METABOLISM OF C^{14}-LABELED GLYCINE, L-HISTIDINE, L-LEUCINE, AND L-LYSINE*

By HENRY BORSOOK, CLARA L. DEASY, A. J. HAAGEN-SMIT, GEOFFREY KEIGHLEY, AND PETER H. LOWY

(From the Kerckhoff Laboratories of Biology, California Institute of Technology, Pasadena)

(Received for publication, July 17, 1950)

A study of the rates of incorporation *in vivo* of labeled amino acids into tissue proteins was undertaken to find the best time at which intermediates in the process might be found. The rates observed were so fast that we decided to investigate the process in some detail. The amino acids were L-α-aminoadipic-6-C^{14} acid, glycine-1-C^{14}, L-histidine-2-C^{14}-imidazole, L-leucine-1-C^{14}, and L-lysine-1-C^{14}.

There have been no studies in which the metabolism of intravenously injected carbon-labeled amino acids has been followed at short time intervals. Olsen *et al.* (1) fed C^{13}-labeled glycine to mice and 16 hours later found questionable amounts of the labeled carbon in the proteins, presumably because the glycine fed contained too little C^{13}. With C^{14}-labeled amino acids there has been no difficulty in finding their incorporation into tissue proteins (2–6). With the exception of the study by Greenberg and Winnick (3) the shortest time interval at which observations were made after the injection or feeding of an amino acid was 6 hours. Greenberg and Winnick made their first set of observations $\frac{1}{4}$ hour after injection of C^{14}-labeled glycine and their next 6 hours later. The observations reported below indicate that at such time intervals the rapidity of the incorporation into the visceral and serum proteins is missed.

Labeled Amino Acids

Syntheses of the following amino acids have been described: L-α-aminoadipic-6-C^{14} acid (7), glycine-1-C^{14} (8), L-leucine-1-C^{14} (9), L-lysine-1-C^{14} (9).

The synthesis of L-histidine-2-C^{14}-imidazole was a modification of Tesar and Rittenberg's (10) synthesis of L-histidine labeled with N^{15}, which in turn was a modification of Ashley and Harington's procedure (11). $NaSC^{14}N$ is condensed with α,δ-diamino-γ-ketovaleric acid dihydrochlo-

* This work is a part of that done under contract with and joint sponsorship of the United States Atomic Energy Commission and the Office of Naval Research, United States Navy Department. The C^{14} used in this investigation was supplied by the Carbide and Carbon Chemicals Corporation, Oak Ridge, Tennessee, and obtained on allocation from the United States Atomic Energy Commission.

ride to give thiol-L-histidine, which on oxidation with ferric sulfate gives histidine. The amino acid was purified via the diflavianate.

$NaSC^{14}N$ was prepared by the following adaptation of the method of Castiglioni (12). 56 mg. of $NaC^{14}N$ and 40 mg. of sulfur were refluxed with 1.7 ml. of acetone for 45 minutes. The acetone was then drawn off and the insoluble residue refluxed again with 20 mg. of sulfur in 1.5 ml. of acetone for 45 minutes. The combined acetone solutions were evaporated in a 3 ml. centrifuge tube with a stream of nitrogen. The residue, which consisted of $NaSC^{14}N$ and some sulfur, was dried over H_2SO_4. Titration of the thiocyanate in similar test runs showed that the $NaC^{14}N$ is converted quantitatively to $NaSC^{14}N$.

With four 0.5 ml. portions of water (centrifuging each time to remove the sulfur) the $NaSC^{14}N$ was transferred to a flask containing 835 mg. of α,δ-diamino-γ-ketovaleric acid dihydrochloride and 296 mg. of non-isotopic KSCN. The mixture was heated on a steam bath for 1 hour. Then it was poured dropwise and with constant mixing into a 10 per cent solution of $HgSO_4$ in 5 per cent H_2SO_4 and the mixture allowed to stand 20 hours at room temperature. The precipitate was collected, washed three times with water, suspended in 150 ml. of water, and decomposed with H_2S. The filtrate from the HgS was heated to remove H_2S and then brought to pH 8 with $Ba(OH)_2$. The $BaSO_4$ was filtered and the thiol-histidine solution concentrated *in vacuo* to about 2 ml.

