Studies on Metabolism of Vitamin A

2. ENZYMIC SYNTHESIS AND HYDROLYSIS OF PHENOLIC SULPHATES IN VITAMIN-A-DEFICIENT RATS

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The work of Fell, Mellanby & Pelc (1956) had indicated that vitamin A is involved in sulphate metabolism in animal tissues, because excess of vitamin A, when added to the tissue culture of bone cells, inhibited the cartilage synthesis and caused the production of soluble sulphate. Subsequent work has substantiated this. Thus Wolf & Varandani (1960) claimed a lowering of sulphate uptake into the mucopolysaccharides of vitamin-A-deficient rats; also, higher concentrations of serum inorganic sulphate (Dziewiatkowski, 1954) and increased urinary excretion of sulphate (Balakhovskij & Kuznetsova, 1958) were reported in vitamin-A-deficient rats. However, the results of Frape, Allen, Speer, Hays & Carton (1959) tend to disagree with the general trend of the observations made by the above-mentioned workers, because an increased uptake of ^{35}S -labelled sulphate *in vivo* was observed in the tissues of vitamin-A-deficient rats.

The existing information in this regard therefore appears to be inconclusive and rather scanty. It is shown here that in vitamin-A-deficient rats there is a slight increase in the urinary excretion of inorganic sulphate, whereas that of the ethereal sulphates is markedly reduced. It is also shown here that the activity of enzymes responsible for the sulphurylation of phenols is markedly lowered in the tissues of the deficient rats.

MATERIALS AND METHODS

$\text{Na}_2^{35}\text{SO}_4$ was obtained from the Atomic Energy Establishment, Trombay, India. ATP, *p*-nitrophenol and *p*-nitrophenol sulphate were gift samples from Sigma Chemical Co. Nitrocatechol was kindly supplied by Dr A. B. Roy, and nitrocatechol sulphate by Dr K. S. Dodgson.

Collection and analysis of urine. Only male rats were used in this study. Vitamin-A-deficient rats and pair-fed controls were obtained as described by Malathi, Subba Rao, Seshadri Sastry & Ganguly (1963). Rats were maintained on the vitamin-A-free diet in separate cages and their daily weights were recorded. At the time when they were considered to be nearing the plateau stage they were removed to metabolic cages placed on funnels provided with metal sieves for the retention of the faeces; the urine drained into

glass bottles containing 3% (w/v) of boric acid in water. One pair-fed control was used for each deficient rat. $\text{Na}_2^{35}\text{SO}_4$ was injected intraperitoneally and the urine was collected for 24 hr., since most of the injected sulphate is excreted within this time (Dziewiatkowski, 1956; Lloyd, 1961). The cages and funnels were then thoroughly washed and the washings were collected in the bottle with the urine. The samples were stored in the cold for analysis.

The distribution of the labelled sulphate in the urine samples as inorganic, ethereal and neutral sulphates was measured according to the method of Lloyd (1961), with the modification that the total ^{35}S content was estimated after the whole urine was oxidized with Benedict's reagent (Benedict, 1909), instead of HNO_3 . The radioactivity was measured in a windowless internal gas-flow proportional counter and appropriate corrections for background, decay and self-absorption were applied.

Preparation, kinetic data and assay of the enzymes for the sulphurylation of phenols. Rats were killed by decapitation. Tissues were immediately removed into beakers kept in crushed ice and 10% homogenates in 0.15M-KCl were prepared with a Potter-Elvehjem homogenizer. The homogenates were centrifuged at 100 000g for 1 hr. in a Spinco model L ultracentrifuge and the particle-free supernatant was used as the source of the sulphurylating enzymes.

For the routine assay, the reaction mixture consisted of 8 μmoles of K_2SO_4 , 12 μmoles of cystine, 4 μmoles of MgCl_2 , 4 μmoles of ATP as sodium salt, 0.1 μmole of *p*-nitrophenol and 80 μmoles of phosphate buffer, pH 7.8, in a total volume of 1 ml. Time of incubation was 1 hr. at 37°. The sulphurylating activity was determined from the disappearance of the added *p*-nitrophenol according to the method of Hilz & Lipmann (1955), except that 4 ml. of 0.5N-NaOH was added directly to the enzyme digest after the incubation and the colour was directly read against a blank.

