able, the question of codon universality can be more thoroughly and explicitly evaluated by the binding assay. Nevertheless, the various species examined represent an ever-increasing range of evolutionary diversity. Of the 20 naturally occurring amino acids so far studied (Table 3) probably 16 are universally determined by the same code words as found for the respective amino acids when the *E. coli* system is used. Of these amino acids leucine is the only one which clearly shows the unique feature of multiple codons. Further data is required to compare the initiation, termination, and nonsense code words which exist in various phylogenetically divergent species.

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References and Notes

1. M. W. Nirenberg and O. W. Jones, Jr., in *Symposium on Informational Macromolecules*, H. J. Vogel, V. Bryson, J. O. Lampen, Eds. (Academic Press, New York, 1963), p. 451; R. Brimacombe, J. Trupin, M. W. Nirenberg, P. Leder, M. Bernfield, T. Jaouni, *Proc. Nat. Acad. Sci. U.S.* **54**, 954 (1965).
2. J. J. Protass, J. F. Speyer, P. Lengyel, *Science* **143**, 1174 (1964); R. Sager, I. B. Weinstein, Y. Ashkenazi, *ibid.* **140**, 304 (1963); J. V. Etten, B. Parisi, O. Ciferri, *Nature* **212**, 932 (1966).
3. C. Basilio, M. Bravo, J. E. Allende, *J. Biol. Chem.* **241**, 1917 (1966).
4. I. B. Weinstein and A. N. Schechter, *Proc. Nat. Acad. Sci. U.S.* **48**, 1686 (1962); E. S. Maxwell, *ibid.*, p. 1639; R. S. Gardner, A. J. Wahba, C. Basilio, R. S. Miller, P. Lengyel, J. F. Speyer, *ibid.*, p. 2087; M. Ochoa, Jr., and I. B. Weinstein, *ibid.* **52**, 470 (1964).
5. J. Marmur and P. Doty, *J. Mol. Biol.* **5**, 109 (1962).
6. M. W. Nirenberg and P. Leder, *Science* **145**, 1399 (1964).
7. M. W. Nirenberg, in *Methods Enzymol.* **6**, 17 (1963).
8. G. Zubay, *J. Mol. Biol.* **4**, 347 (1962).
9. M. W. Nirenberg, J. H. Matthaei, O. W. Jones, Jr., *Proc. Nat. Acad. Sci. U.S.* **48**, 104 (1962).
10. D. Nathans and F. Lipmann, *ibid.* **47**, 497 (1961).
11. M. F. Singer and J. K. Guss, *J. Biol. Chem.* **237**, 182 (1962).
12. O. W. Jones, Jr., and M. W. Nirenberg, *Proc. Nat. Acad. Sci. U.S.* **48**, 2115 (1962).
13. Abbreviations used: tRNA, transfer RNA; Phe-, Arg-, Gly-, Leu-, Val-, Ser-, and Lys-tRNA, phenylalanyl-, arginyl-, glycyl-, leucyl-, valyl-, seryl-, and lysyl-tRNA, respectively; U, uridine; C, cytidine; A, adenosine; G, guanosine; tris, tris(hydroxymethyl)aminomethane; GTP, guanosine triphosphate; *A*, absorbancy.
14. We thank Dr. M. W. Nirenberg, in whose laboratory this week was performed, for many helpful discussions.
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Hydrocarbons in Digestive Tract and Liver of a Basking Shark

Abstract. *The hydrocarbons of zooplankton pass through the digestive tract of the basking shark without fractionation or structural modification. They are resorbed in the spiral valve and deposited in the liver. In contrast to unsaturated fatty acids, the olefinic hydrocarbons are not decreased in concentration. The hydrocarbon assemblage in the digestive tract and in the liver is indicative of the food sources and feeding grounds of the shark. Squalene, abundant in shark liver, occurs only in traces in zooplankton; phytane, if present at all, constitutes less than 0.005 percent of the hydrocarbons of zooplankton and of shark liver.*

A juvenile male basking shark [*Cetorhinus maximus (Gunnerus)*], 3.9 m, nose to caudal notch, was harpooned off Menemsha, Martha's Vineyard, Massachusetts, in Vineyard Sound on 7 July 1966. The animal was bled and brought into Eel Pond, Woods Hole, Massachusetts, on the same day. It was dissected, and the contents of the digestive tract and several sections from the same lobe of the liver were taken for analysis. Samples for organic analysis were frozen and maintained at −15°C (see Table 1). The basking shark feeds on plankton; therefore, a sample of mixed zooplankton from the Gulf of Maine was analyzed for comparison with the samples from the shark.

