($R = CH(CH_3)CH_3$), leucine ($R = CH_2CH(CH_3)CH_3$) and isoleucine ($R = CH(CH_3)CH_2CH_3$) was also studied.

The results failed to demonstrate a definite pattern of increasing L/D values as the size of the sidechain of the resin-bound L-amino acid increased. (In each case, D,L-leucine was the free amino acid used.) A mean value of 1.0 with an average deviation of $\pm$ 0.2 was observed. There was no apparent correlation of resultant values with the size of the sidechain involved. (Appropriate control experiments for the experimental method used have already been reported[6].) The positive results noted with the leucyltyrosine system may involve the additional factor of the polar nature of the sidechain of tyrosine, although this remains to be examined more thoroughly.

In order to be certain that the results noted with the resin-bound procedure were also applicable to unbound systems, the combination yielding the least number of different dipeptides (leucyl-leucine) was studied. With the use of D,L-leucine-4,5-H^3 and L-leucine-1-C^{14}, as well as leucine aminopeptidase (LAP), the following analytical scheme, outlined in terms of the labeled compounds involved, was devised. (The word 'leucine' has been omitted.):

(^{3}H): L* + D* $\xrightarrow{\text{DCA}}$ L*-L* + L*-D* + D*-L* + D*-D* (1)

L* + D*, L* + D*-L* + D*-D* (2) — LAP

(^{14}C): L* + D $\xrightarrow{\text{DCA}}$ L*-L* + L*-D + D-L* + D-D (3)

L* + L*+D + D-L* + D-D (4) — LAP

In this scheme, labeled substances are indicated with an asterisk (*). Since in the ^{14}C experiments (Nos. 3 and 4) D-leucine is unlabeled, D-leucyl-D-leucine (D-D) is not observed by radiotracer techniques. Scintillation spectrometry can readily differentiate between tritium and carbon-14 in the same sample.

An aqueous solution was prepared containing D,L-leucine, HCl, and sodium dicyanamide (DCA) as before. The only exceptions were that the HCl concentration was 0.12*N*, the D,L-leucine concentration (all unbound) was 0.02*M*, and both ^{14}C-L-leucine and ^{3}H-D,L-leucine were included together in the same reaction mixture. The enzyme step (LAP) was carried out by first neutralizing the product solution with $NaHCO_3$ and then placing it in *Tris* buffer (pH 8.0) containing the leucine aminopeptidase. Aliquots of the products, both after reaction with DCA (Nos. 1 and 3) and after subsequent treatment with LAP (Nos. 2 and 4), were resolved by paper electrophoresis in borate buffer at pH 9.2. The dimer bands, detected by autoradiography, were cut out and analyzed by scintillation spectrometry. By the electrophoretic method used, all the diastereomeric dipeptide products appeared together as one band and the monomers as another.

This method led to the following calculations (P = dimer product; example, 1P = dimer band from experiment No. 1):

$$\left.\begin{aligned}(1P) &= [L\text{-}L] + [L\text{-}D] + [D\text{-}L] + [D\text{-}D]\\ (2P) &= [D\text{-}L] + [D\text{-}D]\end{aligned}\right\}\ ^3H$$

$$\left.\begin{aligned}(3P) &= [L\text{-}L] + [L\text{-}D] + [D\text{-}L]\\ (4P) &= [D\text{-}L]\end{aligned}\right\}\ ^{14}C$$

$(2P)\text{-}(4P) = [D\text{-}D]$ Nos. 2, 4 = LAP-treated

$$L/D = \frac{4P}{2P\text{-}4P} = \frac{[D\text{-}L]}{[D\text{-}D]}$$

Thus, appropriate analysis of the LAP-treated dimer product plus knowledge of the total activity employed would provide the desired data. The results gave an L/D value of 1.2, which compares quite well with the 1.1 figure found with the resin-bound system. This would further corroborate the conclusion that little, if any, stereoselectivity is evident at the oligopeptide level on the basis of preferential interactions.

In the context of possible events which may have occurred on the primitive Earth, these results suggest that the synthesis of stereohomogeneous polypeptides would have had to depend on chance associations at the simple peptide level and then on stabilization of homopolymers by the α-helix at higher degrees of polymerization[7]. (See reference [2] for a more detailed discussion of the problem of prebiological stereoselectivity.)