The activity was linear up to a protein concentration of 6 mg./ml. of reaction mixture, and up to 2 hr. of incubation. The pH optimum was at pH 7.6–8.0 (Fig. 1), with a minor peak at pH 6.0. The optimum concentrations for *p*-nitrophenol, sulphate, cysteine, Mg^{2+} ions and ATP were 0.1, 10, 15, 4 and 4 μmoles respectively (Fig. 2).

Preparation of arylsulphatases. Three arylsulphatases (A, B and C) have been shown (Dodgson, Spencer & Thomas, 1955) to exist in animal tissues, A and B being of mitochondrial origin, whereas C is a microsomal enzyme. The mitochondrial fractions isolated from 10% homogenates of tissues in 0.25M-sucrose, prepared according to Schneider & Hogeboom (1950), were used as the source of arylsulphatases A and B. For obtaining the microsomal fraction, which is the source of arylsulphatase C, 10% homogenates

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of tissues in 0.15M-KCl were centrifuged for 10 min. at 8000g. The supernatant fraction obtained at this step was then further centrifuged at 100 000g for 60 min. in a Spinco model L ultracentrifuge; the sedimented particles, representing the microsomal fraction, were redispersed in 0.15M-KCl and were used as the source of the enzyme.

Assay of arylsulphatases. Arylsulphatases A and B were assayed according to the method of Roy (1953), but proportionally smaller amounts of the enzyme and the substrate were used. The reaction mixture contained 5 μ moles

of nitrocatechol sulphate, 200 μ moles of acetate buffer, pH 6.0, and 2–3 mg. of enzyme protein. Total volume was 1 ml. and the incubation was for 30 min. at 37°.

The arylsulphatase C activity was measured according to the method of Dodgson *et al.* (1955), again with the slight modification that proportionally less enzyme and substrate were used. The reaction mixture contained 0.5 μ mole of *p*-nitrophenol sulphate, 200 μ moles of acetate buffer, pH 6.0, and 1–2 mg. of enzyme protein. The total volume was 1 ml. and the incubation was carried out for 30 min. at 37°.

Proteins were estimated by the biuret method (Gornall, Bardawill & David, 1949).

RESULTS

Tissue distribution of sulphurylating activity and arylsulphatases A, B and C in the normal rat. There is no information from a systematic study of the relative distribution of the sulphurylating activity for phenols in various tissues of the rat, most of the work being confined to the rat liver. This enzyme is widely distributed in rat tissues, with the liver having the highest activity (Table 1).

Existing literature shows that the relative distribution of the arylsulphatases A, B and C was studied with whole homogenates or with acetone-dried preparations of animal tissues (Robinson, Smith & Williams, 1951; Dodgson *et al.* 1955; Dodgson, Spencer & Wynn, 1956; Pulkinen, 1961). A reinvestigation of their distribution in rat tissues, the mitochondrial fraction being used for the sulphatases A and B and the microsomal fraction for sulphatase C, instead of the whole homogenates, was therefore undertaken (Table 2). The relative values obtained for the sulphatases A and B with the mitochondrial fraction compare favourably with those reported with whole-tissue extracts by the earlier workers (Robinson *et al.* 1951). In

Fig. 1. Effect of pH on sulphurylation of *p*-nitrophenol by rat-liver supernatant. Reaction mixture contained 0.1 μ mole of *p*-nitrophenol, 10 μ moles of K_2SO_4 , 10 μ moles of cysteine, 5 μ moles of $MgCl_2$, 5 μ moles of ATP as sodium salt, 80 μ moles of phosphate buffer, pH 7.4, and enzyme protein, 2.4 mg., in a total volume of 1 ml. Incubation time 1 hr. at 37°. Activity was assayed as described in the Materials and Methods section.

Fig. 2. Sulphurylation of *p*-nitrophenol by rat-liver supernatant. Conditions were the same as for Fig. 1, except that, where indicated, the concentration of the given compound was changed, the concentrations of the rest of the compounds being kept constant. (a) Effect of *p*-nitrophenol concentration (protein, 2.0 mg.); (b) effect of sulphate concentration (protein, 2.1 mg.); (c) effect of cysteine concentration (protein, 1.9 mg.); (d) effect of ATP and Mg^{2+} ion concentration (protein, 1.8 mg.; at each point the concentrations of both components were identical).

contrast with the wide distribution of the arylsulphatases A and B, we could find the sulphatase C activity only in rat-liver microsomes.