The concentrate was heated on a steam bath for 1 hour after the addition of 8 gm. of ferric sulfate and 40 ml. of water. 200 ml. of hot water were added and, while hot, an excess of $Ba(OH)_2$. The precipitate was filtered and extracted with boiling water, and the combined filtrates were brought to pH 4.0 with H_2SO_4. After the $BaSO_4$ was removed, the solution was concentrated to 5 ml. and 550 mg. of flavianic acid were dissolved in it with heating. The diflavianate was allowed to separate first at room temperature, then at 0° overnight. It was washed twice with absolute ethanol and twice with ether. M.p., 240–245°; yield, 486 mg.

484 mg. of radioactive and 760 mg. of non-radioactive L-histidine diflavianate were dissolved in 12 ml. of 2 N H_2SO_4 and extracted seven times with warm *n*-butanol. The aqueous phase was boiled with norit, filtered, and brought to pH 7.1 with $Ba(OH)_2$. The filtrate from the $BaSO_4$ was concentrated *in vacuo*, transferred to a small centrifuge tube, and dried in a stream of nitrogen. The crystalline L-histidine was dissolved in 0.5 ml. of hot water and allowed to cool slowly to 0°; 2 to 3 volumes of absolute ethanol were added after the bulk of the crystals had separated A main crop of 210 mg. of L-histidine-2-C^{14}-imidazole was obtained after washing three times with absolute ethanol and once with ether. M.p., 272° (decomposition).

Procedure

Our first experiments were carried out on guinea pigs and rabbits, and some of the results obtained with them are presented. In most of the experiments mice were used in order to conserve labeled amino acids. A single experiment consisted of a group of six mice. The labeled amino acid was injected into the tail vein and the animals were killed singly 10, 20, 30, 60, 120, and 240 minutes later.

Radioactivity measurements were made on the expired CO_2, on the proteins of the blood and viscera, on the CO_2 evolved in ninhydrin treatment of blood and non-protein filtrates before and after complete acid hydrolysis, on the CO_2 from the wet combustion of whole blood and from the wet combustion of the non-protein filtrate of blood and viscera.

The blood was caught, after the animal was killed by a blow on the head, in a 20 ml. beaker containing 0.02 ml. of heparin (1000 units per ml.). A measured volume of the blood was pipetted into a 25 ml. volumetric flask containing 0.01 N NaOH and made up to the mark with the latter solution. One aliquot was taken for precipitation of the proteins; on another 2 ml. aliquot a wet combustion was carried out with the oxidizing mixture of Van Slyke and Folch (13), the oxidation chamber and trap assembly of McCready and Hassid (14), and the CO_2 absorption tower of Weinhouse (15); a 10 ml. aliquot was treated with ninhydrin for the determination of free amino acids by the method of Van Slyke *et al.* (16). The combined thoracic and abdominal viscera were weighed, minced, boiled for 15 minutes in 20 ml. of water, and homogenized; the suspension was brought to pH 5, boiled again for 15 minutes, and filtered; the coagulum was extracted exhaustively with boiling water and the combined filtrate and washings were made up to 100 ml. with water. The coagulum was dried at 100° and weighed. A 2 ml. aliquot of the non-protein filtrate was taken for wet combustion, a 20 ml. aliquot for determination of radioactive free amino acids by the ninhydrin method, and a 10 ml. aliquot was hydrolyzed for 20 hours with 20 per cent HCl; after removal of the excess HCl by distillation, the total radioactive amino acids were measured by the ninhydrin method. The increase in radioactivity in the CO_2 evolved by ninhydrin after acid hydrolysis was taken as a measure of labeled amino acid in combination other than with protein.

In all of the experiments with mice the carcass, after bleeding and removal of the viscera, was dropped into 100 ml. of 30 per cent KOH, allowed to stand at room temperature until the carcass was dissolved, then refluxed at a bath temperature of 150° overnight, cooled, and made up to 150 ml. with water. A 2 ml. aliquot was taken for wet combustion. In

test runs we recovered only 50 to 75 per cent of the radioactivity of added amino acids. We have not investigated at what point the losses occurred. One of the difficulties was that the specific activity of the resulting $BaCO_3$ was so low. The results obtained are considered as indicating roughly only whether there was a large or small fraction or practically none of the injected radioactivity in the carcass.

7 per cent trichloroacetic acid was used to precipitate the blood proteins and to suspend a small portion of the heat-coagulated visceral proteins. After standing overnight at room temperature, the proteins were washed, dried, and their radioactivity was measured by the methods previously described (17).

TABLE I

Metabolism of Glycine-1-C^{14} in Mice

Distribution of radioactivity as per cent of quantity injected.

