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[1] H. HOLDEN AND N. W. PIRIE, *Biochem. J.*, 60 (1955) 39.
[2] R. MARKHAM AND J. L. STROMINGER, *Biochem. J.*, 64 (1956) 46.
[3] W. FRISCH-NIGGEMEYER AND K. K. REDDI, *Biochim. Biophys. Acta*, 26 (1957) 40.
[4] K. REDDI, *Biochim. Biophys. Acta*, 28 (1958) 386.
[5] L. SHUSTER, H. G. KHORANA AND L. A. HEPPEL, *Biochim. Biophys. Acta*, 33 (1959) 452.
[6] R. MARKHAM, *Modern Methods of Plant Analysis*, Vol. 4, 1955 p. 246.

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Biochim. Biophys. Acta, 38 (1960) 552–553

The reactivation of the succinate-cytochrome *c* reductase system by a hydrocarbon residue

There are many reports[1–12] on the reactivation of isooctane-extracted cytochrome preparations by fat-soluble vitamins and a variety of lipids. From the present communication it should be recognized that following the evaporation or redistillation of practical or spectral-grade isooctane (2,2,4-trimethylpentane, No. P2396 and No. S2396, Eastman Organic Chemicals, Distillation Products Industries and Phillips Petroleum Company) a lipid-like residue has been found which is fully able to reactivate isooctane-extracted succinate-cytochrome *c* reductase preparations. Column- and paper-chromatographic analysis, chemical tests and spectral analysis demonstrate that this active residue is a non-polar, branched hydrocarbon.

In the course of the determination of the succinate-cytochrome *c* reductase reactivating ability of various lipid fractions obtained by silicic acid column chromatography[13] of the isooctane-extracted lipids from particulate cytochrome preparations, it was found that the most marked reactivating activity occurred in a lipid fraction (Fraction C, Table I) which was eluted by petroleum ether and which constituted less than 1 % of the total lipid. This lipid fraction was observed on paper chromatograms (carried out on paper impregnated with silicic acid, using as solvent *n*-heptane–diisobutyl ketone (96 : 6)[14]) of the isooctane-extracted lipids of the cytochrome preparations as a rapid-moving spot with an R_F value of 0.85. The spot appeared yellow when viewed under u.v. light following staining with rhodamine-G. However, this lipid fraction was not present when the particulate cytochrome preparations were extracted by the conventional chloroform–methanol method[15] rather than with isooctane. An examination of the active lipid material obtained by isooctane extraction of the cytochrome preparations showed that this material was initially present in the practical-grade isooctane which was used to extract the enzyme system and that it was also present to a lesser extent in spectral grade and redistilled practical grade isooctane.

A sample of the material present in the isooctane was obtained by the distillation of 4000 g of practical-grade isooctane. The resulting yellow oily residue (41 mg) was

TABLE I

REACTIVATION OF ISOOCTANE-EXTRACTED SUCCINATE–CYTOCHROME *c* REDUCTASE PREPARATIONS

*System**	*Per cent restoration of succinate-cytochrome c reductase activity***
Non-extracted preparation – control	100
Extracted preparation	18
Extracted preparation + (A)	46
Extracted preparation + (B)	36
Extracted preparation + (C)	108
Extracted preparation + (D)	95

* (A) 100 μg α-D-tocopherol.

(B) 100 μg of total lipid obtained by isooctane extraction of a cytochrome *c* reductase preparation.

(C) 100 μg of a fraction obtained by silicic acid column chromatography of the isooctane lipid extract (lipid B) of a cytochrome *c* reductase preparation. Fraction (C) is eluted from silicic acid by petroleum ether.

(D) 100 μg of a fraction obtained by silicic acid column chromatography of the residue remaining after distillation of isooctane alone. Fraction (D) is eluted from silicic acid with petroleum ether.

** The preparation, extraction, and assay of the enzyme system were performed as previously described[7,8].

dissolved in 5 ml petroleum ether and placed on a 10-g silicic acid column and then eluted with 20 ml petroleum ether. Following solvent evaporation a colorless oily residue (32 mg) was obtained which was able fully to reactivate an isooctane-extracted succinate-cytochrome *c* reductase preparation (Fraction D, Table I). Paper-chromatographic analysis, chemical tests, and the u.v. spectrum gave strong evidence for the identity of the isooctane residue with the active lipid fraction obtained by column fractionation of isooctane extracts of particulate cytochrome preparations. This material was free from P and N, gave negative tests with ninhydrin and with reagents for the detection of ketones, aldehydes, sugars, esters, unsaturation, choline and cholesterol[13,15,16]. The infrared spectrum* of the active material was in agreement with that of a branched, saturated, non-polar, long-chain hydrocarbon. The major absorption bands were at 3.40 μ, 3.48 μ, 6.83 μ, 7.25 μ and 13.85 μ. The absence of absorption bands characteristic of reactive functional groups was in agreement with the findings of the chemical tests. The u.v. spectrum of this material revealed a broad peak at 250 mμ. A dimerization product of isooctane fits the available evidence.