Urinary excretion of sulphates during vitamin-A deficiency. Table 3 shows the urinary excretory

Table 1. *Distribution of the sulphurylating activity in various tissues of rats*

Activities were assayed as described in the Materials and Methods section, except that the enzyme protein concentrations were changed according to the activity of the tissue. Activity in the liver is taken as 100% and those in the other tissues are represented relatively.

Tissue	Activity (%)
Liver	100
Brain	61.5
Heart	61.5
Pancreas	61.5
Lung	53.8
Kidney	30.7
Intestinal mucosa	30.7
Spleen	23.0
Intestinal muscle	15.0

Table 2. *Distribution of sulphatase A, B and C activities in various tissues of rats*

Enzyme activities were assayed as described in the Materials and Methods section. The mitochondrial fraction was used for the assay of sulphatases A and B and the microsomal fraction for the sulphatase C. Sulphatase A and B activity in the spleen is taken as 100% and those in other tissues are expressed relatively.

Tissue	Activity	
	Sulphatases A and B (%)	Sulphatase C (μ m-moles of <i>p</i> -nitrophenol formed/mg. of protein/30 min.)
Spleen	100	0
Lung	92	0
Kidney	77.6	0
Liver	65.0	22.9
Heart	60.0	0
Brain	49.0	0
Pancreas	49.0	0
Intestinal mucosa	41.0	0

Table 3. *Urinary excretion of injected $\text{Na}_2^{35}\text{SO}_4$ in vitamin-A-deficient rats*

Carrier-free $\text{Na}_2^{35}\text{SO}_4$ (0.3 μ m-mole) in 0.1 ml. of 0.9% NaCl soln. containing 6×10^5 counts/min. was injected intraperitoneally into each rat. All values are averages from five rats.

		Percentage of injected $^{35}\text{SO}_4$ in urine in 24 hr.		
		Inorganic sulphate	Ethereal sulphate	Neutral sulphate
Plateau stage	{ Pair-fed normal	60.0	9.3	3.7
	{ Deficient	73.1	1.9	3.5
Acute deficiency	{ Pair-fed normal	65.6	9.5	2.5
	{ Deficient	88.2	1.6	3.9

pattern of the injected labelled sulphate in the vitamin-A-deficient and pair-fed normal rats during the first 24 hr. after the injection of $\text{Na}_2^{35}\text{SO}_4$. There is a slight increase in the labelled inorganic sulphate, a drastic reduction in the ethereal sulphates and very little change in neutral sulphate in the urine of the vitamin-A-deficient, as compared with the pair-fed control, rats. Injection of vitamin A acetate or acid into the deficient rats restores almost to normal this changed urinary excretory pattern of sulphate (Table 4).

Changes in sulphurylating enzymes during the deficiency. The urinary ethereal sulphates usually consist of phenolic sulphates, and phenols are known to be sulphurylated in the liver through the 'active sulphate' adenosine 3'-phosphate 5'-sulphatophosphate (De Meio, Wizerkaniuk & Schreiberman, 1955; Spencer, 1960). These results therefore suggested that the enzymes responsible for the sulphurylation of phenols might be affected during vitamin-A deficiency. It is demonstrated in Table 5 that even at the plateau stage about 60% of the sulphurylating activity of both liver and intestinal mucosa of rats was lost; however, no further loss occurred during the acute stage of deficiency.

It has already been shown (Table 4) that an injection of vitamin A acetate or acid into the deficient rats rapidly corrects their abnormal urinary sulphate excretion. Table 6 shows that addition of vitamin A alcohol or acid to the reaction mixture *in vitro* fully restores the lost sulphurylating activities of liver and intestinal mucosa of the deficient rats.

Changes in arylsulphatases during the deficiency. The above results have demonstrated that during vitamin-A deficiency the sulphurylation of phenols is markedly reduced, which might explain the lowered excretion of the ethereal sulphates and concomitant increase in the inorganic sulphate in the urine of such rats. However, since the arylsulphatases of animal tissues can hydrolyse such sulphated compounds, it appeared possible that the lowered urinary concentrations of ethereal sulphates in the deficient rats could be due, at

least partly, to an increase in the arylsulphatase activities of their tissues. Table 7 shows that throughout the entire period of deficiency the sulphatase C of the deficient rats, as compared with the pair-fed controls, did not show any change;

sulphatases A and B were unchanged at the plateau stage, and during acute deficiency they increased significantly; addition of vitamin A acetate or acid to the reaction mixture *in vitro* had no effect.

