

From the standpoint of theory, the important observation has been made that the perrhenate ion does not inhibit, although its gross properties are otherwise very similar to those of the perrhenate ion. Results of several studies will be presented soon in detail.

CHEMISTRY DIVISION
THE OAK RIDGE NATIONAL LABORATORY
OAK RIDGE, TENN.

G. H. CARTLEDGE

RECEIVED MARCH 21, 1955

THE METABOLISM OF THIOCTIC ACID IN ALGAE Sir:

Chromatographic separation of extracts from various photosynthetic organisms have shown several compounds that have the biological activity of thioctic acid (6T). The criterion for biological activity of these compounds was the response of propionate-inhibited *S. faecalis* grown on an acetate-free medium.¹ Chromatography in a mixture of butanol-ethanol-water gave the major biologically-active compounds at R_f 's of 0.4, 0.7 and 0.9. The compounds at $R_f = 0.4$ and 0.7 were identified as thioctic acid sulfoxide and thioctic acid, respectively. The very lipid soluble compound at the front was not identified.

The synthesis of S^{35} -labeled 6T² has made possible a further investigation of thioctic acid metabolism in photosynthetic organisms.

0.5 g. of *Scenedesmus obliquus* suspended in 25 cc. 0.01 M phosphate buffer, pH 6.75, and containing 0.25 mg. of S^{35} 6T, was incubated in the dark aerobically. The distribution of the thioctic acid between algae and medium changed with time as follows: after 1 min., 16% in the cells; 10 min., 24%; 30 min., 42%; and 1 hour, 47%.

Such cells treated for at least one hour were extracted with ethanol and water and these extracts chromatographed in butanol saturated with 0.5 N ammonia. Five radioactive compounds were observed in the butanol-ammonia solvent at the R_f values: 0.98, 0.51, 0.33, 0.17, 0.1. The majority of activity was observed at R_f 0.98, 0.51 and 0.17. The latter two spots were identified as 6T and 6T sulfoxide. In a butanol-ethanol-water solvent system at least seven radioactive components could be observed. In long-term experiments the high R_f thioctic acid compound appeared to be a major component of the cells. To demonstrate that this compound was truly a metabolic product and not an artifact of the killing of the cells, extraction procedure, or chromatography, the S^{35} 6T was added to living algae which were then killed immediately and chromatographed. The results shown in Table I indicate clearly that this compound is a major metabolic constituent of the cell. An important observation is that it is not formed in any significant degree by cells under anaerobic conditions.

It has been shown by Bradley and Calvin³ that thioctic acid must be metabolized aerobically by

(1) M. W. Bullock, John A. Brockman Jr., E. L. Patterson, J. V. Pierce, M. H. von Saltza, F. Sanders and E. L. R. Stokstad, THIS JOURNAL, **76**, 1828 (1954).

(2) P. Adams, Univ. Cal. Rad. Lab. Rep. U.C.R.L.-2949.

(3) D. F. Bradley and M. Calvin, Arch. Biochem. Biophys., **53**, 99 (1954).

TABLE I

Compound	S^{35} 6T	S^{35} 6T added and cells killed at once	5.25 hr. uptake in dark, aerobic
6T	91.4 ^a	85	6
6T sulfoxide	5.4	5	14
Front	0.85	2.2	36
Origin	2.3	5.3	41

^a Percentage distribution of total activity on chromatogram.

Scenedesmus cells before any stimulation of the Hill reaction could be observed. It was therefore of interest to see if there was any localization of this lipid compound in the cell in relation to the photochemical apparatus. S^{35} 6T-fed *Chlorella* (we have not yet succeeded in obtaining good plastid preparations from *Scenedesmus* but the total thioctic acid distribution in the two organisms is very similar) were ruptured by ultrasonication and chloroplast fragments isolated and washed. Both an extract of the plastids and the plastid-free cellular supernatant material were chromatographed. The results in Table II show clearly that, of the material in the chloroplast fragments, a large amount is aerobically-formed thioctic lipid.

Table II

COMPOUNDS	DISTRIBUTION OF COMPOUNDS IN CELL FRACTIONS	
	Chloroplast fragments	Plastid-free supernatant
6T	7 ^a	15
6T sulfoxide	5	8
Front	45	18

^a Percentage distribution of total activity on chromatogram.

The very high R_f values and the behavior on alumina-column chromatography indicate that this conjugated thioctic acid is closely associated with the most lipid-soluble (hydrophobic) compounds in the cell and is readily converted back to thioctic acid by 4.0 N HCl, 1 hr., 120°. Further studies are underway to determine the structure of this thioctic-containing lipid and its possible relationship to photosynthesis.

RADIATION LABORATORY AND
DEPARTMENT OF CHEMISTRY⁶
UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA

R. C. FULLER⁴
H. GRISEBACH⁵
M. CALVIN

RECEIVED MARCH 11, 1955

(4) Biology Department, Brookhaven National Laboratory, Upton, L.I., New York.

(5) F.O.A. Fellow, 1954-1955, from University of Tübingen, Germany.

(6) The work described in this paper was sponsored by the U. S. Atomic Energy Commission.

THE METABOLISM OF PROPIONATE BY RAT LIVER SLICES AND THE FORMATION OF ISOSUCCINIC ACID¹

Sirs:

The pathways of propionate metabolism in mammalian tissues have not as yet been definitely established. According to one view, propionate is metabolized by conversion to acrylate and lactate,²

(1) Aided by a grant from the American Cancer Society.

(2) H. R. Mahler and F. M. Huennekens, Biochim. Biophys. Acta, **11**, 575 (1953).