ted for each sample (Fig. 2). The control, treated with normal mouse serum, showed the characteristic double peak. Treatment with *H-2*^{a} antiserum to H-2^b (reactive with both host and tumor cells) abolished both peaks, a demonstration that both classes of cells were sensitive to cytotoxic antibody. However, the antiserum to H-2^a (reactive only with host cells), killed only the smaller cells, and the antiserum to Moloney antigen (reactive only with tumor cells) killed only the larger ones. Thus, tumor cells are confined to the second peak, and the first peak represents cells derived from host tissues.

Similar second peaks also appear in the curves from spleen and thymus cells of mice inoculated with lymphoma. We have used this fact to study the growth of YHA in *H-2*^{a}*H-2*^{b} (F_1) hybrids (C3H × C57B1 or C3H × A.BY). The results, similar with both genotypes, were pooled. Groups of mice, inoculated from the same suspension with 10^6 tumor cells, were killed at intervals. Size-distribution curves plotted for cells from spleen, pooled peripheral lymph nodes, and thymus of each mouse, were used together with the curves from corresponding tissues from untreated animals, to calculate the percentage of neoplastic cells within each organ. We have used this value, rather than the absolute number of lymphoma cells in the preparations, in order to reduce errors due to variation in the proportion of cells recovered from the organs, the number of cells lost upon removal of dead cells by trypsin, and other nonspecific factors. We have expressed only the percentage of tumor cells within the organs and have taken no account of the probable changes in the number of host cells within these organs during growth of the lymphoma.

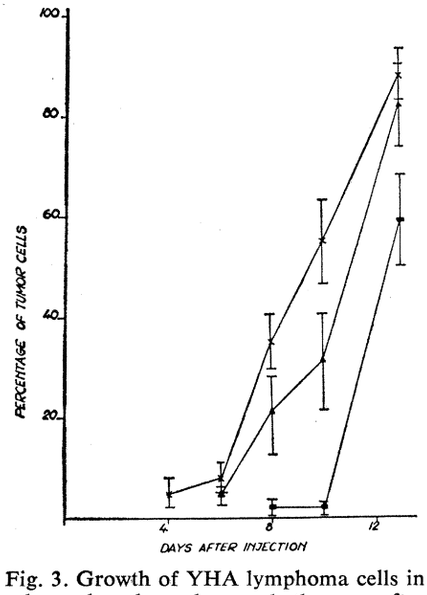

Fig. 3. Growth of YHA lymphoma cells in spleen, lymph nodes, and thymus after intravenous injection (10^6 cells per mouse) into (C3H × C57B1) or (C3H × A.BY)F_1 mice. Proportions of normal and tumor cells were calculated from the heights of the corresponding peaks in size distribution curves (Fig. 1). A correction was applied for overlapping of the peaks by reference to size distribution curves of cell suspensions prepared from corresponding organs of uninoculated mice. Arithmetical mean and standard error of the mean are given for each point; groups of five to seven mice were used. Spleen (×), lymph nodes (▲), and thymus (■) of each mouse were examined.

The three curves are displaced in time (Fig. 3) and indicate that lymphoma cells increase in the spleen about 1 day sooner than in the lymph nodes. Tumor cells appeared in the thymus 3 to 4 days later and were only found in large numbers 13 days after inoculation. At this late stage of tumor growth, other tissues of the host were also invaded and neoplastic cells could be detected, for example, in cell suspension prepared from lung. The data suggest that multiplication of the lymphoma cells takes place within the spleen and lymph nodes, but the late increase in the thymus may be accounted for by massive invasion from without.

Samples of the cell suspensions prepared from the organs of F_1-hybrid hosts inoculated with YHA were also treated with cytotoxic *H-2*^{k} antiserum to H-2^b (reactive only with host cells) in an attempt to detect the first appearance of lymphoma cells. Details of the technique were as described for treatment of Y7A. Less than 5 percent of lymphoma cells admixed with stroma cells can be detected in this way, but more precise quantitation is unreliable. Table 1 shows the ratio of the number of mice with detectable tumor cells to the number of mice examined. These figures also show earliest appearance of tumor cells in the spleen, followed by lymph nodes, followed by thymus (Fig. 3).

