synthesis of the more saturated polyenes (phytofluene, etc.) without affecting growth of *P. blakesleeanus* (Goodwin, 1952a). It has now been shown to have the same effects on *P. blakesleeanus* grown on the acetate medium *C*.

*Effect of di- and tri-carboxylic acids on growth
and carotenogenesis in acetate media*

Leonian & Lilly (1940) noted that the addition of various carboxylic acids (0.02M) to a glucose- NH_4NO_3 medium greatly stimulated growth of *P. blakesleeanus*. These results have now been confirmed, but it was found that acetate and succinate always stimulated carotenogenesis to a greater extent than the other acids. This now appears due to differences in the final pH of the media. Table 8 shows that the higher the final pH the less β -carotene produced, that of the media containing 0.02M succinate or acetate being lowest. Reduction of the concentration of fumarate by one-half increases carotene production, because the final pH

Table 7. *The effect of different nitrogen sources on growth, lipogenesis and carotenogenesis in Phycomyces blakesleeanus*

Amount produced per 100 ml. medium in penicillin flasks; temp. 25°; 7-day cultures; mats from two flasks bulked for each analysis. Medium *C* + 1.0% (w/v) Na acetate anhyd. (corresponding to a total content of 1.12%, w/v, acetate) with varying nitrogen sources 0.2% (w/v). Yeast concn. 0.1% (w/v).

Nitrogen source	Total dry wt. (mg.)	Lipid		β -Carotene	
		Amount (mg.)	Concn. (%)	Amount (μ g.)	Concn. (p.p.m.)
		Without yeast			
Ammonium acetate	57.0	14.9	26.2	32.7	573
L-Asparagine	49.6	10.7	21.6	8.7	176
Glycine	41.6	12.0	28.9	15.8	380
L-Leucine (0.1%, w/v) + L-asparagine (0.1%, w/v)	59.9	18.6	31.1	23.7	396
With yeast					
Ammonium acetate	117.7	30.2	25.7	111.0	943
L-Asparagine	91.6	19.4	21.2	45.8	500
Glycine	76.6	19.4	25.4	44.8	585
L-Leucine (0.1%, w/v) + L-asparagine (0.1%, w/v)	122.7	26.9	21.9	77.5	632

*The effect of β -ionone and diphenylamine on
carotenogenesis in acetate media*

Mackinney, Nakayama, Buss & Chichester (1952) showed that the addition of small quantities of β -ionone (about 10 $\mu\text{l.}/100\text{ ml.}$) to the standard (glucose/asparagine) medium greatly stimulated carotenogenesis in *P. blakesleeanus*. The same effect has now been observed on the acetate medium *C*, with or without the addition of yeast extract. Stimulations of 200–300% are regularly obtained; e.g. in one experiment the mean value per flask in

is lower than with the higher concentration. When the concentration of citrate is reduced, however, no change in pH results and there is also no change in β -carotene synthesis. The reduced synthesis using 0.005M acetate is due to the fact that this concentration is insufficient to produce maximal growth stimulation; the concentration of the β -carotene in the mat remains at its high level.

The final pH has no obvious effect on the amount of growth of the organism but has a profound effect on its appearance. At low pH values, the mats are bright orange with little or no production

of sporangia. At the higher pH values, the mats are greenish grey owing to the synthesis of large amounts of an alkali-soluble green pigment, and sporangia production is considerable. These mats are very similar in appearance to those produced on agar slants, where the pH is maintained around 6.

The above observations agree with a previous investigation (Goodwin & Willmer, 1952) in which it was found that when the standard glucose-asparagine medium was buffered so that the final pH could not drop to 3.2 or below, carotenogenesis, but not growth, was considerably inhibited.

slight during growth, reaching its maximal value only after completion of growth (Goodwin & Willmer, 1952); (ii) although optimum synthesis occurs at about the same concentration of acetate and glucose (2.0%), increasing the concentration of acetate above this level reduces synthesis, whilst concentrations of glucose up to 10% (w/v) have no deleterious effect (Garton *et al.* 1951).

When relative yields on the two media are compared, 100 μ g. of β -carotene are produced on the average from 0.75 g. of acetate or 0.35 g. of glucose; this is assuming that all the carotene arises from

Table 8. *The effect of the addition of carboxylic acids to a glucose-NH₄NO₃ medium on growth and carotenogenesis by Phycomyces blakesleeanus*

Standard glucose-asparagine medium (Garton *et al.* 1951) with NH₄NO₃ (0.25%, w/v) replacing asparagine. Amounts produced in 8 oz. medicine bottles containing 15 ml. medium; mats from three bottles bulked for each analysis: 7-day cultures at 25°; all media originally at pH 5.5.