Table 4. *Effect of intraperitoneal injection of vitamin A acetate or acid into vitamin-A-deficient rats on the urinary excretion of injected $^{35}\text{SO}_4$*

Dispersions of vitamin A acetate or acid were prepared as described by Malathi *et al.* (1963). The labelled sulphate (6.0×10^5 counts/min.) was injected as described in Table 3 and immediately after this 0.1 ml. of the dispersion containing 200 $\mu\text{g.}$ of either vitamin A acetate or acid was similarly injected. The control normal rats received no injection, and the control deficient rats received an injection of 0.1 ml. of a dispersion containing all the ingredients except the vitamin A acetate or acid. Values are averages from six rats.

Status of rat		Supplement	Percentage of injected $^{35}\text{SO}_4$ in urine in 24 hr.		
			Inorganic sulphate	Ethereal sulphate	Neutral sulphate
Plateau stage	Pair-fed normal	—	60.2	10.8	1.2
	Deficient	—	72.1	2.7	2.1
	Deficient	200 $\mu\text{g.}$ of vitamin A acetate	58.6	8.2	2.2
	Deficient	200 $\mu\text{g.}$ of vitamin A acid	61.3	9.0	1.8

Table 5. *Changes in sulphurylating activity in the liver and intestinal mucosa of vitamin-A-deficient rats*

Enzymes were assayed as described in the Materials and Methods section. Values are expressed as $\mu\text{m-moles}$ of *p*-nitrophenol disappeared/mg. of protein/hr. and are averages from six rats, given $\pm$ S.E.M.

Status of rat		Liver	Intestinal mucosa
Plateau stage	Pair-fed normal	10.9 $\pm$ 1.117	3.65 $\pm$ 0.023
	Deficient	3.74 $\pm$ 0.10	1.2 $\pm$ 0.021
Acute deficiency	Pair-fed normal	10.7 $\pm$ 0.163	*
	Deficient	3.6 $\pm$ 0.129	*

* During the acute deficiency stage there was practically no mucosa.

Table 6. *Effect of addition of vitamin A alcohol or acid to the reaction mixture in vitro on the sulphurylation of p-nitrophenol by liver or intestinal mucosa of vitamin-A-deficient rats*

Enzymes were assayed as described in the Materials and Methods section. The given amounts of vitamin A alcohol or acid were added to the reaction mixture. Values are expressed as $\mu\text{m-moles}$ of *p*-nitrophenol disappeared/mg. of protein/hr. and are averages from six rats, given $\pm$ S.E.M.

Status of rat		Additions	Liver	Intestinal mucosa
Plateau stage	Pair-fed normal	None	11.3 $\pm$ 1.01	3.65 $\pm$ 0.023
	Deficient	None	3.8 $\pm$ 0.11	1.2 $\pm$ 0.021
	Deficient	10 $\mu\text{g.}$ of vitamin A alcohol in 0.1 ml. of 1% ethanol	9.8 $\pm$ 0.252	3.95 $\pm$ 0.102
	Deficient	10 $\mu\text{g.}$ of vitamin A acid in 0.1 ml. of phosphate buffer, pH 10.0	10.43 $\pm$ 0.177	3.23 $\pm$ 0.028
	Deficient	0.1 ml. of 1% ethanol	3.96 $\pm$ 0.02	1.1 $\pm$ 0.011
	Pair-fed normal	None	10.6 $\pm$ 0.12	*
Acute deficiency	Deficient	None	3.5 $\pm$ 0.01	*
	Deficient	10 $\mu\text{g.}$ of vitamin A alcohol in 0.1 ml. of 1% ethanol	10.1 $\pm$ 0.20	*
	Deficient	10 $\mu\text{g.}$ of vitamin A acid in 0.1 ml. of phosphate buffer, pH 10.0	10.7 $\pm$ 0.23	*
	Deficient	0.1 ml. of 1% ethanol	3.8 $\pm$ 0.10	
	Deficient			

* Practically no mucosa could be obtained at this stage.

Table 7. *Changes in sulphatases A, B and C activities of rat liver during vitamin-A deficiency*

Enzymes were assayed as described in the Materials and Methods section, except that, where stated, the given amounts of vitamin A alcohol or acid were added. All values are averages from six rats and are given $\pm$ S.E.M.