Weight of animal	Time after injection	Blood			Viscera					Expired CO_2, cumulative	Total in blood, viscera, and expired CO_2, cumulative
		Total	Protein	Non-protein fraction	Protein	Non-protein fraction					
						Total	Liberated by ninhydrin reagent		Bound		
							Before hydrolysis	After hydrolysis			
gm.											
23	10 min.	1.4	0.1	1.3	6.7	54.2	23.1	26.0	2.9	2.6	64.9
24	20 "	2.1	0.3	1.7	20.9	48.2	19.9	24.0	4.1	9.5	80.7
22	30 "	2.1	2.1	0	34.3	47.3	20.7	25.2	4.5	15.2	98.9
17	1 hr.	2.3	2.3	0	36.8	34.0	6.6	17.6	11.0	22.4	95.5
19	2 hrs.	2.2	2.2	0	45.4	15.1	3.9	7.0	3.1	22.9	85.6
24	4 "	3.2	3.2	0	46.3	10.9	2.1	8.6	6.7	28.4	88.8

The glycine gave 55,000 c.p.m. per mg. Injected 0.7 mg. = 38,500 c.p.m.

Results

Tables I to V summarize the main results of the experiments on mice. The features common to the metabolism of glycine, histidine, leucine, and lysine were the rapid disappearance of the labeled amino acid from the blood (so rapid that 10 minutes after injection less than 3 per cent was in the blood), the appearance of radioactivity in the blood proteins later than in the visceral proteins, the very rapid incorporation of labeled amino acids into the visceral proteins, attaining at least 75 per cent of the maximum ½ hour after injection, the more rapid decrease of labeled amino acid in the non-protein fraction of the viscera than in their proteins, the small fraction of bound labeled amino acids, and the rapid appearance of

labeled CO_2 in the expired air, attaining nearly its maximum 1 hour after injection.

TABLE II

Metabolism of L-*Histidine-2-*C^{14}*-Imidazole in Mice*

Distribution of radioactivity as per cent of quantity injected.

Weight of animal	Time after injection	Blood			Viscera		Expired CO_2, cumulative	Total in blood, viscera, and expired CO_2, cumulative
		Total	Protein	Non-protein fraction	Protein	Non-protein fraction*		
gm.								
24	10 min.	2.3	0.1	2.2	8.5	29.8	1.3	41.9
25	20 "	2.6	0.8	1.8	17.8	20.3	9.3	50.0
24	30 "	2.3	1.6	0.7	40.8	10.5	26.1	79.7
26	1 hr.	3.5	2.6	0.9	45.4	6.5	35.6	91.0
24	2 hrs.	4.8	4.8	0	40.4	6.2	39.8	91.2
22	4 "	4.9	4.9	0	38.3	5.8	43.3	92.3

* Ninhydrin determinations were carried out on the non-protein filtrate before and after hydrolysis. The liberated CO_2 gave no counts.

The histidine gave 35,900 c.p.m. per mg. Injected 1.47 mg. = 52,800 c.p.m.

TABLE III

Metabolism of L-*Leucine-1-*C^{14} *in Mice*

Distribution of radioactivity as per cent of quantity injected.

Weight of animal	Time after injection	Blood			Viscera					Expired CO_2, cumulative	Total in blood, viscera, and expired CO_2, cumulative
		Total	Protein	Non-protein fraction	Protein	Non-protein fraction			Bound		
						Total	Liberated by ninhydrin reagent				
							Before hydrolysis	After hydrolysis			
gm.											
28	10 min.	1.9	0.2	1.7	16.7	7.2	7.1	7.1	0	3.3	28.9
19	20 "	1.6	0.1	1.5	26.1	12.9	5.0	5.2	0.2	18.6	59.2
24	30 "	1.0	0.7	0.3	46.8	5.2	2.3	3.4	1.1	39.8	92.8
24	1 hr.	2.0	2.0	0	33.3	3.4	0.8	1.1	0.3	58.3	97.0
23	2 hrs.	2.8	2.8	0	35.6	5.2	1.9	2.2	0.3	54.2	97.8
22	4 "	3.7	3.7	0	38.2	7.0	2.8	2.9	0.1	56.8	105.7

The leucine gave 15,000 c.p.m. per mg. Injected 2.0 mg. = 30,000 c.p.m.

Analysis of the carcass indicated the presence of a large fraction of the injected amino acid in the first 10 to 20 minutes after the injection, after which time it rapidly decreased; therefore after 1 hour little or no radioactivity could be detected.