Acid added (as Na salt)	Concentration (M)	Total dry wt. (mg.)	β -Carotene		Final pH of medium
			Amount (μ g.)	Concn. (p.p.m.)	
—	Control	14.6	14.2	973	2.70
Acetate	0.005	39.0	57.6	1476	2.75
	0.01	69.0	73.3	1062	2.70
	0.02	98.6	82	832	2.78
	0.04	104.5	18	17	7.0
Succinate	0.02	85.4	109	1276	2.75
Citrate	0.01	96.1	57	593	3.65
	0.02	96.8	49.5	511	3.75
α -Oxoglutarate	0.02	93.8	52	554	3.70
Fumarate	0.01	75.2	54	718	3.85
	0.02	93.6	25	266	4.80
DL-Malate	0.04	119.4	31	259	5.50

No stimulatory effect on either growth or carotenogenesis was obtained when the carboxylic acids (0.02M) were added either to the acetate medium *C* or to the standard glucose/asparagine medium. Tested at higher concentrations (1%, w/v) in medium *C* all the compounds of the tri-carboxylic acid cycle, except citrate, produced some inhibition of growth and carotenogenesis (about 40%); citrate at this level completely inhibited growth. Furthermore, complete replacement of the glucose in the standard medium by any of these acids (except acetate) resulted in failure of the mould to germinate, and numerous attempts to demonstrate a carotenogenic action of these acids in vegetative mats were always unsuccessful.

DISCUSSION

β -Carotene synthesis by *P. blakesleeanus* on acetate media differs from that on the standard glucose medium in two main ways: (i) carotene synthesis closely parallels growth on the acetate medium (Fig. 1), whilst on the glucose medium it is

glucose on the standard (glucose-asparagine) medium, an assumption which may not be correct (Chichester, Wong & Mackinney, 1954).

The present results indicate that the optimal medium for incorporation of acetate into β -carotene is medium *C* plus 0.5% (w/v) Na acetate (anhyd.) (corresponding to a content of 0.722%, w/v, total acetate) and plus 0.1% (w/v) yeast extract, the efficiency of conversion being of the order of 0.05% (on a carbon basis). This value could be perhaps increased to about 0.15% in the presence of β -ionone, assuming the action of this substance is indirect and not due to its incorporation into the β -carotene molecule. Its effect is certainly indirect on a glucose medium in so far as it is not incorporated *in toto*, for neither [6-¹⁴C]- nor [9-¹⁴C]- β -ionone (using the carotene numbering scheme (Goodwin, 1952b)) is incorporated into β -carotene (Mackinney, Nakayama, Chichester & Buss, 1953; Engel, Würsch & Zimmermann, 1953). In either case, the calculation serves to emphasize one of the difficulties of investigations on carotene synthesis, for even in *P. blakesleeanus*, considered

a good pigment producer, carotene synthesis represents only a very minor part of the total metabolic activity of the organism.

Although members of the tricarboxylic acid cycle will stimulate growth and carotenogenesis on a glucose-NH₄NO₃ medium, it is not possible from these experiments to distinguish a specific role of this cycle in the biosynthesis of β -carotene, as it is considered to play in *Sarcina lutea* (Arnaki & Stry, 1952). The different levels of β -carotene synthesis obtained in the presence of equimolar quantities of the various members of the cycle is almost certainly due to the different final pH of the medium in each case. The reason for the stimulation of growth in these experiments is not exactly known. Foster (1949) suggests that the carboxylic acid is metabolized to the corresponding keto acid, which then undergoes amination, thus stimulating the utilization of nitrogen. Whatever the mechanism is, it cannot be direct because the growth increment is much greater than the amount of tricarboxylic acid added.

SUMMARY

1. *P. blakesleeanus* cultured on a medium containing acetate as the sole carbon source grows better and produces more β -carotene than on media containing lactate or pyruvate as the sole carbon source.

2. In acetate media, yeast extract (0.05–0.1%, w/v) stimulates both growth and carotenogenesis. Similar results are obtained with lactate media, although the stimulation of carotene synthesis is not so great. Yeast extract has no effect on growth and carotenogenesis in media containing glucose.

3. Substitution of ammonium acetate by ammonium nitrate in the acetate medium does not stimulate carotene synthesis, whilst substitution of asparagine reduces synthesis. The carotenogenic effect of leucine which occurs on glucose media is also observed on acetate media.

4. Addition of either acetate or any member of the tricarboxylic acid cycle (0.02M) to a medium containing glucose and ammonium nitrate stimulates growth equally in all cases, but β -carotene

synthesis to varying extents. This is mainly due to the variation in the final pH values of the media rather than to any specific carotenogenic activity of a single acid.

5. Addition of β -ionone or diphenylamine to an all-acetate medium stimulates and inhibits carotenogenesis respectively in the same manner as on glucose media.

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