The cardiac stomach of the shark contained large quantities of copepods, few larger planktonic animals, and only traces of filamentous algae. Many recognizable specimens of growth stages III and IV of *Calanus hyperboreus* and *C. finmarchicus* were present. Both are abundant at this time of the year in the deeper waters of the Gulf of Maine. A single *Centropages typicus* (adult male) was identified. This neritic copepod is common to the surface waters of the area where the shark was encountered.

The material in the pyloric stomach contained much less water than that in the cardiac stomach. Individual copepods were no longer recognizable. Small amounts of free, red copepod oil appeared in the bursa entiana. No free oil and little extractable lipid material were found in the light tan paste contained in the spiral valve.

The lipids were extracted by homogenizing the samples with anhydrous sodium sulfate under pentane. Fractions (10 μl) of the solvent-free lipids were chromatographed on a 1-ml bed of silica gel (*1*) deactivated with 10 percent water. The saturated hydrocarbons together with the mono-, di-, and triolefins were quantitatively eluted by 1.5 ml of *n*-pentane. This fraction was analyzed by gas chromatography on FFAP (*2*). Duplicate analyses of the same sample of basking shark liver oil (Table 1, last two columns) indicate good analytical reproducibility. Normal heptadecane, whose elution follows the very large pristane peak, is determined with less accuracy than the remaining compounds. Squalene was determined by hydrogenation of the lipids in isooctane with platinum oxide, followed by chromatographic recovery of the saturated hydrocarbons and by gas chromatography as squalane.

To search for phytane, a larger quantity of the hydrocarbon concentrate obtained by chromatography was chromatographed again over activated silica gel (120°C). The saturated hydrocarbon fraction containing pristane and possibly phytane, together with the normal paraffins, was collected and analyzed by gas chromatography. Phytane would have been detected at a ratio of 1 part phytane to 20,000 parts pristane. None was found, either in the oil of the copepods collected in the Gulf of Maine or in the liver oil of the basking shark.

Structures, gas chromatographic retention data, and infrared band positions of the C_{19} monoolefins and of the C_{20} diolefins have been reported (*2*). The structures of the olefins D and E (Table 1) are still unknown. Both compounds have the carbon skeleton of pristane to which they are converted by hydrogenation. Gas chromatographic retention data suggest that compound D is possibly a diolefin, and compound E a triolefin.

The analytical data indicate little or no differentiation between saturated and olefinic hydrocarbons in the digestive tract and in the liver of the basking shark. The drop in concentration of C_{20} diolefins between the pyloric limb of the stomach and the spiral valve is not reflected in the content of C_{19} di- and triolefins. Thus, it may show differences in the food rather than a gradual hydrogenation or destruction of the more unsaturated compounds during digestion. The relative

Table 1. Hydrocarbons in zooplankton in the organs of the basking shark. Mixed zooplankton was obtained from Gosnold cruise 50, at 42°38'N, 69°35'W, on 9 September 1964 with a No. 2 net. A: 2,10,14-trimethyl-6-methylenepentadecane. B: 2,6,10,14-tetramethylpentadecene-1 (norphytene). C: 2,6,10,14-tetramethylpentadecene-2. D: presumed C_{19} diolefin with pristane skeleton; retention index on Carbowax C20M, 1832. E: presumed C_{19} triolefin with pristane skeleton; retention index on Carbowax C20M, 1885. F: 3-methylene-7,11,15-trimethylhexadecene-1. G: 3,7,11,15-tetramethylhexadecadiene-1,3 (*cis*). H: 3,7,11,15-tetramethylhexadecadiene-1,3 (*trans*). I: 3,7,11,15-tetramethylhexadecadiene-2,4.

Squalene (%)	Pristane (%)	Other hydrocarbons (in parts per 10,000 parts of pristane)									
		Alkanes		C_{19} olefins				C_{20} diolefins			
		$C_{15}H_{32}$	$C_{17}H_{36}$	A	B+C	Di (D)	Tr (E)	F	G	H	I
				Zooplankton, lipids							
0.014	1.0	11	7	4	65	7	36	3	10	66	
				Cardiac stomach of shark, lipids							
	0.73	23	15	4	39	5	55	2	14	31	14
				Pyloric stomach of shark, lipids							
	0.40	21	30	9	43	9	41	3	11	13	
				Bursa entiana of shark, lipids							
	0.86	17	24	6	39	6	40	2	13	18	1
				Spiral valve of shark, lipids							
	0.77	18	9	5	48	5	51	2	11	18	
				Sample 1 of shark liver oil							
20.7	1.11	12	4	9	57	8	51	4	22	60	
				Sample 2 of shark liver oil							
11.8	1.31	11		8	61	7	47	5	21	56	
				Sample 2 (rerun) of shark liver oil							
		12	4	8	57	6	41	4	21	49	

stability of the olefinic hydrocarbons in the digestive system of the shark is surprising, in view of the deep alteration of the fatty acids. The polyunsaturated C_{20} and C_{22} acids typical of the copepod lipids are almost entirely replaced in the shark liver by the corresponding monounsaturated acids. Thus, of the total C_{22} acids, polyunsaturates $C_{22:2}$ through $C_{22:6}$ amount to 53 percent in the copepod oil but to only 2 percent in the basking shark liver oil.