Table VI shows more clearly than Tables I to IV the initially more rapid incorporation of labeled amino acids into the visceral than into the

TABLE IV

Metabolism of L-*Lysine-1-*C^{14} *in Mice*

Distribution of radioactivity as per cent of quantity injected.

Weight of animal	Time after injection	Blood			Viscera					Expired CO_2, cumulative	Total in blood, viscera, and expired CO_2, cumulative
		Total	Protein	Non-protein fraction	Protein	Non-protein fraction					
						Total	Liberated by ninhydrin reagent		Bound		
							Before hydrolysis	After hydrolysis			
gm.											
25	10 min.	2.2	0	2.2	20.0	20.9	17.1	17.1	0	2.7	45.8
29.5	20 "	3.5	0.1	3.4	32.8	11.3	9.1	9.5	0.4	8.8	56.4
27	30 "	1.0	0.2	0.8	17.9	7.8	3.8	5.8	2.0	14.3	41.0
20	1 hr.	0.6	0.2	0.4	19.4	6.9	2.2	3.8	1.6	21.4	48.3
23	2 hrs.	2.7	0.8	1.5	24.0	6.0	2.7	2.9	0.2	25.6	58.3
26.5	4 "	2.8	0.8	1.6	23.0	4.4	1.7	2.3	0.6	27.5	57.7

The lysine gave 19,500 c.p.m. per mg. Injected 1.46 mg. = 28,500 c.p.m.

TABLE V

Metabolism of L-α-*Aminoadipic-6-*C^{14} *Acid in Mice*

Distribution of radioactivity as per cent of quantity injected.

Weight of animal	Time after injection	Blood			Viscera		Expired CO_2, cumulative	Total in blood, viscera, and expired CO_2, cumulative
		Total	Protein	Non-protein fraction	Protein	Non-protein fraction*		
gm.								
22	10 min.	0.6	0	0.6	0	6.6	1.2	8.4
	20 "						6.0	
25	30 "	0.5	0	0.5	0	19.7	14.9	35.1
26	1 hr.	0.07	0	0.07	0	12.5	31.5	44.1
20	2 hrs.						46.6	
	4 "	0	0	0	0	2.4	55.3	57.7

* Ninhydrin determinations were carried out on the non-protein filtrate before and after hydrolysis. The liberated CO_2 gave no counts.

The α-aminoadipic acid gave 14,450 c.p.m. per mg. Injected 1.2 mg. = 17,350 c.p.m.

plasma proteins, followed by an approach to a balance between them. In every case the ratio of the specific activity of the visceral to that of the plasma proteins was high at first, greatly in excess of 1, and later, after 1 to 2 hours, approached a value near 1.

The specific activity of the plasma proteins was not measured directly; all of the proteins of the blood were precipitated with trichloroacetic acid. It was assumed that none of the labeled amino acids had been incorporated into the hemoglobin and that all the radioactivity in the precipitate of mixed proteins was in the plasma proteins. The total blood in the animal and the fraction, as plasma proteins, was calculated as follows: weight of blood = 6.4 per cent of body weight (18), specific gravity of blood = 1.06; the blood contained 23 per cent of proteins, of which 30 per cent was

TABLE VI

Incorporation in Mice of C^{14}-Labeled Amino Acids into Proteins of Viscera and of Blood at Different Time Intervals

Time after injection	Specific activity of proteins of			Visceral protein / Plasma protein calculated	Time after injection	Specific activity of proteins of			Visceral protein / Plasma protein calculated
	Viscera	Blood	Plasma, calculated			Viscera	Blood	Plasma, calculated	
	Counts per min. per mg. protein					Counts per min. per mg. protein			
	Glycine					L-Leucine			
10 min.	2.2	0.1	0.3	7.3	10 min.	2.7	0.2	0.7	3.8
20 "	6.9	0.4	1.3	4.6	20 "	5.9	0.1	0.3	19.6
30 "	10.6	1.5	5.0	2.1	30 "	7.1	0.8	2.6	2.7
1 hr.	10.7	1.7	5.6	1.9	1 hr.	7.1	2.5	8.3	0.85
2 hrs.	9.3	2.1	6.9	1.3	2 hrs.	7.9	2.5	8.3	0.95
4 "	9.6	2.6	8.6	1.1	4 "	8.5	3.5	11.6	0.73
	L-Histidine					L-Lysine			
10 min.	3.6	0.2	0.7	5.1	10 min.	3.3	0	0	
20 "	7.3	1.1	3.6	2.0	20 "	4.9	0.07	0.02	24.5
30 "	16.2	2.6	8.6	1.9	30 "	4.7	0.2	0.7	6.7
1 hr.	17.5	5.2	17.2	1.0	1 hr.	5.5	1.0	3.3	1.7
2 hrs.	16.1	7.6	25.1	0.64	2 hrs.	5.9	1.1	3.6	1.6
4 "	17.6	8.3	27.4	0.64	4 "	5.2	0.9	3.0	1.7

