2. The effect of varying the conditions of isolation of the microsomal fraction and the determination of enzymic activity has been studied.

3. The influence of other univalent ions (Li^+ , Rb^+ , Cs^+ , NH_4^+ and choline) has been determined.

4. The possible connexion between this adenosine triphosphatase and sodium transport is discussed.

I am indebted to Mr B. W. Street for skilled technical assistance throughout this work.

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Carbohydrate Substrates for Skin Cells

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(Received 1 December 1961)

Thin slices of mammalian skin can be maintained *in vitro* on an appropriate buffer solution to which various sugars, e.g. glucose or fructose, have been added. Respiration proceeds at a steady rate and the sugars are utilized with the formation of lactic acid and carbon dioxide (Cruickshank & Trotter, 1956). When [^{32}P]orthophosphate is added to the medium the phosphorylated intermediates of glycolysis become labelled. After separation by two-dimensional paper chromatography these phosphate esters, though present in amounts too small for detection chemically, are visible on a radioautograph of the chromatogram. In previous work these methods were applied in a study of skin maintained on glucose and the effects of various

deoxyglucoses were examined; the inhibition of respiration by 2-deoxyglucose was measured and the formation of 2-deoxyglucose 6-phosphate by skin cells was observed (Brooks, Lawrence & Ricketts, 1959). The present work considerably extends the range of carbohydrates tested as substrates. The effects of 4-, 5- and 6-deoxyglucose and 2-deoxygalactose have been studied.

MATERIALS

Sugars were of the D-series except where stated and all were laboratory reagents except for 4- and 5-deoxyglucose [recently synthesized by Hedgerly & Overend (1960)], and 6-deoxyglucose [synthesized by Dr Sheila Brooks by the

method of Compton (1938)]. The purity of all sugars was examined by paper chromatography with butan-1-ol-ethanol-water (5:1:4, by vol.) and by paper electrophoresis in borate buffer. Sugars were detected with aniline hydrogen phthalate (Partridge, 1949) or alkaline silver nitrate (Trevelyan, Procter & Harrison, 1950). Traces of glucose were found in the samples of cellobiose, isomaltose and 6-deoxyglucose but not in amounts sufficient to cause a significant increase in respiration (see below). Three of four samples of maltose tested contained a trace of glucose; the fourth sample, free from glucose, was used for experiments on the maltase of skin. The other sugars were free from glucose. The galactose contained a trace of lactose and the allose a trace of either gulose or altrose. A small amount of impurity was detected in the erythrose and in the L-arabinose.

METHODS

Guinea-pig skin was obtained from the ear of the adult animal as described by Cruickshank (1954). Measurements of respiration, maintenance of skin slices on media containing [32 P]orthophosphate, preparation of skin extracts, two-dimensional paper chromatography and preparation of radioautographs were made as described by Brooks *et al.* (1959).

RESULTS

Without substrate skin showed a steady rate of oxygen uptake between 0.65 and 0.70 μ l./mg. wet wt./hr. for 1.5 hr., after which the respiration fell gradually, the mean rate during the period from 1.5 to 3.5 hr. being 85 % and that during the period from 22 to 24 hr. only 37 % of the original rate. This effect is probably due to utilization of endogenous substrates. In contrast, glucose added 1.5–2 hr. after the start of incubation maintains the rate of oxygen uptake at its original value for the next 2 hr. and at 85 % of the original value at 22–24 hr. However, if the glucose is added 3–4 hr. after the start of incubation, respiration is not maintained. Thus it is possible to test the efficacy of a substrate by adding it to the culture fluid at the end of a 1.5 hr. control period and measuring the rate of oxygen uptake subsequently. Table 1 shows respiration measurements made in this way. The last two columns of this Table provide some indication of the number and intensity of the spots seen on the radioautograph of a paper chromatogram separating the phosphate esters formed by the skin cells on each substrate. On the whole, the intensity is in agreement with the respiration percentage.

Ability of a given substrate to support skin respiration was assessed by comparing the residual respiration after 24 hr. with that of skin on glucose and on 0.9 % sodium chloride. Results may be classified into three groups: (a) sugars that are as effective as glucose in maintaining respiration; (b) sugars that maintain respiration at a higher rate than skin incubated without substrate but at

a significantly lower rate than glucose; (c) sugars that do not give a significant increase in respiratory rate compared with skin maintained without substrate. A fourth group might be expected with toxic substances where the residual respiration would be significantly lower than the rate obtained without substrate; however, no such substances were encountered among the carbohydrates tested. Results denoted by an asterisk in the Tables have been included from a previous publication (Brooks *et al.* 1959) for comparison with current findings.