The above findings are in accord with the suggestion that a long hydrocarbon chain is a common feature of and may be responsible for the ability of various substances to reactivate an extracted cytochrome *c* reductase preparation[8,11,12]. The branched isoprenoid-like character of the hydrocarbon may be of particular interest since the structural unit occurs in several biologically active compounds. The above findings also suggest that caution must be exercised in evaluating the activity of any lipid residue which is obtained by isooctane extraction.

*Spectral analysis was done by Dr. W. B. MASON and M'. A. BEHRINGER of the University of Rochester Atomic Energy Project and were made possible in part by funds from the U.S. Atomic Energy Commission. The spectrum was done in KBr using a Perkin-Elmer model no. 21 double-beam spectrophotometer.

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1 A. NASON AND I. LEHMAN, *Science*, 1122 (1955) 19.
2 A. NASON, B. AVERBACH AND A. TERRELL, *Biochim. Biophys. Acta*, 19 (1956) 395.
3 I. LEHMAN AND A. NASON, *J. Biol. Chem.*, 222 (1956) 497.
4 A. NASON AND I. LEHMAN, *J. Biol. Chem.*, 222 (1956) 511.
5 K. DONALDSON AND A. NASON, *Proc. Natl. Acad. Sci. U.S.*, 43 (1957) 364.
6 K. DONALDSON, A. NASON, K. MOORE AND R. GARRETT, *Biochim. Biophys. Acta*, 26 (1957) 665.
7 M. MORRISON, R. CRAWFORD AND E. STOTZ, *Biochim. Biophys. Acta*, 22 (1956) 579.
8 R. CRAWFORD, M. MORRISON AND E. STOTZ, *Biochim. Biophys. Acta*, 33 (1959) 543.
9 J. BOUMAN AND E. SLATER, *Biochim. Biophys. Acta*, 26 (1957) 624.
10 D. DUEL, E. SLATER AND L. VELDSTRA, *Biochim. Biophys. Acta*, 27 (1958) 133.
11 F. WEBER, U. GLOOR AND O. WISS, *Helv. Chim. Acta*, 41 (1958) 1038, 1046.
12 E. R. REDFEARN AND A. M. PUMPHREY, *Biochim. Biophys. Acta*, 30 (1958) 437.
13 G. V. MARINETTI, D. SCARAMUZZINO AND E. STOTZ, *J. Biol. Chem.*, 224 (1957) 819.
14 G. V. MARINETTI, J. ERBLAND AND H. STOTZ, *J. Biol. Chem.*, 233 (1958) 562.
15 G. V. MARINETTI, J. ERBLAND AND J. KOCHEN, *Federation Proc.*, 16 (1957) 837.
16 G. ROUSER, G. V. MARINETTI, R. F. WITTER, J. F. BERRY AND E. STOTZ, *J. Biol. Chem.*, 223 (1956) 485.

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Synthesis of amino acids from carboxylic acids by isolated rat diaphragm

MANCHESTER AND KRAHL[1] have recently shown that isolated rat diaphragm will incorporate ^{14}C from a variety of carboxylic acids into its protein. This communication describes the results of experiments in which, by acid hydrolysis of such protein samples and separation of amino acids by column chromatography, the location and nature of the ^{14}C incorporated into diaphragm protein from the various carboxylic acids has been determined.

The samples of protein had been prepared in experiments reported elsewhere[1]. The method for the hydrolysis and separation of amino acids was as described by MANCHESTER AND YOUNG[2], except that the 1.5 *N* HCl used for elution was replaced by 1 *N* HCl. The results are shown in Table I.

No significant radioactivity was found in any fraction other than those described below. ^{14}C from [1,5-$^{14}C_2$]citrate and [1-^{14}C]acetate was found largely in the glutamic acid fraction and to a less extent in the aspartic acid fraction. ^{14}C from [2-^{14}C]succinate, [1-^{14}C]isobutyrate and [1-^{14}C]propionate was found mainly in the aspartic acid and glutamic acid fractions to a roughly equal extent, but a small portion was also detected