plasma proteins. The specific activity of the plasma proteins was, then, taken as 3.3 × the observed specific activity of the total blood protein.

Although each of the experiments of Tables I to VI was carried out twice and gave the general metabolic picture described above, we draw no specific conclusions from the quantitative differences found among the rates of metabolism of the amino acids except that of α-aminoadipic acid. This amino acid was different from the other four in that, although the L form was injected, none was incorporated into the visceral or plasma proteins. It was oxidized to yield its C^{14} in the expired air approximately as fast as was lysine, if we take into account that there was less of the

lysine available for oxidation since 20 per cent had been incorporated into the visceral proteins. Our findings with α-aminoadipic acid are in accord with that of Miller and Bale (19) who fed DL-lysine-6-C^{14} to a dog and found 24 hours later no evidence of α-aminoadipic acid in the proteins. It appears, therefore, that α-aminoadipic acid is a catabolic product of lysine in animal tissues (7, 20) and is not a normal constituent of animal proteins.

In order to compare the rates of incorporation of the labeled amino acids observed in mice with those in other animals and under other conditions, the rates of incorporation into the visceral proteins are summarized in Table VII in terms of micromoles of amino acid incorporated per gm. of protein (dry weight) per hour. The data show that the incorporation

TABLE VII

Amounts of Labeled Amino Acids Found in Visceral Proteins of Mice at Successive Time Intervals after Intravenous Injection

The amounts are expressed as rates per hour in the interval.

Time	Glycine	Histidine	Leucine	Lysine
	μM per gm. protein per hr.			
min.				
10	3.2	3.4	8.2	6.9
20	4.3	3.9	9.0	5.1
30	5.2	5.8	7.2	3.4
60	2.5	3.1	3.6	1.9
120	1.1	1.4	2.0	1.0
240	0.6	0.8	1.0	0.4

of glycine and of histidine proceeded unslackened for 30 minutes after the injection; with leucine and lysine the maximum rates came earlier. 30 minutes after injection the rates diminished progressively, until after 4 hours there was practically no further increase in labeled amino acids in the proteins.

The rates of incorporation were similar in rabbits 30 minutes after intravenous injection (40 mg. per kilo) of leucine. Expressed in Table VII as micromoles per gm. of protein per hour, representative rates were as follows: in heart 2.4, small intestine 6.6, kidney 4.0, liver 5.6, skeletal muscle 1.8, red blood corpuscles (washed) 0.26, serum 1.0, and spleen 5.4.

Somewhat slower rates were found in guinea pigs 30 minutes after the intravenous injection of 16 mg. per kilo of glycine, histidine, leucine, and lysine; the rates found for glycine, histidine, leucine, and lysine, respectively, in small intestine were 1.3, 0.90, 3.5, and 2.3, and in liver 0.80, 1.7, 2.3, and 2.0 μM per gm. of protein per hour.

We have reported (9) rates of incorporation *in vitro* in different fractions of guinea pig liver homogenate. It was interesting to compare these rates with those *in vivo* 30 minutes after injection of 16 mg. per kilo of the amino acid. The units are the same as above; the values in parentheses are those previously observed *in vitro*: glycine, "nuclear" fraction 0.56 (0.52); mitochondria 0.6 (0.40); microsomes 1.2 (0.075); supernatant 0.69 (0); histidine "nuclear" fraction 1.5 (0.32);[1] mitochondria 1.2 (0.15);[1] microsomes 3.1 (1.5);[1] supernatant 1.2 (3.7);[1] leucine, "nuclear" fraction 2.3 (0.61); mitochondria 1.1 (?); microsomes 4.3 (0); supernatant 1.8 (0); lysine, "nuclear" fraction 1.3 (4.1); mitochondria 1.6 (3.2); microsomes 2.9 (0.9); supernatant 1.6 (3.2). *In vivo* the greatest incorporation of every amino acid was in the microsome fraction and it was much greater than that observed in that fraction *in vitro*. The one other consistent difference between the findings *in vivo* and *in vitro* was that there was considerable incorporation *in vivo* of each of the amino acids in every fraction. *In vitro* there was little or no incorporation of glycine and leucine in the microsome and supernatant fractions.