Attempts to apply the same technique for the detection of lymphoma cells to bone marrow have failed, because there are two sets of cells in normal bone marrow; the larger of these cells are similar in size to the tumor cells and are also resistant to the cytotoxic action of antibody to H-2 in the presence of complement. Lymphoma cells are thus masked.

This technique for detection and counting of tumor cells within organs offers (i) objectivity; (ii) sensitivity; (iii) speed; (iv) a method for measuring rate of tumor increase within the tissues of the host; and (v) wide applicability for study of tumor growth. The cells of sarcomas, carcinomas, and similar tumors are even easier to detect than are leukemic cells because of their even larger volume.

In order to avoid possible misinterpretations of size-distribution curves, it is, however, important that neoplastic cells be positively identified as described for each new tumor examined.

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Absence or Singular Specificity of Carotenoids in Some Lower Fishes

Abstract. *No colored carotenoids were recovered from the scales or liver of the coelacanth,* Latimeria chalumnae, *or from skin, immature eggs, or liver of the Pacific hagfish,* Eptatretus stoutii. *Zeaxanthin, present as esters in the skin and largely unesterified in the liver, was the only carotenoid identified in the thornback ray,* Platyrhinoidis triseriata, *and in the horned shark,* Heterodontus francisci, *whereas the skin of the Pacific mako shark,* Isurus glaucus, *yielded no carotenoids.*

An earlier review (*1*), surveying the wide occurrence of carotenoids in fishes, indicated the preponderance of the oxygenated or xanthophyllic class, while the hydrocarbon or carotene type was represented uncommonly and in minor proportions. Two xanthophylls resembling taraxanthin and lutein, which are common in plants, were encountered most frequently; astaxanthin

was placed third in order; and zeaxanthin was listed as being of rare or questionable incidence.

Among the lower fishes, the larvae and the adult skin of two cyclostome lampreys, *Lampetra fluviatilis* and *L. planeri*, yielded lutein, which was reported also to be present in the skin of two elasmobranch species, the rays *Raja batis* and *R. clavata*.

More recently, Fisher (*2*) reported concentrations of total lipids, vitamin A, and collective carotenoids in livers from 17 species of sharks and dogfishes, 13 species of skates and rays, and from the chimaerid species, *Chimaera monstrosa*. He found wide variations, average values equivalent to the extremes of 0.06 to 2.3 mg/100 g wet weight for the sharks and dogfishes, and from 0.08 to 1.3 mg/100 g for the livers of the skates, rays, and chimaerid. The greater average figure for the latter group was considered as due, perhaps, to the composition of the natural food consumed. Fisher did not identify the carotenoids present, but measured the light-absorption of the collective yield at 450 mμ in normal hexane, a maximum close to that of β-carotene and to that of its mono- and dihydroxy derivatives, cryptoxanthin and zeaxanthin.

We report here on two species, widely separated in the evolutionary procession, that apparently are without colored carotenoids and two elasmobranchs whose liver and skin yielded only zeaxanthin.

Specimens of the hagfish, *Eptatretus stoutii*, were captured in baited screen-traps at depths of about 200 m a few kilometers seaward from San Diego (*3*). We received scales and liver tissue from a specimen of the coelacanth, *Latimeria chalumnae*, which was nearly 2 m in length, taken on 11 December 1964 from waters off Mutsamudu, Anjouan, Isles Comores (*4*). The tissues had been preserved in 10 percent formaldehyde promptly after capture of the animal. A piece of the preserved liver, sectioned on 8 February 1965, was analyzed 2 days later. The thornback ray, *Platyrhinoidis triseriata*, the horned shark, *Heterodontus francisci*, and the Pacific mako shark, *Isurus glaucus*, were caught in neighboring waters.

Each tissue to be analyzed (except the scales) was minced with scissors and finely comminuted in several volumes of ethanol in a Waring Blendor. The solid and fluid materials were transferred to glass-stoppered flasks and were covered with an atmosphere of nitrogen; they were then stored overnight or longer in a dark refrigerator before being clarified through celite and washed until the filtrate showed no more pigment. All lipids and lipid-soluble pigments were transferred to *n*-hexane. Carotenoids were resolved into chromatographically separable fractions on columns of MgO and celite (1 : 1); they were eluted individually with hexane that contained graded concentrations of methanol from 0.1 to 5.0 percent.