In an experiment in which guinea-pig skin (36 mg.) was incubated at 37° for 24 hr. floating on an approximately isotonic (9.8 %, w/v) maltose solution (3 ml.), free from glucose, and containing 50 μ g. of streptomycin sulphate/ml., the production of glucose was clearly demonstrated by paper chromatography. A control experiment in which the same solution was incubated without skin showed no hydrolysis of the maltose.

DISCUSSION

Polysaccharides, such as dextran, starch or glycogen, seem unlikely to enter skin cells from the surrounding medium, so that the possibility of their supporting cell respiration would depend on the presence of extracellular hydrolytic enzymes. Kiverin (1957) found amylase and maltase in extracts of skin from various species including the guinea pig. In our experiments neither starch nor glycogen supported the respiration of guinea-pig skin, and so it would appear that the amylase is either present in too small an amount or located within the cells. Maltose (α -1 $\rightarrow$ 4), which would be the product of amylase action, supported skin-cell respiration just as well as glucose, but cellobiose (β -1 $\rightarrow$ 4) was quite ineffective (see Table 1). It seemed likely that the maltose was being hydrolysed to glucose. Skin floating on an approximately isotonic solution of maltose produced glucose in excess of its metabolic requirement, and this glucose was detected chromatographically. Thus the report by Kiverin (1957) of a maltase in skin is confirmed. The phosphate esters found in skin maintained on maltose were the same as with glucose as substrate, no new ester being formed. Neither isomaltose (α -1 $\rightarrow$ 6), the disaccharide from dextran, nor gentiobiose (β -1 $\rightarrow$ 6) supported skin-cell respiration. The phosphate esters found in skin maintained on these sugars were reduced in number and in radioactivity; the pattern on the radioautograph resembled that obtained without substrate. Lactose provided significant support for skin-cell respiration though it was not nearly so effective as glucose; the sample used was shown to be free from galactose. Presumably skin contains a small amount of lactase. Sucrose was completely

Table 1. *Respiration of guinea-pig-skin slices on various carbohydrates*

The concentration of each substrate was 0.02M (calculated as anhydroglucose for dextran, starch and glycogen), except that for DL-glyceraldehyde, which was 0.08M. Respiratory rates are given $\pm$ S.E.M.