SUMMARY

1. The synthesis of L-histidine-2-C^{14}-imidazole is described.

2. C^{14}-Labeled L-α-aminoadipic acid, glycine, L-histidine, L-leucine, and L-lysine were injected intravenously into mice. Within less than 10 minutes nearly all of the amino acid had disappeared from the blood into the carcass. Within the next 30 minutes 18 to 47 per cent of the injected amino acid (except α-aminoadipic acid) was incorporated into the visceral proteins; after an hour both the incorporation into the visceral proteins and the initial burst of oxidation of the amino acid had attained nearly their maxima. 1 hour after the injection onward a significant amount of labeled amino acid appeared in the plasma proteins, and within 2 hours the visceral and plasma proteins were in balance with respect to labeled amino acid content.

3. No α-aminoadipic acid was incorporated into either the visceral or serum proteins. It was oxidized at approximately the same rate as lysine.

4. Similarly rapid rates of incorporation were found in the guinea pig and rabbit.

5. Rates of incorporation are given of the labeled amino acids *in vivo* into the proteins of different fractions of guinea pig liver.

BIBLIOGRAPHY

1. Olsen, N. S., Hemingway, A., and Nier, A. O., *J. Biol. Chem.*, **148**, 611 (1943).
2. Winnick, T., Friedberg, F., and Greenberg, D. M., *J. Biol. Chem.*, **173**, 189 (1948).

[1] The details of these determinations will be reported in a later communication.

3. Greenberg, D. M., and Winnick, T., *J. Biol. Chem.*, **173,** 199 (1948).
4. Reid, J. C., and Jones, H. B., *J. Biol. Chem.*, **174,** 427 (1948).
5. Altman, K. I., Casarett, G. W., Masters, R. E., Noonan, T. R., and Salomon, K., *J. Biol. Chem.*, **176,** 319 (1948).
6. Goldsworthy, P. D., Winnick, T., and Greenberg, D. M., *J. Biol. Chem.*, **180,** 341 (1949).
7. Borsook, H., Deasy, C. L., Haagen-Smit, A. J., Keighley, G., and Lowy, P. H., *J. Biol. Chem.*, **176,** 1383 (1948).
8. Sakami, W., Evans, W. E., and Gurin, S., *J. Am. Chem. Soc.*, **69,** 1110 (1947).
9. Borsook, H., Deasy, C. L., Haagen-Smit, A. J., Keighley, G., and Lowy, P. H., *J. Biol. Chem.*, **184,** 529 (1950).
10. Tesar, C., and Rittenberg, D., *J. Biol. Chem.*, **170,** 35 (1947).
11. Ashley, J. N., and Harington, C. R., *J. Chem. Soc.*, 2586 (1930).
12. Castiglioni, A., *Gazz. chim. ital.*, **63,** 171 (1933).
13. Van Slyke, D. D., and Folch, J., *J. Biol. Chem.*, **136,** 509 (1940).
14. McCready, R. M., and Hassid, W. Z., *Ind. and Eng. Chem., Anal. Ed.*, **14,** 525 (1942).
15. Weinhouse, S., in Calvin, M., Heidelberger, C., Reid, J. C., Tolbert, B. M., and Yankwich, P. E., Isotopic carbon, New York, 95 (1949).
16. Van Slyke, D. D., Dillon, R. T., MacFadyen, D. A., and Hamilton, P., *J. Biol. Chem.*, **141,** 627 (1941). Van Slyke, D. D., MacFadyen, D. A., and Hamilton, P., *J. Biol. Chem.*, **141,** 671 (1941).
17. Borsook, H., Deasy, C. L., Haagen-Smit, A. J., Keighley, G., and Lowy, P. H., *J. Biol. Chem.*, **179,** 689 (1949).
18. Skipper, H. E., White, L., Jr., and Bryan, C. E., *J. Biol. Chem.*, **180,** 1187 (1949).
19. Miller, L. L., and Bale, W. F., *Federation Proc.*, **8,** 230 (1949).
20. Borsook, H., Deasy, C. L., Haagen-Smit, A. J., Keighley, G., and Lowy, P. H., *J. Biol. Chem.*, **176,** 1395 (1948).