- Goodwin, T. W. (1952*b*). *The Comparative Biochemistry of the Carotenoids*, p. 163. London: Chapman and Hall Ltd.
- Goodwin, T. W. (1953). *Biochem. J.* **53**, 538.
- Goodwin, T. W. (1954). *Biochem. J.* **58**, 90.
- Goodwin, T. W. & Jamikorn, M. (1956). *Biochem. J.* **62**, 275.
- Goodwin, T. W., Jamikorn, M. & Willmer, J. S. (1953). *Biochem. J.* **53**, 531.
- Goodwin, T. W. & Morton, R. A. (1946). *Analyst*, **71**, 15.
- Goodwin, T. W. & Osman, H. G. (1953). *Arch. Biochem. Biophys.* **47**, 215.
- Goodwin, T. W. & Osman, H. G. (1954). *Biochem. J.* **56**, 222.
- Goodwin, T. W. & Srisukh, S. (1949). *Biochem. J.* **45**, 268.
- Grundmann, C. & Takeda, Y. (1937). *Naturwissenschaften*, **25**, 27.
- Haas, H. F. & Bushnell, L. D. (1944). *J. Bact.* **48**, 219.
- Ingraham, M. A. & Steenbock, H. (1935). *Biochem. J.* **29**, 2553.
- Kharasch, M. S., Conway, E. A. & Bloom, W. (1936). *J. Bact.* **32**, 535.
- Kuhn, R. & Lederer, E. (1931). *Ber. dtsch. chem. Ges.* **65**, 637.
- Takeda, Y. & Ohta, T. (1939). *Hoppe-Seyl. Z.* **258**, 6.
- Takeda, Y. & Ohta, T. (1940). *Hoppe-Seyl. Z.* **265**, 233.
- Takeda, Y. & Ohta, T. (1944). *J. pharm. Soc. Japan*, **64**, 67.
- Tischer, J. (1938). *Hoppe-Seyl. Z.* **251**, 109.
- Tischer, J. (1939). *Hoppe-Seyl. Z.* **260**, 257.
- Turian, G. (1950*a*). *Helv. chim. acta*, **33**, 13.
- Turian, G. (1950*b*). *Helv. chim. acta*, **33**, 1303.
- Turian, G. (1950*c*). *Helv. chim. acta*, **33**, 1988.
- Turian, G. (1951). *Helv. chim. acta*, **34**, 1060.
- Turian, G. (1953). *Helv. chim. acta*, **36**, 937.
- Turian, G. & Haxo, F. T. (1952). *J. Bact.* **63**, 690.
- Turian, G. & Haxo, F. T. (1954). *Bot. Gaz.* **115**, 254.
- Zechmeister, L. (1944). *Chem. Rev.* **34**, 267.
- Zechmeister, L. (1954). *Experientia*, **10**, 1.
- Zechmeister, L. & Wallcave, L. (1953). *J. Amer. chem. Soc.* **75**, 5341.

Studies in Carotenogenesis

18. CAROTENOID PRODUCTION BY A STRONGLY CHROMOGENIC BACTERIUM ISOLATED FROM BUTTER

BY T. W. GOODWIN AND MALINI JAMIKORN
Department of Biochemistry, The University, Liverpool 3

(Received 20 April 1955)

The nature of the carotenoid pigments and the factors controlling their production have been investigated in a strongly chromogenic bacterium isolated from butter by Dr A. Lutz (Strasbourg). It was originally designated *Mycobacterium phlei* strain Pelligrino, but Dr Ruth Gordon (Rutgers University, N.J.), who recently kindly examined the strain, reported that it is not a typical *Myc. phlei*. 'It is not acid-fast but resembles strains coming to us as *Nocardia* spp.' As Topley & Wilson (see Wilson & Miles, 1946) do not recognize the genus *Nocardia*, and as Dr Gordon is 'inclined to agree with them', the difficult taxonomic problem of its complete identification remains unsolved and there does not appear any immediate possibility of its solution. A culture of this organism has been lodged with the DSIR National Collection of Industrial Bacteria, Teddington, Middlesex.

In spite of this difficulty of identification, the results of our investigation are now presented mainly because of their relevance to the general problem of carotenogenesis, but also because the information may eventually help to identify the organism.