Substrate	No. of expts.	Uptake of oxygen				Percentage of initial rate	22-24 hr. after substrate was added (μ l./mg. wet wt./hr.)	Percentage of initial rate	Chromatogram	
		0-1½ hr. before substrate was added (μ l./mg. wet wt./hr.)	0-2 hr. after substrate was added (μ l./mg. wet wt./hr.)	Percentage of initial rate	22-24 hr. after substrate was added (μ l./mg. wet wt./hr.)				No. of compounds	Intensity of ester pattern
None*	9	0.70 $\pm$ 0.02	0.60 $\pm$ 0.02	86	0.23 $\pm$ 0.01	33			6	+
Dextran	6	0.62 $\pm$ 0.03	0.56 $\pm$ 0.03	90	0.24 $\pm$ 0.02	39§			12	+
Starch	6	0.63 $\pm$ 0.03	0.56 $\pm$ 0.03	89	0.22 $\pm$ 0.01	35§			7	+
Glycogen	6	0.66 $\pm$ 0.02	0.57 $\pm$ 0.01	86	0.25 $\pm$ 0.02	38§			12	+
Maltose	5	0.66 $\pm$ 0.03	0.68 $\pm$ 0.04	103	0.60 $\pm$ 0.02	91†			18	+
Cellobiose	6	0.68 $\pm$ 0.03	0.60 $\pm$ 0.03	88	0.24 $\pm$ 0.02	35§			11	+
Isomaltose	5	0.63 $\pm$ 0.03	0.55 $\pm$ 0.02	87	0.22 $\pm$ 0.02	35§			14	+
Gentiobiose	6	0.64 $\pm$ 0.02	0.53 $\pm$ 0.02	83	0.23 $\pm$ 0.02	36§			13	+
Lactose	4	0.61 $\pm$ 0.01	0.61 $\pm$ 0.02	100	0.28 $\pm$ 0.03	46†			7	+
Sucrose	5	0.66 $\pm$ 0.02	0.58 $\pm$ 0.01	88	0.26 $\pm$ 0.03	39§			15	+
Allose	3	0.61 $\pm$ 0.05	0.53 $\pm$ 0.04	87	0.23 $\pm$ 0.01	38§			5	+
Glucose + allose	5	0.66 $\pm$ 0.03	0.65 $\pm$ 0.03	98	0.54 $\pm$ 0.03	82†			19	+
Altrose	3	0.62 $\pm$ 0.04	0.54 $\pm$ 0.04	87	0.23 $\pm$ 0.01	37§			7	+
Glucose + altrose	4	0.66 $\pm$ 0.03	0.63 $\pm$ 0.04	95	0.56 $\pm$ 0.03	85†			18	+
L-Sorbose	9	0.66 $\pm$ 0.02	0.60 $\pm$ 0.02	91	0.26 $\pm$ 0.02	39§			14	+
Glucose + L-sorbose	4	0.61 $\pm$ 0.01	0.61 $\pm$ 0.01	100	0.51 $\pm$ 0.02	84†			—	—
Sorbitol	11	0.64 $\pm$ 0.02	0.69 $\pm$ 0.02	108	0.52 $\pm$ 0.02	81†			16	+
Mannitol	6	0.62 $\pm$ 0.06	0.57 $\pm$ 0.06	92	0.21 $\pm$ 0.03	34§			16	+
Dulcitol	5	0.65 $\pm$ 0.02	0.58 $\pm$ 0.03	89	0.22 $\pm$ 0.02	34§			10	+
D-Arabinose	4	0.65 $\pm$ 0.02	0.60 $\pm$ 0.02	92	0.30 $\pm$ 0.04	46†			13	+
L-Arabinose	6	0.66 $\pm$ 0.04	0.57 $\pm$ 0.02	86	0.35 $\pm$ 0.03	53†			16	+
Xylose	6	0.66 $\pm$ 0.05	0.62 $\pm$ 0.04	94	0.26 $\pm$ 0.06	39§			18	+
Ribose	6	0.68 $\pm$ 0.02	0.56 $\pm$ 0.05	82	0.29 $\pm$ 0.03	43§			17	+
Erythrose	6	0.67 $\pm$ 0.02	0.62 $\pm$ 0.03	93	0.24 $\pm$ 0.03	36§			3	+
DL-Glyceraldehyde	5	0.62 $\pm$ 0.04	0.58 $\pm$ 0.04	94	0.30 $\pm$ 0.08	48§			7	+

* From Brooks, Lawrence & Ricketts (1959).

† Not significantly different from cultures maintained on glucose.

‡ Significantly lower than cultures maintained on glucose but significantly higher than cultures without substrate.

§ Not significantly different from cultures without substrate.

ineffective in supporting respiration and, since glucose and fructose are very effective, there appears to be no invertase or sucrase activity associated with skin.

Since both glucose and mannose are substrates for skin-cell hexokinase, it was of interest to test the C-2-epimeric pair allose and altrose which differ from glucose and mannose respectively in having the opposite configuration at C-3; neither supported respiration. Phosphorylation of allose and altrose by hexokinases from various sources has been reported, e.g. by Sols & Crane (1954) and by Lange & Kohn (1961). No compounds which might have been allose 6-phosphate or altrose 6-phosphate were observed on chromatograms from skin cells maintained on Krebs-Ringer phosphate buffer containing allose or altrose (0.02M). The presence of either of these sugars in equimolar concentration (0.02M) with glucose did not inhibit respiration. Thus allose and altrose resemble 3-deoxyglucose (3-deoxyallose) in not being substrates for skin-cell hexokinase (Brooks *et al.* 1959) so far as our experiments show.

It was thought that L-sorbose might show some interference with glucose metabolism since α -L-sorbopyranose (see Fig. 1) closely resembles D-glucopyranose and 1,5-anhydro-D-glucitol, i.e. '1-deoxyglucose'. Experiments showed, however, that L-sorbose neither supported skin-cell respiration itself nor inhibited respiration in the presence of glucose.

Sorbitol appeared to be an adequate substrate for skin cells since respiration was fully maintained; the radioautograph showed the recognized phosphate esters in the expected proportions as far as could be determined by inspection. It seems likely that skin, like liver (Blakley, 1951), contains a

sorbitol dehydrogenase which could lead to the formation of fructose within the cells. Whatever the mechanism it is specific, since neither mannitol nor dulcitol supported cell respiration.

