

Table 1. DISTRIBUTION OF CARBON-14 IN POLYSACCHARIDE GLUCOSE FORMED DURING PHOTOSYNTHESIS USING *A. nidulans* AND *C. pyrenoidosa*

Time (sec)	Relative specific activity of the carbons of glucose on the basis of C-4 = 100					
	C-1	C-2	C-3	C-4	C-5	C-6
	<i>Chlorella pyrenoidosa</i>					
5	5.0	5.4	73	100 (1.0)	0.8	0.8
10	5.2	5.4	80	100 (7.5)	1.5	2.4
20	14	12	86	100 (21)	8.5	9.6
30	20	17	86	100 (19)	14	18
	<i>Anacystis nidulans</i>					
5	5.6	1.6	55	100 (0.66)	0.9	0.9
10	9.3	5.5	68	100 (6.1)	0.8	2.1
20	18	8.0	77	100 (11)	3.9	9.0
30	26	21	73	100 (27)	11	19

Value in parenthesis is the specific activity of C-4 expressed in  $\mu\text{mc./mg}$  carbon.

water (52 : 32 : 16). Only one band of radioactivity was found. This band corresponded to glucose and was identified by cochromatography with authentic sugar and by its colour reaction with aniline hydrogen phthalate. Each glucose sample together with 600  $\mu\text{moles}$  of carrier glucose was degraded by the *Leuconostoc mesenteroides* method<sup>5</sup>. Table 1 shows the results. The asymmetric pattern of distribution of carbon-14 in the glucose of the polysaccharide isolated from both algae is almost identical at any given time.

These results and those of Kandler<sup>4</sup> suggest the following: (1) aldolase is present in *Anacystis* and *Chlorella* and the reductive pentose phosphate cycle functions in both organisms, but the enzyme is not detected in extracts of *Anacystis* for various reasons<sup>5</sup>; (2) aldolase is not involved with the conversion of carbon dioxide to carbohydrate during photosynthesis; (3) different mechanisms of photosynthetic assimilation of carbon dioxide operate in the two algae but both mechanisms give rise to the same distribution of carbon-14 pattern.

This work was supported by grants from the U.S. National Science Foundation and from the U.S. Air Force through the Air Force Office of Scientific Research of the Air Research and Development Command, under contract AF49(638)798.

PAUL KINDEL\*  
MARTIN GIBBS

Department of Biochemistry,  
Cornell University,  
Ithaca, New York.

\* Predoctoral Fellow of the U.S. Public Health Service. Present address: Max-Planck-Institut für Zellchemie, Karlstrasse 23, Munich, Germany.

<sup>1</sup> Bassham, J. A., and Calvin, M., *The Path of Carbon in Photosynthesis* (Prentice Hall, Inc., Englewood Cliffs, N.J., 1957).

<sup>2</sup> Richter, G., *Naturwiss.*, **46**, 604 (1959).

<sup>3</sup> Fewson, C. A., Al-Hafidh, M., and Gibbs, M., *Plant Physiol.*, **37**, 402 (1962).

<sup>4</sup> Kandler, O., *Naturwiss.*, **48**, 604 (1961).

<sup>5</sup> Gibbs, M., Kindel, P. K., and Busse, M., in *Methods in Carbohydrate Chemistry*, edit. by Whistler, R. L., and Wolfrom, M. L., **2**, 496 (Academic Press, New York, 1963).

### Natural Occurring Variations in Rat Liver DNA Content

THE content of deoxyribonucleic acids (DNA) in non-dividing somatic cells is believed to be constant<sup>1,2</sup>. For this reason the nucleic acid content is a reference standard in several biochemical investigations. On the other hand, there are few data which lead to the conclusion that DNA content is not very constant<sup>3</sup> and one has to assume that DNA occurs also in a metabolic form<sup>4</sup>.

In an investigation of the influence of sex and season on the diurnal rhythm of rat liver content, nucleic acids were biochemically and histologically determined. This investigation was carried out with untreated animals of an inbred Wistar strain of both sexes kept under constant conditions (temperature, humidity, free access to food and water). The determination of nucleic acids after fractionated extraction and ultra-violet spectrography and the Dische test has shown that the DNA-content varies very little during day-time in the spring. The content is about 18  $\gamma/100$  mg liver wet weight. In the night there is a strong increase of the DNA with a maxi-

mum of 73  $\gamma/100$  mg. There are no differences between sexes by the time the peak is reached. Assays carried out in winter (January 1963) show quite different patterns of amount of DNA when estimated every 2 h for 24 h. Here I found two marked peaks (12 h and 18 h) during the day, while at night the content is low and without very strong variations. Sexual differences are marked in winter by a time difference of 2 h reaching the maximum peaks. The proportions of the values are the same when DNA-content is calculated for the dry weight of total protein content.

This leads to the conclusion that DNA changes not only in a diurnal rhythm pattern, but also that this pattern is influenced by the sex and season.

GABRIELLE HORVATH

Laboratorium voor Cyto-Histologie,  
Universiteit Nijmegen, Holland.

<sup>1</sup> Davidson, J. N., and Leslie, I., *Nature*, **165**, 49 (1950).

<sup>2</sup> Davidson, J. N., and Leslie, I., *Cancer Res.*, **10**, 587 (1951).

<sup>3</sup> Halberg, F., *Twelfth Ann. Conf. Electrical Techniques in Medicine and Biology* (1959).

<sup>4</sup> de Faria, H. Lima, *Prog. Biophys. and Biophys. Chem.*, **12**, 282 (1962).

### Disassociation of Heart Cells by Collagenase

THE disassociation of cells from various parent tissues by digestion with trypsin has been often applied by workers on tissue cultures<sup>1-3</sup>. There are, however, several defects in the trypsinization method when the goal is use of the cells for physiological investigations. We have examined various possible methods for avoiding these difficulties and have found that the substitution of collagenase for trypsin satisfied most of our requirements.

The criteria for a suitable disassociating enzyme were: (a) cell damage must be minimized; (b) cell yield should be high; (c) the mucus-like strands which appear during trypsinization should be absent; (d) the ionic environment during disassociation should be normal. It was observed that, using trypsin, the proportion of injured or dead cells as indicated by the nigrosin uptake method<sup>4</sup> was fairly high, particularly when old embryonic hearts (11-16 days) were used. It was also noted that quite large amounts of non-cellular material and ropes of mucus-like substance were always present following tryptic digestion. Furthermore, many workers<sup>2</sup> when using trypsin have found it desirable to reduce the concentrations of divalent cations in the digestion medium. In the work recorded here it was felt that the physiologically important factors to be studied (ion transport, transmembrane potentials, etc.) might be seriously, possibly irreversibly, damaged by exposure to such an abnormal environment.

The mucoid material in trypsinized suspensions suggested that collagen was present; accordingly, collagenase



Fig. 1. Collagenase. 24 h culture. ( $\times c. 245$ )