EXPERIMENTAL

Culture. The culture of the unidentified organism was kindly supplied by Dr A. Lutz, Strasbourg. In this paper it will be called Pelligrino.

Culture conditions and media. The standard solid medium and standard liquid medium used were those described in the previous paper (Goodwin & Jamikorn, 1956). All media were normally sterilized by autoclaving (15 lb./sq.in./15 min.), although the iron salts used in an attempt to reverse the effect of diphenylamine were sterilized by filtration. Washed suspensions were prepared as described previously for *Rhodospirillum rubrum* (Goodwin & Osman, 1954).

Analytical methods. The methods used for determination of dry weight lipid and total carotenoids and for chromatographically separating and identifying the pigments were those described in the previous paper (Goodwin & Jamikorn, 1956).

RESULTS

Pigments present

Pigment extracts from Pelligrino cultured on the standard solid medium for 10 days were first chromatographed on deactivated alumina. Fraction A ran straight through the column and was

Table 1. *First separation of carotenoids of Pelligrino*

Adsorbent, deactivated alumina; developer, light petroleum containing varying amounts of diethyl ether; zones in order of increasing adsorptive power.

Zone	Description	Absorption maxima (m μ .) (light petroleum)	Concn. (v/v) of ether required to elute zone	Provisional identification
A	Yellowish, passes straight through to column	—	0	Mixture
B	Yellowish pink	448*	10–20	Pigments B and C of <i>Myco. phlei</i> (Goodwin & Jamikorn, 1956)
C	Brownish yellow	452*	60–80	
D	Orange-red	439, 458, 488	Ether + 5% ethanol	(?) Dihydroxy-carotenoid, similar to that in <i>Bacillus lombardo pelligrini</i> (Chargaff & Lederer, 1935)

* Only one main band.

Table 2. *Separation of Fraction A (Table 1) of polyenes of Pelligrino*

Adsorbent, activated + deactivated alumina (4:1); developer, light petroleum containing varying amounts of diethyl ether; zones in order of increasing adsorptive power.

Zone	Description	Absorption maxima (m μ .) in light petroleum	Concn. (v/v) of diethyl ether required to elute zone	Probable identification
A ₁	Colourless, trace of green fluorescence in u.v. light	—	3	Traces of phytofluene
A ₂	Orange-yellow	~ 425, 450, 475	5	β -Carotene
A ₃	Yellow-brown	~ 428, 452, 479	10	Leprotene
A ₄	Trace of pink	435, 461, 490	15–20	γ -Carotene

~ Denotes inflexion.

collected in the filtrate. Development of the chromatogram with light petroleum (light petroleum b.p. 40–60° was used throughout this investigation) containing varying amounts of ethyl ether resulted in a chromatogram described in Table 1. Fraction A was rechromatographed on a 4:1 mixture of activated and deactivated alumina and a typical separation is given in Table 2. A₁, A₂ and A₃ were identified as phytofluene, β -carotene, and leprotene respectively. They had the correct adsorptive and spectral properties and did not separate on mixed chromatography with specimens of leprotene obtained from various strains of *Myco. phlei* (Goodwin & Jamikorn, 1956). Fraction A₄, which occurs only in traces, has the very characteristic spectrum of γ -carotene. This, together with the fact that its adsorptive power is very similar to that of γ -carotene, makes it reasonably certain that it is this rather characteristic pigment.

Pigments B and C (Table 1) appear to be identical with those previously observed in three strains of *Myco. phlei* (Goodwin & Jamikorn, 1956). They have similar adsorptive and spectral properties and cannot be separated on mixed chromatography. The concentration of ether required to elute C from a column can vary con-

Fig. 1. Absorption spectrum of pigment D: in light petroleum, —; in benzene, ---.

Table 3. *Separation of hydrocarbon polyenes from diphenylamine cultures of Pelligrino*

Adsorbent, activated + deactivated alumina (4:1); developer, light petroleum containing varying amounts of ethyl ether; polyenes in order of increasing adsorptive power.