Among the pentoses listed only D- and L-arabinose showed any sign of supporting respiration. In agreement with the rather higher respiration on L-arabinose, the spots corresponding to the recognized phosphate esters were more intense on this radioautograph. In a carefully comparative experiment, this result was confirmed by cutting out equal areas for corresponding spots from the chromatograms, dissolving the paper in hot 60% (v/v) perchloric acid, making up to a known volume and measuring the radioactivity in a Geiger-counter tube for liquid samples. Compounds 1, 3, 8, 9A, 9B, 11, 14, 17 and 26 [numbered according to Brooks *et al.* (1959)] from the D-arabinose experiment gave 2, 4, 5, 6, 8, 4, 2, 3 and 91 counts/min. respectively, and the same compounds from the experiment with L-arabinose gave 11, 12, 10, 14, 10, 14, 10, 8 and 272 counts/min. respectively.

The only examples of tetroses and trioses tested, D-erythrose, DL-glyceraldehyde and glycerol, appeared to be quite inert.

Glucose and mannose support skin-cell respiration and presumably enter metabolism through phosphorylation at C-6 by the hexokinase of skin cells. It was found that respiration was inhibited in cells maintained on these sugars by the presence of equimolar (0.02M) 2-deoxyglucose (see Table 2). The respiration of cells maintained on galactose appeared to be unaffected by the presence of 2-deoxyglucose. Probably this is because galactose is phosphorylated by a different enzyme, galactokinase, yielding galactose 1-phosphate. When skin cells were maintained on galactose and the

Fig. 1

Table 2. *Effects of various deoxy sugars on the respiration of guinea-pig-skin slices*

The concentration of each substrate was 0.02 M, except that for sodium pyruvate (when used alone), which was 0.04 M. Respiratory rates are given $\pm$ S.E.M.

Substrate	No. of expts.	Uptake of oxygen			Chromatogram		
		0-1½ hr. before substrate was added (μ l./mg. wet wt./hr.)	0-2 hr. after substrate was added (μ l./mg. wet wt./hr.)	Percentage of initial rate	22-24 hr. after substrate was added (μ l./mg. wet wt./hr.)	Percentage of initial rate	No. of compounds
Glucose*	11	0.69 $\pm$ 0.03	0.69 $\pm$ 0.04	100	0.58 $\pm$ 0.03	84	22
Glucose + 2-deoxyglucose*	4	0.66 $\pm$ 0.08	0.58 $\pm$ 0.09	88	0.31 $\pm$ 0.05	47†	5
Mannose*	11	0.71 $\pm$ 0.03	0.70 $\pm$ 0.04	99	0.57 $\pm$ 0.03	80†	15
Mannose + 2-deoxyglucose	5	0.61 $\pm$ 0.02	0.55 $\pm$ 0.02	90	0.35 $\pm$ 0.01	57†	14
Galactose*	8	0.68 $\pm$ 0.03	0.69 $\pm$ 0.04	102	0.52 $\pm$ 0.06	76†	16
Galactose + 2-deoxyglucose	6	0.68 $\pm$ 0.02	0.66 $\pm$ 0.03	97	0.54 $\pm$ 0.02	79†	13
2-Deoxygalactose	6	0.60 $\pm$ 0.04	0.51 $\pm$ 0.04	85	0.22 $\pm$ 0.03	37§	10
Galactose + 2-deoxygalactose	8	0.64 $\pm$ 0.02	0.59 $\pm$ 0.01	92	0.38 $\pm$ 0.02	59†	12
Glucose + 2-deoxygalactose	6	0.66 $\pm$ 0.03	0.64 $\pm$ 0.02	97	0.37 $\pm$ 0.02	86†	24
4-Deoxygalactose	6	0.69 $\pm$ 0.05	0.60 $\pm$ 0.06	87	0.27 $\pm$ 0.01	39§	22
Galactose + 4-deoxygalactose	5	0.68 $\pm$ 0.04	0.60 $\pm$ 0.04	88	0.32 $\pm$ 0.02	47†	17
Fructose	6	0.68 $\pm$ 0.02	0.78 $\pm$ 0.02	114	0.60 $\pm$ 0.02	77†	13
Fructose + 2-deoxyglucose	9	0.64 $\pm$ 0.02	0.59 $\pm$ 0.02	92	0.32 $\pm$ 0.02	50†	12
5-Deoxyglucose	6	0.67 $\pm$ 0.04	0.57 $\pm$ 0.03	85	0.24 $\pm$ 0.01	36§	22
Fructose + 5-deoxyglucose	6	0.65 $\pm$ 0.02	0.66 $\pm$ 0.02	102	0.52 $\pm$ 0.02	80	15
6-Deoxyglucose	5	0.66 $\pm$ 0.03	0.56 $\pm$ 0.04	85	0.22 $\pm$ 0.02	33§	—
Glucose + 6-deoxyglucose	5	0.65 $\pm$ 0.04	0.62 $\pm$ 0.03	95	0.42 $\pm$ 0.08	65	—
Sodium pyruvate	5	0.64 $\pm$ 0.02	0.66 $\pm$ 0.03	103	0.60 $\pm$ 0.03	94†	24
Sodium pyruvate + 2-deoxyglucose	6	0.61 $\pm$ 0.03	0.63 $\pm$ 0.03	103	0.57 $\pm$ 0.03	93	14