Squalene, which occurs only in small amounts in zooplankton, is synthesized by the shark. The two analyses represent different sections of the same lobe of the liver, which contained 74 and 71 percent pentane soluble oil by wet weight; they indicate a much less even distribution of squalene than of pristane and of the related olefinic hydrocarbons.

In the absence of hydrocarbon differentiation in the digestive tract of the shark, the liver acts as a long-term integrator of the hydrocarbon distribution patterns encountered in the food. Analyses of the hydrocarbons from mixed marine zooplankton show a rapid decrease in pristane and an increase in C_{19} and C_{20} olefins from the Gulf of Maine toward the mid-Atlantic at 42°N (*3*). This is due to the corresponding decrease in the pristane-rich copepods of *C. hyperboreus* and *C. finmarchicus*. The excellent agreement between hydrocarbon-type distribution of the basking shark liver and zooplankton from the Gulf of Maine suggests that the shark spent much of the time required for the accumulation of its liver lipids in the colder waters of the Gulf of Maine or of the North Atlantic.

The small number of local copepods in the stomach of the shark, together with large numbers of *Calanus* and hydrocarbons derived from *Calanus*, suggests that this animal either moved south just before being caught or that it had not recently fed in the local waters.

Hydrocarbon analysis is potentially useful in studies of the movement of marine animals; more knowledge of the relative turnover rates of food, hydrocarbons, and body lipids of these animals is needed.

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References and Notes

1. Silica gel (grade 922, through 200 mesh) was obtained from Davison Chemicals, Baltimore, Md. The FFAP was obtained from Varian Aerograph, Walnut Creek, Calif.
2. M. Blumer and D. W. Thomas, *Science* **147**, 1148 (196➡———, *ibid.* **148**, 370 (1965).
3. M. Blumer and J. Sass, in preparation.
4. Supported by ONR (N0014-66-contract CO-241) and NSF (GA-539). F. G. Carey dissected the basking shark and provided the samples; G. Calef analyzed the plankton. Contribution 1894 of the Woods Hole Oceanographic Institution.

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Gastrin Antibodies: Induction, Demonstration, and Specificity

Abstract. *Antibodies to the antral hormone gastrin have been induced in the rabbit and detected by passive hemagglutination. Specificity of the antibody, as determined by three methods, is directed to gastrins I and II, gastrin pentapeptide, and gastrin tetrapeptide, as well as to the stage-I gastrin used for immunization.*

Determination of the biologic activity of gastrin since its postulation as a gastric acid secretagogue in 1905 (*1*) has been limited to assays in animals in vivo. Complete chemical definition of the gastrin molecule (*2*) has made it possible to investigate the use of immunologic procedures for determining gastrin, similar to those used for other peptide hormones (*3*). Antibodies to gastrin have already been demonstrated by inhibition of the biologic activity of the hormone; the antibody was not detected immunologically, however, and the specificity of the antibody was not determined (*4*). We now describe the induction and detection of antibodies specific for gastrin.

Partially purified gastrin (stage I), prepared from hog antrum by the method of Gregory and Tracy (*5*), was chosen for immunization since it appeared that pure gastrin probably would not be antigenic by itself because of its low molecular weight. Pure gastrin has been repeatedly administered to human subjects over a period of 12 months without evident production of antibody (*6*), and it was reasoned that the impurities contained in the partially purified gastrin preparation (stage I) would perform a carrier function. Albino rabbits were immunized by subcutaneous and intramuscular injection of 50 mg of gastrin (stage I, in Freund adjuvant) weekly for 9 weeks. Blood was obtained for determination of antibody titers at 3 and 6 weeks, and weekly thereafter.

Antibody titers were determined by use of a system of passive hemagglutination (*7*). Human group-O erythrocytes treated with bis-diazotized benzidine (*7*) were coupled with each of the following as detector antigen: stage-I gastrin, a lyophilized preparation of gastrins I and II (*8*), gastrin pentapeptide (*9*), and gastrin tetrapeptide (*9*). The erythrocytes were then washed three times with Sorenson phosphate buffer (*p*H 7.3, 0.15*M*), and there followed