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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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

a single washing with normal, heat-inactivated, absorbed rabbit serum diluted 1:100 in Sorenson phosphate buffer. The washed erythrocytes were resuspended in rabbit serum similarly diluted with Sorenson phosphate buffer.

Erythrocytes exposed to antigen without pretreatment with bis-diazotized benzidine did not show hemagglutination in the presence of antibody, presumably because of failure of the cells to bind the antigen. Erythrocytes treated with bis-diazotized benzidine, but not coupled with antigen, also showed no hemagglutination. When gastrins I and II were used as the detector antigen, complete antibody titers of 256, 128, and 512, respectively, were determined in three rabbits 6 weeks after initial immunization. Comparable titers were determined by use of stage-I gastrin, gastrin pentapeptide, or gastrin tetrapeptide as detector antigen coupled to the erythrocytes. The use of goat anti-rabbit gamma globulin in the detection procedure increased the titers mentioned to 1024, 512, and 2048, respectively.

Since a pure antigen was not used to incite antibody production, it was necessary to establish the specificity of the antibody by several different methods. This specificity was first demonstrated by hemagglutination with gastrin pentapeptide, gastrin tetrapeptide, and gastrins I and II, as well as stage-I gastrin, as detector antigens on the erythrocytes. For hemagglutination to occur with the peptides as detector antigens, specificity must necessarily be directed to this portion of the gastrin molecule.

Specificity of the antibody was also demonstrated by inhibition of passive hemagglutination (*7*); a titer of antiserum to gastrin gave 4+ hemagglutination. When gastrins I and II were used as detector antigen coupled to the erythrocytes, hemagglutination was completely inhibited when less than 0.25 μg of stage-I gastrin, gastrins I and II, gastrin pentapeptide, or gastrin tetrapeptide was preincubated with 0.1 ml of the antiserum (Table 1). The relative effectiveness of gastrins I and II, pentapeptide, and tetrapeptide as inhibitors of hemagglutination appears to be related to their molecular weights. The lesser amounts of the two peptides (that of gastrins I and II) necessary for complete inhibition of hemagglutination is noted as evidence of the specificity of the antibody for a specific portion of the gastrin molecule.

One cannot compare the relative effectiveness of stage-I gastrin as an inhibitor on a molar basis because of the impurities contained in the preparation. The increased amounts necessary for complete inhibition of hemagglutination may reflect the impurities contained in the stage-I gastrin preparation. This idea is supported by the data obtained when stage-I gastrin was used on the erythrocytes as the detector: in this case smaller amounts of stage-I gastrin than of gastrins I and II were required to inhibit hemagglutination completely (Table 1).

Table 1. Specificity of antibody as determined by inhibition of passive hemagglutination. The inhibitor was preincubated with 0.1 ml of antiserum before reaction in the passive hemagglutination system. Detector was the antigen coupled to the erythrocytes by bis-diazotized benzidine. Complete inhibition is expressed as the least amount of inhibitor necessary to inhibit hemagglutination completely. Each value is the mean of six determinations.

Inhibitor	Complete inhibition (μg)
Detector: gastrins I and II	
Stage-I gastrin	0.25
Gastrins I and II	.16
Gastrin pentapeptide	.10
Gastrin tetrapeptide	.05
Detector: stage-I gastrin	
Stage-I gastrin	0.12
Gastrins I and II	.33
Detector: gastrin pentapeptide	
Stage-I gastrin	0.08
Gastrins I and II	.10
Gastrin pentapeptide	.14
Detector: gastrin tetrapeptide	
Stage-I gastrin	0.11
Gastrins I and II	.12
Gastrin tetrapeptide	.20

Specificity of the antibody was further determined by experiments in which antibody activity was completely absorbed by erythrocytes coupled with gastrins I and II, gastrin pentapeptide, or gastrin tetrapeptide. The absorption was complete when stage-I gastrin, gastrins I and II, or gastrin pentapeptide was used as the detector antigen coupled to the erythrocytes.

Each of these three methods served to establish that the specificity of the antibody is directed not only toward the original antigen (stage-I gastrin), but also to the pure gastrins I and II. In addition, specificity of the antibody is directed toward a portion of the gastrin molecule: that is, the gastrin pentapeptide and the gastrin tetrapeptide.

In fractionation of the antiserum on a column of Sephadex G-200, the antibody was eluted in the fractions corresponding to a 19*S* immunoglobulin (*10*).

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Viral Hemorrhagic Encephalopathy of Rats

Abstract. *A virus has been isolated and serially passed in suckling rats; it causes an acute fatal paralysis associated with hemorrhage and necrosis in the brain and spinal cord. The agent is relatively resistant to heat and ether, is about 20 millimicrons in diameter, and is antigenically closely related to rat virus. Its isolation resulted from the study of occasional cases of paralysis in adult rats after administration of cyclophosphamide.*

In order to produce transient immunosuppression, 500 adult Lewis rats were given cyclophosphamide in a single intraperitoneal dose of 100 to 150 mg per kilogram body weight. Paralysis, usually of the hind limbs, and usually beginning 2 to 3 weeks after cyclophosphamide injection, was observed in less than 2 percent of treated animals, but never in untreated animals.

Four affected rats were killed, under pentobarbital anesthesia, by perfusion with sterile balanced saline, within 1 to 3 days after onset of paralysis. The brain and cord were combined and homogenized in balanced saline containing