Zone	Description	Absorption maxima (m μ .) in light petroleum	Concn. of diethyl ether (% v/v) required for elution	Identification
A	Colourless; non-fluorescent in u.v. light	282	1-2	Phytoene
B	Colourless, green fluorescence in u.v. light	332, 348, 368	3-5	Phytofluene
C	Brownish	428, 450, 475	5	β -Carotene
D	Lemon-yellow	380, 400, 421	10	ζ -Carotene
E	Yellow	424, 456	10	Pigment X*
F	Khaki	441.5, 470	15	Neurosporene
G	Brown	429, 452, 478	15-20	Leprotene

* (Goodwin & Osman, 1953b).

Table 4. *Relative and absolute amounts of constituent polyenes of Pelligrino in presence and absence of diphenylamine*

7-day cultures; standard solid medium.

Polyene	Normal cultures		Diphenylamine cultures	
	Concn. (μ g./g. dry wt.)	% of total pigments	Concn. (μ g./g. dry wt.)	% of total pigments
Phytofluene	Trace	—	31	10.8
β -Carotene	7.5	2.2	Trace	—
ζ -Carotene	0	0	3	1.1
Pigment X	0	0	34	11.9
Neurosporene	0	0	7	2.4
γ -Carotene	3.7	1.1	3	1.1
Leprotene	110.2	32.4	85	29.5
Pigment B	129.5	38.1		
Pigment C	55.1	16.2	123	43.0
Pigment D	34.0	10.0		
Total	340		286	

siderably from experiment to experiment [compare Table 1 with Table 1 in the previous paper (Goodwin & Jamikorn, 1956)]; this is often the case with compounds requiring a considerable proportion of ether for elution]. Pigment D, which has not been observed in the *Myco. phlei* strains examined, has the adsorptive power of a dihydroxycarotene and an absorption spectrum (Fig. 1) very similar to that of γ -carotene. It may, therefore, be a dihydroxy- γ -carotene. It appears to be very similar to the xanthophyll obtained from *Bacillus lombardopelligrini* by Chargaff & Lederer (1935).

In diphenylamine cultures of Pelligrino, the synthesis of the more unsaturated polyenes (β -carotene, etc.) is inhibited, whilst that of the more saturated polyenes is stimulated. The usual members of these latter series have been identified (Table 3) with those obtained from diphenylamine cultures of *P. blakesleeana* (Goodwin, 1952). A typical quantitative distribution of pigments in normal and diphenylamine cultures is given in Table 4. It will be noted that both the relative and absolute amounts of the unsaturated polyenes,

especially the more strongly adsorbed components, are reduced in the presence of diphenylamine.

Quantitative experiments on liquid media

Reproducibility of results. Table 5 indicated the adequate degree of reproducibility with replicate liquid cultures of Pelligrino under our standard conditions. The concentration of pigments can, however, vary to a greater degree from one experiment to another.

Table 5. *Reproducibility of determination of dry weight and carotenoids in Pelligrino*

Standard liquid medium; amounts per 50 ml. of medium in 150 ml. Erlenmeyer flasks; 8-day cultures; temp. 37°.

Flask no.	Total dry wt. (mg.)	Total carotenoids	
		Amount (μ g.)	Concn. (p.p.m.)
1	321	16.0	49.6
2	319	15.3	47.9
3	321	15.7	49.0
4	303	14.8	48.9

Glucose concentration and carotenogenesis. Table 6 shows that growth increases with increasing glucose concentration up to the highest examined (8%, w/v); carotenogenesis, on the other hand, ceases to increase to any great extent above a glucose concentration of 1% (w/v). This results in a lowered concentration of pigments in cultures grown in the presence of high concentrations of glucose. These effects are observed with both 6-

carotenogenesis (Table 7); there is, therefore, a higher concentration of carotenoids in the glycerol cultures. This agrees with the observations of Ingraham & Steenbock (1935) on *Myco. phlei*. Table 7 also shows that maximal carotenoid production is not reached until the culture is 8–10 days old. Growth is also generally completed by 10 days, but occasionally increases in dry weight have been observed up to 14 days.

Table 6. *Effect of glucose concentration on growth and carotenogenesis in Pelligrino*

Amounts produced in 50 ml. of medium in 150 ml. Erlenmeyer flasks; standard liquid medium containing varying amounts of glucose. Temp. 37°; each value represents the mean of two flasks.

