This work was supported in part by grants (2063 and 1322) from the National Heart Institute, National Institutes of Health, U.S. Public Health Service.

Department of Biochemistry, University of Rochester, School of Medicine and Dentistry, Rochester, N.Y. (U.S.A.)

J. KOCHEN
R. B. CRAWFORD*
G. V. MARINETTI
M. MORRISON**
E. STOTZ

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Received August 24th, 1959

* Present address: Department of Microbiology, School of Dentistry, University of Pennsylvania.

** M.M. was supported by a Senior Research Fellowship, SF 47, of the U.S. Public Health Service.

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

TABLE I

LOCATION AND DISTRIBUTION OF ^{14}C IN PROTEIN OBTAINED FROM RAT DIAPHRAGM WHICH HAD BEEN INCUBATED *in vitro* WITH ^{14}C-LABELLED CARBOXYLIC ACIDS

Diaphragm incubated with	*Radioactivity in hydrolysate from 10 mg protein*					
	Total counts/min eluted from column	*Percentage of ^{14}C found in*				
		Aspartate	*Serine + threonine*	*Glutamate*	*Alanine*	*Other amino acids*
[1,5-$^{14}C_2$]citrate	214	34	0	66	0	0
[2-^{14}C]succinate	1139	44	0	49	7	0
[1-^{14}C]isobutyrate	187	40	0	47	13	0
[1-^{14}C]propionate	6981	51	0	41	8	0
[1-^{14}C]acetate	5570	24	0	76	0	0
[^{14}C]formate	319	0	100	0	0	0

in the alanine fraction. ^{14}C from [^{14}C]formate was found only in the fraction containing serine plus threonine.

The incorporation of ^{14}C from formate into serine would be consistent with the formation of an active one-carbon fragment[3] from formate in diaphragm and the synthesis of serine from glycine by addition of an active one-carbon fragment, as had been previously found[2,4]. Both propionate[5] and isobutyrate[6] are believed to be broken down in the animal body to succinate. That the distribution of ^{14}C incorporated into protein derived from these three substrates is similar is compatible with this view. Aspartic acid is most probably formed from succinate by operation of the tricarboxylic acid cycle and subsequent amination of oxaloacetate so formed. The mechanism whereby ^{14}C from succinate enters glutamic acid is not known with certainty, but might be the reverse of the ketoglutarate dehydrogenase reaction in which CO_2 is fixed with succinyl-CoA or succinic semi-aldehyde to form ketoglutarate, which can then be aminated to glutamic acid[2]. As is to be expected, entrance of labelled intermediates of the tricarboxylic acid cycle between ketoglutarate and oxaloacetate leads to a different pattern of labelling of the glutamic and aspartic acids from that which is observed when diaphragm is incubated with acetate or citrate.

The author wishes to thank Dr. M. E. KRAHL of the Department of Physiology, University of Chicago, for his constant encouragement and for the provision of facilities for the performance of this work.

K. L. MANCHESTER*

Department of Physiology, University of Chicago, Chicago, Ill. (U.S.A.)

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Received September 9th, 1959

* Permanent address: Department of Biochemistry, University of Cambridge, Cambridge (Great Britain).