Zusammenfassung. Die Umsetzung von D,L-Leucin mit verschiedenen L-Aminosäuren und Dicyanamid als Kondensationsmittel zu Oligopeptiden verläuft nicht stereoselektiv. Die Bedeutung dieser Beobachtung in bezug auf den Ursprung des Lebens wird diskutiert.

G. STEINMAN

Department of Biochemistry, The Pennsylvania State University, University Park (Pennsylvania 16802, USA), 5th September 1966.

[6] G. STEINMAN, Archs Biochem. Biophys., in press.

[7] The author is indebted to the College of Science and the Department of Biochemistry of the Pennsylvania State University for providing funds with which this investigation was carried out. The skillful technical assistance of Mr. W. DRESSELHAUS, Mrs. CAROL ESHELMAN and Mr. A. TISCHLER is gratefully acknowledged. Mr. R. COTNER graciously helped with the scintillation counting.

Dietary Cholesterol and Activity of Enzymes Hydrolyzing and Synthesizing Cholesterol Ester in Pancreatic Juice of Rats

Dietary cholesterol esters are hydrolyzed, presumably in the lumin of the small intestine, before absorption into the intestinal mucosa[1]. Free cholesterol is esterified prior to the transfer of cholesterol into the intestinal lymphatics, maintaining a ratio of esterified to free cholesterol of approximately 2–3:1 in intestinal lymph[1]. Enzymes derived from the pancreas are believed to be involved in both the hydrolysis of the esters and the esterification of free cholesterol[1]. Feeding rats a high cholesterol diet does not increase the activity of these enzymes in the pancreas[2]. The present study was undertaken to determine

[1] DEW. S. GOODMAN, Physiol. Rev. *45*, 747 (1965).

[2] S. K. MURTHY, S. MAHADEVAN, and J. GANGULY, Archs. Biochem. Biophys. *95*, 176 (1961).

whether or not an adaptation of the exocrine secretion of the rat pancreas to a high cholesterol diet could be observed by testing the activity of enzymes synthesizing and hydrolyzing cholesterol esters in pancreatic juice collected from rats fed a cholesterol-free diet and one containing 2% cholesterol.

The constituents of the cholesterol-free diet were: sucrose, 50.5%; casein, 25%; tripalmitin, 3%; triolein, 2.9%; trilinolein, 0.1%; vitamin mixture (Calbiochem diet fortification in dextrose), 2.5%; salt mixture (U.S.P. XIV, Nutritional Biochemicals, Cleveland, Ohio), 6.0%; and Alphacel (Nutritional Biochemicals), 10%. In the diet containing 2% cholesterol, the cholesterol was added at the expense of the Alphacel. These diets were fed for 5 weeks for 3 separate feeding periods. At the end of each week pancreatic juice was collected from 2–3 rats of each group through a polyethylene cannula inserted into the common bile duct as closely as possible to the duodenum, after another cannula had first been inserted into the duct to drain off the bile secretions. The samples of juice used for the measurement of enzyme activity were taken during a 3 h period 16–20 h after cannulation, at which time a steady flow of juice had been established.

The incubation mixture for the experiments measuring the esterification of free cholesterol consisted of 1 mg cholesterol (commercial grade purified by crystallization of the bromide[3]), 1.5 mg of oleic acid (Calbiochem, Los Angeles, Calif., A grade), plus 10,000 cpm (4 ^{14}C) cholesterol (New England Nuclear Corp., Boston, Mass., s.a. 21.1 mC/mM), added in 0.1 ml of acetone; 5 mg sodium taurocholate (Nutritional Biochemicals); 0.1 ml of pancreatic juice (diluted 1:1 with distilled water); and 0.1 M phosphate buffer, pH 5.8 (the pH optimum for the reaction under these conditions, as determined in preliminary experiments) to make a final volume of 3.0 ml. In the mixture for the assay of the hydrolytic reaction 0.25 mg of cholesteryl oleate (Calbiochem, A grade) plus 10,000 cpm (4 ^{14}C) cholesteryl oleate (New England Nuclear Corp., s.a. 11.7 mC/mM) was substituted for free cholesterol and oleic acid in the reaction mixture, and 0.1 M phosphate buffer of pH 7.6 (the pH optimum determined in preliminary experiments) was used. The incubations were carried out for 1 h at 37 °C with continuous shaking. Lipids were extracted as previously described[4] or by the procedure of DOLE and MEINERTZ[5]. Free and esterified cholesterol were separated on silicic acid columns[6] and assayed for ^{14}C as previously described[4]. Protein concentration of the pancreatic juice was determined by the biuret reaction[7].