Concn. of glucose (%, w/v)	6-day cultures			10-day cultures		
	Total dry wt. (mg.)	Carotenoids		Total dry wt. (mg.)	Carotenoids	
		Amount (μ g.)	Concn. (p.p.m.)		Amount (μ g.)	Concn. (p.p.m.)
0.5	138.2	11.6	84	154.2	13.4	87
1.0	192.9	12.0	62	247.4	24.7	99
2.0	238.9	12.2	51	315.5	24.2	77
4.0	286.9	13.4	47	418.7	27.7	66
8.0	333.5	14.2	43	563.5	29.9	53

Table 7. *Growth and carotenogenesis in Pelligrino in presence of glucose or glycerol*

Amounts produced in 50 ml. of medium in 150 ml. Erlenmeyer flasks; standard liquid medium with either glucose or glycerol as carbon source. Temp. 37°; each value represents the mean of two flasks.

Age of culture (days)	Glucose (2%, w/v) medium			Glycerol (2%, w/v) medium		
	Total dry wt. (mg.)	Carotenoids		Total dry wt. (mg.)	Carotenoids	
		Amount (μ g.)	Concn. (p.p.m.)		Amount (μ g.)	Concn. (p.p.m.)
2	68	1.0	15	18	1.0	56
4	188	5.0	27	49	3.0	61
6	261	11.1	48	82	5.9	72
8	308	19.0	62	106	10.4	98
10	333	23.2	70	120	11.9	99

and 10-day cultures. This is rather different from that observed with *P. blakesleeanus* (Garton, Goodwin & Lijinsky, 1951), where growth ceases at a glucose concentration of 1% (w/v) whilst β -carotene synthesis continues with concentrations of glucose up to 2.0–2.5% (w/v). It will also be seen that, with the exception of the 0.5% (w/v) glucose medium, where the glucose is probably exhausted after 6 days carotenoid synthesis is relatively greater in old than in young cultures; this is similar to that observed with *P. blakesleeanus* (Goodwin & Willmer, 1952) and *Rsp. rubrum* (Goodwin & Osman, 1953a).

Effect of glycerol. In the case of Pelligrino replacement of glucose by glycerol in the standard liquid medium results in a considerable reduction of growth, accompanied by a smaller decrease in

Effect of acetate. The effect on carotenogenesis of the replacement of part of the glucose in the standard liquid medium by acetate has been investigated because of the claim of Schopfer & Grob (1950, 1952) that acetate is the primary precursor of carotene in *P. blakesleeanus* and *Mucor hiemalis*. Under our conditions no stimulatory effect of acetate could be demonstrated but both growth and carotenogenesis were reduced to about one-third when acetate completely replaced glucose (Table 8). These observations are in marked contrast to the statement of Barbier & Lederer (1953) that increased growth and lipid production was obtained with *Myco. phlei* by the addition of acetate to Sauton's medium. *iso*Propanol, which Ingraham & Steenbock (1935) found to stimulate pigment synthesis in *Myco. phlei*, reduces it somewhat in Pelligrino (Table 8).

Effect of different nitrogen sources. The substitution of asparagine in the standard liquid medium by either $(\text{NH}_4)_2\text{SO}_4$ or NH_4NO_3 results in a reduction of growth in both cases (Table 9); carotenogenesis is reduced similarly on $(\text{NH}_4)_2\text{SO}_4$; but, in contrast, it is reduced to a considerably greater extent on NH_4NO_3 ; this results in a much lower concentration of carotenoid on the latter medium; on this medium, however, the cells were much more pigmented than usual, being bright red. It was not possible using usual carotenoid solvents to extract most of the red pigments present, indicating that one component was, in all probability, not a carotenoid. Further attempts to extract this residual pigment using hot or cold water or dilute acid or alkali were unsuccessful.

previous observations with *P. blakesleeanus* (Goodwin *et al.* 1953) and *Rsp. rubrum* (Goodwin & Osman, 1954), but contrary to those of Turian (1950a) using his strain of *Myco. phlei*.