Status of rat	Additions	Sulphatase A and B (μ m-moles of nitrocatechol formed/mg. of protein/30 min.)	Sulphatase C (μ m-moles of <i>p</i> -nitrophenol formed/mg. of protein/30 min.)
Plateau stage	Pair-fed normal	None	170 $\pm$ 19.5
	Deficient	None	22.9 $\pm$ 2.05
Acute deficiency	Pair-fed normal	None	178 $\pm$ 20.0
	Deficient	None	169.8 $\pm$ 16.0
	Deficient	10 μ g. of vitamin A alcohol in 0.1 ml. of 1% ethanol	235.0 $\pm$ 21.2
	Deficient	10 μ g. of vitamin A acid in 0.1 ml. of acetate buffer, pH 6.0	233.0 $\pm$ 24.6
			18.5 $\pm$ 2.4
			17.9 $\pm$ 3.14
			—
			—

DISCUSSION

The results of this investigation demonstrate that sulphate metabolism is affected in vitamin-A deficiency. We have shown here that even at the plateau stage of deficiency, rats excrete significantly less amounts of the ethereal sulphates and slightly increased amounts of inorganic sulphate, when compared with the pair-fed controls. This is in general agreement with the earlier observations of Balakhovskij & Kuznetsova (1958), who had shown that vitamin-A-deficient rats excrete more sulphate in their urine. We have also shown here that, on injection of vitamin A acetate or acid into the deficient rats, the changed pattern of urinary excretion of the sulphates can be restored to normal within 24 hr. of the injection. At the same time it has been demonstrated here that the activities of the tissue enzymes sulphurylating phenols are also markedly reduced at the plateau stage itself and that the lost activities could be fully restored by the addition of vitamin A alcohol or acid to the reaction mixture *in vitro*.

The reduced urinary excretion of ethereal sulphates is therefore directly due to the loss of the sulphurylating activities, because at the plateau stage the urinary ethereal sulphates and also the tissue sulphurylating activities had shown marked reduction, whereas no further changes in either occurred on prolonging the deficiency. Moreover, work by Meunier, Zwingelstein & Jouanneteau (1953) has shown that vitamin-A-deficient rats excrete increased amounts of phenolic compounds in their urine. This also appears to be due to the lowering of the sulphurylating activity, because phenolic compounds are known to be normally excreted in the urine as ethereal sulphates.

There was, however, some increase in the urinary amounts of inorganic sulphate at the plateau stage, which increased further with continuing deficiency. But we found practically no

change in the activities of all the three sulphatases at the plateau stage, and at the acute stage only the sulphatases A and B had increased significantly. It is quite possible that the elevated inorganic sulphate concentration at the plateau stage reflects the decreased activity of the sulphurylating enzymes and is not caused by any increase in the hydrolytic activities of the arylsulphatases; further increase in the urinary inorganic sulphate at the acute stage might be the result of the combined effect of the increased sulphatases A and B activities and the lowered sulphurylating activities.

Wolf & Varandani (1960) have claimed that the sulphate incorporation into the mucopolysaccharides of the colon homogenates of vitamin-A-deficient rats is decreased; their later work has implied that this is caused by a reduction in the activities of the enzymes synthesizing adenosine 3'-phosphate 5'-sulphatophosphate and not by any change in the transferring enzyme (Varandani, Wolf & Johnson, 1960). However, existing literature indicates that enzymes taking part in the transfer of sulphate from adenosine 3'-phosphate 5'-sulphatophosphate to phenolic compounds and to mucopolysaccharides are quite different (Gregory & Robbins, 1960). We have shown here that the total transferring activity for phenols is affected in vitamin-A deficiency. It is therefore possible that the formation of adenosine 3'-phosphate 5'-sulphatophosphate alone or both the formation of this compound and the transfer of sulphate from it to phenols are affected in such cases.

SUMMARY

1. In 24 hr. after the intraperitoneal injection of $\text{Na}_2^{35}\text{SO}_4$ into vitamin-A-deficient and pair-fed control rats, the deficient rats, even at the plateau stage, excreted in their urine slightly more inorganic sulphate and markedly less ethereal sul-

phates. Injection of vitamin A acetate or acid restored this changed pattern to normal.

2. The enzyme activities of the liver and intestinal mucosa for the sulphurylation of phenols were significantly reduced in the deficient animals and the lost activity could be restored by the addition of vitamin A alcohol or acid to the reaction mixture *in vitro*.