Table 1. Assays for carotenoids in five species of lower fishes.

Tissue and wet weight	Color of ethanol extract	Total carotenoids (mg/100 g)	Identity and proportions of carotenoids (%)
	Cyclostome: *Eptatretus stoutii* (hagfish)		
Captive 3 mo at 7°C			
Liver (1.99 g)	Colorless	None	
Skin (4.60 g)	Colorless	None	
Captive 1 wk at 7°C			
Liver (1.51 g)	Colorless	None	
Eggs	Colorless	None	
	Crossopterygian: *Latimeria chalumnae* (coelacanth)		
Preserved 2 mo with formaldehyde			
Scales	Colorless	None	
Liver piece (33.56 g)	Pale cloudy yellow	None; much lipid; typical reactions for sterols by Salkowski's and Liebermann-Burchard tests	
	Elasmobranch: *Platyrhinoidis triseriata* (thornback ray)		
Skin (10.85 g)	Yellow	0.17	Zeaxanthin esters, 100
Liver (3.47 g)	Yellow	7.86	Free zeaxanthin, 93; zeaxanthin esters, 7
	Elasmobranch: *Heterodontus francisci* (horned shark)		
Skin (6.91 g)	Yellow	1.92	Zeaxanthin esters, 100
Liver (14.12 g)	Yellow	4.57	Free zeaxanthin, 65.3; zeaxanthin esters, 34.7
	Elasmobranch: *Isurus glaucus* (Pacific mako shark)		
Skin (10.44 g)	Colorless	None	

For identification with authentic carotenoids we used thin-layer chromatography with silica gel as adsorbent and with 20 percent acetone in hexane, or equal volumes of ethyl acetate and benzene, as eluants in a modified Davies concentration chamber (*5*). Profiles of the absorption spectra and concentrations of carotenoid were determined with a Bausch & Lomb 505 recording photoelectric spectrophotometer; the factor for β-carotene, the ratio of the molecular weight to the extinction coefficient 0.0041, was used throughout (Table 1).

No traces of pigment soluble in ethanol were found in the skin and liver of one hagfish specimen and the liver of a second one, more recently captured. Also, the pale-yellow or dirty cream-colored maturing eggs from a recently captured specimen yielded no carotenoid after these eggs were ground in ethanol and allowed to stand in it for several days; the solvent was then filtered. *Eptatretus stoutii* thus differs from the two lutein-storing *Lampetra* species.

The coelacanth, *Latimeria chalumnae* (of the crossopterygian order Actinistia which may be dated back to the lower Carboniferous), yielded no carotenoid from its heavy, partially melanized scales overlying the white flesh when these scales were soaked in hot ethanol. A section of the formaldehyde-preserved liver (wet weight, 33.56 g; dry weight, 40 percent of wet weight) contained much waxy lipid material and yielded pale-yellow ethanolic and hexane extracts. Hydrolysis of the whole extract left very little pale-yellow, hexane-soluble material, which showed a green fluorescence under ultraviolet illumination and showed no spectral absorption maxima in the visible portion, but only a steady rise into the blue-violet region. Upon treatment with concentrated H_2SO_4 alone or in the presence of acetic anhydride (Salkowski's and Liebermann-Burchard reactions), chloroform solutions of the hexane extract exhibited series of blue, green, and red colors typical of unsaturated sterols. The liver and other

tissues had been preserved with 10 percent formaldehyde solution for the 2-month period since the animal's capture; this should have denatured proteins, including any lipoxidases that might otherwise have tended to degrade carotenoids. Thus it was surprising to encounter such a firm, lipid-rich section of liver lacking in these pigments.

The skin of both the thornback ray and the horned shark yielded only xanthophyllic esters, while the uncombined xanthophyll (93 percent of total carotenoids) predominated over its esters in the ray's liver and comprised two-thirds of the carotenoids in the horned shark's liver.