The results show that there was no difference between the 2 groups of rats in either the esterification of free cholesterol or the hydrolysis of cholesteryl oleate by pancreatic juice during the 5-week feeding period (Table). In experiments not tabulated, in which $^1/_4$, $^1/_2$ and twice the amount of juice were added to the assay mixture, again no difference between the 2 groups in either reaction was observed.

Adaptation of the exocrine secretion of the pancreas of rats to a high starch diet, with an increase in amylase activity, and to a high casein diet, with an increase in chymotrypsinogen and trypsinogen activity, has been reported[8]. The present experiments did not reveal a similar adaptation to a high cholesterol diet. MURTHY, MAHADEVAN and GANGULY[2] have reported that feeding diets supplemented with 1% cholesterol to rats did not increase the hydrolysis or synthesis of cholesterol ester in preparations of pancreas or intestinal mucosa or the hydrolysis of cholesterol ester by the mucosa of the small intestine but did increase the esterification of free cholesterol by the mucosa of the small intestine. Enzymes present in both the pancreatic juice and in the small intestine probably play a role in the esterification of cholesterol during the absorption of cholesterol from the intestine into the intestinal lymphatics[2,9,10]. The present study, in conjunction with that of MURTHY, MAHADEVAN and GANGULY, indicates that, whereas the intestinal enzyme adapts to changes in the composition of the diet, the enzyme excreted into the pancreatic juice does not[11].

Esterification of free cholesterol and hydrolysis of cholesteryl oleate by pancreatic juice of rats

Weeks on diet	Esterification		Hydrolysis	
	Diet		Diet	
	Cholesterol free	2% cholesterol	Cholesterol free	2% cholesterol
1	32.9 ± 4.1	35.2 ± 2.9	56.2 ± 4.5	59.8 ± 3.6
2	29.0 ± 3.6	29.8 ± 3.9	60.0 ± 4.4	65.3 ± 4.5
3	17.1 ± 4.1	22.4 ± 3.9	70.8 ± 3.6	65.2 ± 5.0
4	21.6 ± 3.5	25.9 ± 3.4	70.2 ± 3.6	62.1 ± 3.7
5	19.6 ± 1.4	21.6 ± 2.6	61.9 ± 4.2	61.9 ± 4.8

Values are means ± S.E.

Zusammenfassung. Cholesterol-Esterifizierung und Hydrolyse des Cholesteryl oleates wurden im Pankreassaft von Ratten bestimmt, deren eine Gruppe mit einer Diät ohne Cholesterol, deren andere mit 2% Cholesterol gefüttert wurden. Es konnten keine Unterschiede zwischen den zwei Versuchsgruppen beobachtet werden.

L. K. FINAGIN[12], W. J. LOSSOW[13], and I. L. CHAIKOFF[14]

Department of Physiology, University of California, Berkeley (California, USA), August 16, 1966.

[3] L. F. FIESER, J. Am. chem. Soc. *75*, 5421 (1953).
[4] W. J. LOSSOW, N. BROT, and I. L. CHAIKOFF, J. Lipid Res. *3*, 207 (1962).
[5] V. P. DOLE and H. MEINERTZ, J. biol. Chem. *235*, 2595 (1960).
[6] B. G. CREECH and B. SEWELL, Analyt. Biochem. *3*, 119 (1962).
[7] A. G. GORNALL, C. J. BARDAWILL, and M. M. DAVID, J. biol. Chem. *177*, 751 (1949).
[8] A. B. ABDELJLIL, A. M. VISANI, and P. DESNUELLE, Biochem. biophys. Res. Commun. *10*, 112 (1963).
[9] W. J. LOSSOW, R. H. MIGLIORINI, N. BROT, and I. L. CHAIKOFF, J. Lipid Res. *5*, 198 (1964).
[10] C. R. BORJA, G. V. VAHOUNY, and C. R. TREADWELL, Am. J. Physiol. *206*, 223 (1964).
[11] This investigation was supported by a grant from the U.S. Public Health Service.
[12] Soviet Union exchange student, academic year 1965–66. Present address: Kiev State University, Institute of Physiology, Kiev 17 (USSR).
[13] Present address: Donner Laboratory of Medical Physics, University of California, Berkeley (California, USA).
[14] Deceased January 25, 1966. This study was initiated before Dr. CHAIKOFF's death; the manuscript was prepared after his death.