Effect of other inhibitors. The following well-known inhibitors were without any obvious effect on either growth or carotenogenesis: malonate (0.005%, w/v), fluoroacetate (0.001%, w/v), arsenite (0.08M), iodoacetate (0.01%, w/v). Phenol (1:2500) also has no effect, although it is inhibitory to Turian's strain of *Myco. phlei* (Turian, 1950b; Goodwin & Jamikorn, 1956). 2,4-Dinitrophenol inhibited both growth and carotenogenesis equally by about 50%; in *Myco. phlei*, 2,4-dinitrophenol stimulates carotenogenesis, according to Turian (1950b). Complete inhibition of growth was

Table 8. *Effect of acetate and isopropanol on growth and carotenogenesis by Pelligrino*

Amounts produced in 50 ml. of medium in 150 ml. Erlenmeyer flasks. Standard liquid medium with changes indicated below; each value represents the mean of two flasks.

Medium	6-day cultures			10-day cultures		
	Total dry wt. (mg.)	Carotenoids		Total dry wt. (mg.)	Carotenoids	
		Amount ($\mu\text{g.}$)	Concn. (p.p.m.)		Amount ($\mu\text{g.}$)	Concn. (p.p.m.)
Standard (glucose 2%, w/v)	237	12.4	52	316	27.5	87
Glucose (1%, w/v) + acetate (1%, w/v)	169	10.9	64	204	14.8	73
Acetate (2%, w/v)	108	6.5	60	112	7.6	67
Glucose (2%, w/v) + iso-propanol (1%, w/v)	218	9.1	42	249	11.9	48

Table 9. *Effect of altering the nitrogen source on growth and carotenogenesis by Pelligrino*

Amounts produced in 50 ml. of medium in 150 ml. Erlenmeyer flasks; standard liquid medium with different N sources. Temp. 37°; each value represents the mean of two flasks.

Nitrogen source (all 0.5%, w/v)	6-day cultures			10-day cultures		
	Total dry wt. (mg.)	Carotenoids		Total dry wt. (mg.)	Carotenoids	
		Amount ($\mu\text{g.}$)	Concn. (p.p.m.)		Amount ($\mu\text{g.}$)	Concn. (p.p.m.)
Asparagine (control)	293	13.7	46	533	36.3	68
$(\text{NH}_4)_2\text{SO}_4$	226	11.4	50	333	23.3	69
NH_4NO_3	158	3.8	29	236	4.6	20

Attempts to reverse the effects of diphenylamine. Neither adenosine 5'-phosphate (AMP) nor riboflavin, both of which reverse the inhibitory action of diphenylamine on carotenogenesis in *P. blakesleeanus* (Goodwin, Jamikorn & Willmer, 1953), has any marked effect in Pelligrino. Riboflavin at a concentration of 1:5000 completely inhibits growth of the organism. Furthermore, it was not possible to reverse the diphenylamine effect with either Fe^{2+} or Fe^{3+} . This is in agreement with our

observed with 0.01% (w/v) fluoride, 0.02% (w/v) iodoacetate and 0.001% (w/v) streptomycin; lower concentrations of streptomycin tend to inhibit carotenogenesis to a greater extent than growth. This is similar to the observations made with *Rsp. rubrum* (Goodwin & Osman, 1953a), but different from the very marked effect observed with *P. blakesleeanus* (Goodwin, Griffiths & Modi, 1956) and *Euglena gracilis* var. *bacillaris* (Goodwin & Jamikorn, 1954).

Effect of Tween 80. In preliminary experiments designed to examine the role of fat-soluble substances in carotenogenesis, the effect of the dispersing agent (Tween 80) was examined as a control. It was found that Tween 80 (0.02%, w/v) completely inhibited growth in *Pelligrino*. This inhibition was completely overcome when a fatty substance such as squalene or liquid paraffin (0.1%, w/v) was dispersed in the medium with the Tween. Furthermore, under these conditions it was found that squalene had no demonstrable effect on the biosynthesis of carotenoids in this organism.

experiments were carried out but no significant additional carotenoid synthesis was ever observed even in resuspended diphenylamine cultures. Studies with labelled acetate, however, show that there is a turnover of pigments in washed suspensions (E. Fisher, personal communication).