* From Brooks, Lawrence & Ricketts (1959).

† Not significantly different from cultures maintained on glucose.

‡ Significantly lower than cultures maintained on glucose but significantly higher than cultures without substrate.

§ Not significantly different from cultures without substrate.

phosphate esters from the cells were subsequently separated by two-dimensional paper chromatography, the radioautograph showed a faint spot with R_f 1.31 in propan-1-ol-aq. ammonia (sp.gr. 0.88)-water (6:3:1, by vol.) and R_f 0.42 in picric acid-2-methylpropan-2-ol-water (1:20:5, w/v/v) [see Table 2 of Brooks *et al.* (1959)]. Evidence in favour of this spot's being galactose 1-phosphate was obtained by adding some of this compound to the skin extract and demonstrating that the galactose 1-phosphate detected on the chromatogram by molybdate spray reagent coincided with the blackening of the radioautograph.

It was found that when skin cells were maintained on 0.02M-galactose in the presence of 2-deoxyglucose (0.02M), not only was respiration fully supported (Table 2) but the spot corresponding to galactose 1-phosphate was very much intensified. In this experiment the coincidence on chromatograms with the galactose 1-phosphate spot was confirmed by the same technique, the R_f values in duplicate experiments being 1.32, 1.42 in propan-1-ol-ammonia-water and 0.37, 0.42 in picric acid-2-methylpropan-2-ol-water. As expected from previous observations, 2-deoxyglucose 6-phosphate was also formed.

The respiration of skin cells was not supported by 2-deoxygalactose, nor did this sugar interfere with the metabolism of glucose so far as could be ascertained by respiration measurements and examination of the phosphate esters formed in the cells. When skin cells were maintained on 0.02M-galactose, however, their respiration was depressed to 59% of the initial rate by the presence of 2-deoxygalactose (0.02M), galactose 1-phosphate again being present on the radioautograph. There was no indication of the presence of 2-deoxygalactose 1-phosphate which might perhaps have resulted from the action of galactokinase on 2-deoxygalactose. A considerable inhibition (30-50%) of the respiration and fermentation of galactose-adapted yeasts in the presence of 2-deoxygalactose was reported by Robichon-Szulmajster (1961).

An interesting result was obtained with 4-deoxyglucose (4-deoxygalactose) (see Fig. 1). When this sugar was present in equimolar amount with galactose the respiration of skin cells was effectively inhibited. On the radioautograph a spot with R_f values corresponding to those of galactose 1-phosphate was found but no other ester which might have been 4-deoxygalactose 1-phosphate was observed.

Skin cells respired well on fructose, and fructose 6-phosphate was found in the cells. Probably fructose is phosphorylated by the hexokinase which phosphorylates glucose and mannose. Experiments showed that 2-deoxyglucose inhibited the

respiration of skin cells maintained on fructose, 2-deoxyglucose 6-phosphate appearing on the radioautograph. The inhibition by 2-deoxyglucose tends to confirm the view that skin-cell hexokinase phosphorylates fructose as well as glucose and mannose.

Alone, neither 5- nor 6-deoxyglucose supported cell respiration. In the presence of equimolar fructose no inhibitory effect was noted with 5-deoxyglucose, the cyclic form of which is necessarily furanose (see Fig. 1). 6-Deoxyglucose was tested in equimolar concentration (0.02M) with glucose and a definite inhibition of respiration was recorded, though the effect was less than that of 2-deoxyglucose. This finding is in accord with the observation by Wick, Brooks, Stewart & Christensen (1961) that 6-deoxyglucose inhibits glucose oxidation in several mammalian tissues. As there is no possibility of a 6-phosphate ester being formed, the mechanism of this effect is a matter of speculation, further experiments being necessary to decide whether the inhibition is competitive. Pyruvate was as effective as glucose in maintaining respiration; 2-deoxyglucose did not inhibit respiration of cells maintained on pyruvate and 2-deoxyglucose 6-phosphate was formed and detected on the chromatogram, as expected.