3. The arylsulphatase C showed no change throughout the deficiency; the arylsulphatases A and B did not change at the plateau stage, but were markedly increased at the acute deficiency stage.

4. It is suggested that the reduced excretion of ethereal sulphates and increased amounts of inorganic sulphate in the urine of vitamin-A-deficient rats are due to the marked loss of the sulphurylating activity caused by the deficiency.

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REFERENCES

- Balakhovskij, S. D. & Kuznetsova, I. V. (1958). *Dokl. Akad. Nauk S.S.S.R.* **118**, 331.
- Benedict, S. R. (1909). *J. biol. Chem.* **6**, 363.
- De Meio, R. H., Wizerkaniuk, M. & Schreibman, I. (1955). *J. biol. Chem.* **213**, 439.
- Dodgson, K. S., Spencer, B. & Thomas, J. (1955). *Biochem. J.* **59**, 29.
- Dodgson, K. S., Spencer, B. & Wynn, C. H. (1956). *Biochem. J.* **62**, 500.
- Dziewiatkowski, D. D. (1956). *J. biol. Chem.* **223**, 239.
- Dziewiatkowski, D. S. (1954). *J. exp. Med.* **100**, 11.
- Fell, H. B., Mellanby, E. & Pele, S. R. (1956). *J. Physiol.* **134**, 179.
- Frape, D. L., Allen, R. S., Speer, V. C., Hays, V. W. & Carton, D. V. (1959). *J. Nutr.* **68**, 189.
- Gornall, A. G., Bardawill, C. J. & David, M. M. (1949). *J. biol. Chem.* **177**, 751.
- Gregory, J. D. & Robbins, P. W. (1960). *Annu. Rev. Biochem.* **29**, 347.
- Hilz, H. & Lipmann, F. (1955). *Proc. nat. Acad. Sci., Wash.*, **41**, 880.
- Lloyd, A. G. (1961). *Biochem. J.* **80**, 572.
- Malathi, P., Subba Rao, K., Seshadri Sastry, P. & Ganguly, J. (1963). *Biochem. J.* **87**, 305.
- Meunier, P., Zwingelstein, G. & Jouanneteau, J. (1953). *Bull. Soc. Chim. biol., Paris*, **35**, 1189.
- Pulkinen, M. O. (1961). *Acta physiol. scand.* **52**, Suppl. 180.
- Robinson, D., Smith, J. N. & Williams, R. T. (1951). *Biochem. J.* **49**, lxxiv.
- Roy, A. B. (1953). *Biochem. J.* **53**, 12.
- Schneider, W. C. & Hogeboom, G. H. (1950). *J. biol. Chem.* **183**, 123.
- Spencer, B. (1960). *Biochem. J.* **77**, 294.
- Varandani, P. T., Wolf, G. & Johnson, B. C. (1960). *Biochem. biophys. Res. Commun.* **3**, 97.
- Wolf, G. & Varandani, P. T. (1960). *Biochim. biophys. Acta*, **43**, 501.

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Studies in Carotenogenesis

29. ATTEMPTS TO DETECT LYCOPERSENE IN HIGHER PLANTS*

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The biosynthesis of carotenoids proceeds from isopent-3-enyl pyrophosphate (Varma & Chichester, 1962) through farnesyl pyrophosphate (Yamamoto, Yokoyama, Simpson, Nakayama & Chichester, 1961). Since squalene, a triterpene, is formed from the condensation of two C_{15} terpenyl pyrophosphate molecules (see Popják & Cornforth, 1960), it is probable that the tetraterpene carotenoids are formed by an analogous condensation of two C_{20} units. Tomato plastids and a soluble carrot-enzyme

system have been shown to form C_{20} terpenyl pyrophosphates from isopentenyl pyrophosphate and farnesyl pyrophosphate (Beeler, Nandi & Porter, 1962). If the condensation of two such units to form carotenoids is completely analogous to triterpene biosynthesis, the first C_{40} compound formed would be lycopersene (I), the C_{40} analogue of squalene. Preliminary investigations (Davies, Goodwin & Mercer, 1961) failed to detect lycopersene in carotenogenic systems, and the suggestion was made that phytoene (15,15'-dehydrolycopersene, II) and not lycopersene is the first

* Part 28: Goodwin & Phagpolngarm (1960).