DISCUSSION

Apart from the relevance of these pigment studies to the general problem of carotenogenesis which has been discussed briefly in the 'results' section, the question arises whether they can help the

Table 10. *Effect of light and temperature on growth and carotenogenesis by Pelligrino*

Amounts produced in 50 ml. of medium in 150 ml. Erlenmeyer flasks; 'Light' cultures were incubated in a thermostatically controlled water bath illuminated at a distance of 10–12 in. by two 80w daylight fluorescent lamps. Each value represents the mean of two flasks.

		6 days		10 days		
Temp. of incubation	Total dry wt. (mg.)	Carotenoid		Total dry wt. (mg.)	Carotenoid	
		Amount (μg.)	Concn. (p.p.m.)		Amount (μg.)	Concn. (p.p.m.)
		Light cultures				
15°	38	1.7	45	51	2.8	55
32°	161	9.1	56.5	246	16.8	68
37°	—	—	—	—	—	—
		Dark cultures				
15°	35	1.8	51	101	5.5	54
32°	171	10.3	60	277	19.3	70
37°	249	16.2	65	327	23.3	71

Effect of light and temperature. Table 10 shows that light has no effect on carotenogenesis either at 15° or 32°. Growth is greater at the higher temperature after 6 and 10 days and carotene concentration is also somewhat increased. *Pelligrino* thus resembles *P. blakesleeanus* in producing less carotenoids at lower temperatures (Friend & Goodwin, 1954), but differs in not being affected by light (Garton *et al.* 1951).

Effect of pH. Growth of *Pelligrino* on the standard liquid medium was obtained only over the narrow pH range 6.0–7.0. There appeared to be slight variations in growth rate after 6 days on media with pH values of 6.0, 6.5 and 7.0, but after 10 days there were no significant differences between these media. In no case was there any significant differences in carotenogenesis.

Experiments with washed suspensions

Six-day cultures grown on the standard medium, either alone or in the presence of diphenylamine, were washed free from original medium and resuspended in the same volume of a medium consisting either of phosphate buffer (M/15, K_2HPO_4 , KH_2PO_4) or phosphate buffer plus glucose (2.0%, w/v) and incubated for 24 hr. A large number of

bacterial taxonomist in assigning the correct classification to this bacterium. *Pelligrino* synthesizes all the pigments found in 'true' strains of *Myc. phlei* except lycopene (Goodwin & Jamikorn, 1956) and synthesizes a further xanthophyll, pigment *D*. These two differences, absence of lycopene and presence of *D*, cannot, however, be taken as a sufficient differentiation, because strain Lombardo *Pelligrino* which is acid-fast, produces the same pigments as *Pelligrino* (Chargaff & Lederer, 1935); furthermore, *Pelligrino* produces leprotene which has not yet been observed outside the *Mycobacteriaceae*. It was suggested by Dr Lutz that 'Pelligrino' and 'Lombardo *Pelligrino*' are the same strain. The only marked difference is that *Pelligrino* is not acid-fast. As acid-fastness is not an immutable property and in some strains is particularly liable to disappear on continual sub-culturing under laboratory conditions (A. W. Downie, personal communication), the possibility remains that these two strains are basically the same. This conclusion would be supported by the identity of pigmentation. It will be interesting to see whether bacteriologists will eventually be able to decide unequivocally that *Pelligrino* is not a species of *Myc. phlei*.

SUMMARY

1. Pelligrino synthesizes phytofluene, β -, ζ - and γ -carotenes, leprotene, and three unknown xanthophylls, two of which also appear in various strains of *Mycobacterium phlei*.

2. Diphenylamine inhibits the synthesis of the more unsaturated polyenes but stimulates the production of phytoene, phytofluene, ζ -carotene, neurosporene and pigment X. The diphenylamine effect is not overcome by riboflavin, adenosine-5'-phosphate, Fe^{2+} , or Fe^{3+} .

3. Maximal carotenoid concentration occurs about 10 days after inoculation and with a glucose concentration of about 1% (w/v). Higher glucose levels increase growth but not carotenogenesis. Replacement of glucose by glycerol reduces growth and enhances carotenogenesis. Acetate reduces growth but has little effect on pigmentation.

4. Replacement of asparagine by $(\text{NH}_4)_2\text{SO}_4$ reduces growth and carotenogenesis equally, whilst NH_4NO_3 inhibits carotenogenesis much more than growth.