SUMMARY

1. Slices of skin weighing a few milligrams were maintained on a buffer solution with the addition of various carbohydrates. Respiration was measured and the phosphate esters formed were examined by paper chromatography.

2. The polysaccharides and disaccharides tested, with the exception of maltose, were unable to maintain the respiration of skin cells. Glucose was formed from maltose during the incubation.

3. Of the monosaccharides tested, glucose, mannose, fructose, galactose, and to some extent D- and L-arabinose, supported respiration; allose, altrose, L-sorbose and a number of pentoses, tetroses and trioses did not. Sorbitol was effective but not mannitol or dulcitol.

4. Respiration of cells maintained on glucose, mannose or fructose was inhibited by equimolar 2-deoxyglucose, but cells on galactose or pyruvate were unaffected.

5. Respiration of cells maintained on galactose was inhibited by 2- and 4-deoxygalactose, the latter being apparently the more powerful inhibitor of galactose metabolism. Glucose utilization was unaffected by 2-deoxygalactose.

6. Galactose 1-phosphate was formed in cells maintained on galactose.

7. None of the carbohydrates tested reduced the endogenous respiration of the skin slices.

Thanks are due to Professor W. G. Overend and Dr W. J. Whelan for the gift of various carbohydrates. The technical assistance of Mrs A. Kidson in making the respiration measurements is gratefully acknowledged.

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Studies on the Biosynthesis of Porphyrin and Bacteriochlorophyll by *Rhodopseudomonas spheroides*

1. THE EFFECT OF GROWTH CONDITIONS

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(Received 28 December 1961)

The various enzymes concerned with the synthesis of porphyrins from glycine and succinyl-coenzyme A (succinyl-CoA) have been found in plants and micro-organisms as well as in animal tissues. Thus the enzyme (aminolaevulinate synthetase) responsible for the formation of δ -aminolaevulinate has been shown to occur in the photosynthetic micro-organism *Rhodopseudomonas spheroides* (Gibson, 1958; Kikuchi, Kumar, Talmage & Shemin, 1958) and the enzyme aminolaevulinate hydratase catalysing the conversion of δ -aminolaevulinate into porphobilinogen has been detected in extracts of *Rps. spheroides* (Neuberger, Scott & Shuster, 1956), of *Chlorella* (Bogorad & Granick, 1953) and of spinach (Bogorad, 1955). The enzymic formation of uroporphyrinogen has been shown in extracts of wheat germ (Bogorad, 1958*a*) and its decarboxylation to coproporphyrinogen in extracts of *Chlorella* (Bogorad, 1958*b*). The conversion of coproporphyrinogen III into protoporphyrin IX has been studied in detail only in liver mitochondria (Sano & Granick, 1961), but it seems reasonable to assume that a similar enzyme is present in all cells containing haem proteins or chlorophyll.

Granick (1948*a, b*; 1950) found that protoporphyrin, magnesium protoporphyrin and magnesium

vinyl phaeoporphyrin a_5 accumulated in different mutants of *Chlorella* which were unable to form chlorophyll. He proposed that these substances were intermediates in the conversion of protoporphyrin into chlorophyll and that their accumulation was caused by the absence of the enzymes required for their further metabolism. Granick (1960, 1961) has identified magnesium protoporphyrin monomethyl ester and protoporphyrin monomethyl ester in another mutant of *Chlorella* and has also detected the former substance in barley seedlings incubated with aminolaevulinate. Although the enzymes involved were not known, Granick (1960) suggested a biosynthetic pathway from protoporphyrin to chlorophyll which fits the evidence at present available. Tait & Gibson (1961) reported briefly on the conversion of magnesium protoporphyrin into magnesium protoporphyrin monomethyl ester by chromatophore preparations of *Rps. spheroides*.

Factors controlling porphyrin and bacteriochlorophyll biosynthesis in *Rps. spheroides* have been studied by Lascelles and by Stanier and his co-workers. Lascelles (1956) found that, on illumination of organisms in a medium containing α -oxoglutarate and glycine, porphyrins (mainly coproporphyrin) were excreted into the medium. Organisms previously grown in a medium deficient in biotin, nicotinic acid or thiamine did not form

* Some of this work forms part of a Ph.D. Thesis submitted to the University of London in July 1961 by G. H. Tait.