5. Concentrations of streptomycin less than 0.001% (w/v) tend to inhibit carotenogenesis to a greater extent than growth, whilst a number of common metabolic inhibitors when they did not inhibit growth completely did not have a preferential effect on carotenogenesis.

6. Tween 80 (0.02%, w/v) completely inhibits growth of Pelligrino; this is overcome when a lipid-soluble material (squalene or liquid paraffin, 0.1%, w/v) is dispersed in the medium with Tween.

7. Washed suspensions of Pelligrino did not synthesize additional amounts of carotenoids.

We wish to thank the Medical Research Council for a grant towards laboratory expenses.

REFERENCES

- Barbier, J. & Lederer, E. (1953). *Commun. 6th Int. Congr. Microbiol., Rome*, 1, 86.
- Chargaff, E. & Lederer, E. (1935). *Ann. Inst. Pasteur*, **54**, 383.
- Friend, J. & Goodwin, T. W. (1954). *Biochem. J.* **57**, 434.
- Garton, G. A., Goodwin, T. W. & Lijinsky, W. (1951). *Biochem. J.* **48**, 154.
- Goodwin, T. W. (1952). *Biochem. J.* **50**, 550.
- Goodwin, T. W., Griffiths, L. A. & Modi, V. V. (1956). *Biochem. J.* **62**, 259.
- Goodwin, T. W. & Jamikorn, M. (1954). *J. Protozool.*, **1**, 216.
- Goodwin, T. W. & Jamikorn, M. (1956). *Biochem. J.* **62**, 269.
- Goodwin, T. W., Jamikorn, M. & Willmer, J. S. (1953). *Biochem. J.* **53**, 531.
- Goodwin, T. W. & Osman, H. G. (1953a). *Biochem. J.* **53**, 541.
- Goodwin, T. W. & Osman, H. G. (1953b). *Arch. Biochem. Biophys.* **47**, 215.
- Goodwin, T. W. & Osman, H. G. (1954). *Biochem. J.* **56**, 222.
- Goodwin, T. W. & Willmer, J. S. (1952). *Biochem. J.* **51**, 213.
- Ingraham, M. A. & Steenbock, H. (1935). *Biochem. J.* **29**, 2553.
- Schopfer, W. H. & Grob, E. C. (1950). *Experientia*, **6**, 419.
- Schopfer, W. H. & Grob, E. C. (1952). *Experientia*, **8**, 140.
- Turian, G. (1950a). *Helv. chim. acta*, **33**, 1303.
- Turian, G. (1950b). *Helv. chim. acta*, **33**, 1988.
- Wilson, G. S. & Miles, A. A. (1946). *Topley and Wilson's Principles of Bacteriology and Immunology*, 3rd ed. London: Arnold.

The Metabolism of Pteroylglutamic Acid by the Rat

By K. GUGGENHEIM, S. HALEVY, HANNAH NEUMANN AND VARDA USIELI

Laboratory of Nutrition, Department of Biochemistry, Hebrew University-Hadassah Medical School, Jerusalem, Israel

(Received 22 August 1955)

From evidence published so far it appears that before pteroylglutamic acid (PGA)* can carry out its metabolic functions the organism must first

* As used by Welch & Nichol (1952) the term 'pteroylglutamic acid' has been restricted to the synthetic compound and 'citrovorum factor' to the substance(s) promoting growth of *Pediococcus cerevisiae* 8081 (*Leuconostoc citrovorum*); the term 'folic acid' has been considered a generic term applicable to substances which stimulate the growth of *Streptococcus faecalis*. The term 'unconverted folic acid' relates to the substance(s) enhancing the growth of *Strep. faecalis* but not of *Pediococcus cerevisiae*.

convert it into the tetrahydro form and that citrovorum factor (CF) may represent one of the metabolically active forms of folic acid (FA). Evidence has been presented (Nichol, 1950; Doctor *et al.* 1953; Doctor, Elam, Sparks, Lyman & Couch, 1954) for the existence in liver of an enzyme that is involved in the conversion of PGA into CF. Moreover, Nichol (1953a) has demonstrated that the liver is the main site of this conversion in the body. Of particular interest is the finding of Welch, Nichol, Anker & Boehne (1951) and of Broquist, Stokstad & Jukes (1951) that more CF is formed