The liver of *Platyrhinoidis* and that of *Heterodontus* each yielded two chromatographically separable esters and the skin of the latter, three ester fractions; but all xanthophyllic material, whether free or esterified, showed the same absorption profile within minor limits of error. The typical values were: 475, 447.3, and an inflection or sloping shoulder at about 424 mμ, but varying between 420 and 425 mμ. All 13 values for the first maximum were located from 474 to 476 mμ except for one fraction which gave a maximum centering at 471, and another, a value of 478 mμ. All loci for the chief maximum not actually at 447 mμ were within 2 or 3 mμ above or below it. Minor departures of this kind, if not indeed within instrumental error, may arise either from a degree of isomerization or from the presence of fatty acid conjugants or incidental lipids.

The spectral absorption maxima of the xanthophyll present in these fishes agreed very closely with those of authentic zeaxanthin: 475, 447, and 422 to 424 mμ in the same solvent (*n*-hexane) and measured with the same instrument. The experimental xanthophyll likewise exhibited the same partition ratio (11 : 89) between hexane and 95 percent methanol as pure zeaxanthin does [as measured by Petracek and Zechmeister (*6*) and by us].

Co-chromatography of a purified sample (from *Platyrhinoidis* liver) with isozeaxanthin, prepared by us from canthaxanthin, showed wide separation between the two xanthophylls. Moreover, Goodwin's test (*7*) for allylic hydroxyl groups resulted in noticeable darkening of isozeaxanthin and reversed the hypophasic behavior, but the shark-liver xanthophyll remained unchanged, as zeaxanthin also does.

Finally, an isolated sample of the xanthophyll, obtained from the hydrolytic treatment of collective carotenoids from *Heterodontus* liver, was co-chromatographed on thin-layered silica gel with some of the authentic, crystalline zeaxanthin (*8*). The two gave identical R_F values on individual chromatograms and a single zone when combined in the same solution, which shows the identity between our xanthophyll and zeaxanthin.

It should be emphasized that contaminating lipids may distort chromatographic zonation as well as behavior on partition and position of absorption maxima. Accordingly, it was necessary sometimes to first adsorb a xanthophyll ester upon powdered MgO or Al_2O_3, discard the supernatant colorless, cloudy solvent, and then rinse the colored powder several times before eluting the carotenoid in preparation for diagnostic examination (*5*).

Our values for the collective liver carotenoid fractions (7.86 and 4.57 mg/100 g, respectively, for the ray and the shark analyzed), although somewhat higher than Fisher's, lie well within the order of magnitude of his analyses, while the skin of the shark yielded more than ten times as much zeaxanthin esters as the skin of the ray. It was surprising that no carotenoid was recovered from the skin of a pelagic shark, *Isurus glaucus*, after a 3-day extraction with ethanol, since Fisher's findings would suggest that carotenoids are present, although in relatively low concentrations, in the livers of all or most sharks, and hence might be expected also, at least in minor quantities, in the integument of this species, as in other fishes (*1*).

Note added in proof: Since this report went to press, Professor C. L. Hubbs kindly made available to us for analysis 70.95 g (wet weight) of liver tissue from a black-skinned chimaerid (*Hydrolagus* sp., being described by him), measuring about 1 m in length, freshly captured off Baja California at a depth of 1200 m. The dark, melanistic, very fatty liver involved less than 0.04 mg of suspected carotenoids, whose recovery was rendered impracticable by the presence of the high concentrations of lipids.

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Gastric Secretion: Mechanism for Production of Hydrogen Ions

Abstract. *The passage of a direct electric current across a fixed-charge membrane interposed between neutral electrolyte solutions can give rise to the production of hydrogen and hydroxide ions at the solution-membrane boundary in equivalent amounts, each of which can approach and equal the number of faradays passed. At this unique boundary, the electric field can inhibit the rate of recombination of hydrogen and hydroxide ions strongly enough to increase the dissociation constant of water and other weak electrolytes by several orders of magnitude. These observations lead to a model of the gastric secretory process wherein the gastric potential, primarily due to the activity of a chloride-ion pump, is applied across an adjacent ion-selective membrane also present in the mucosa. The dissociating weak electrolyte can be water, to produce hydrogen ions in the stomach and hydroxide ions which combine with carbon dioxide in the blood, or it can be carbonic acid to produce bicarbonate directly.*

Gregor and Peterson (*1*) have used a new experimental approach to the study of the production of acid and base at the boundary between a neutral electrolyte solution and an ion-exchange membrane under conditions of electrodialysis. These studies were suggested by observations of Bethe and Toropoff (*2*) who measured small *p*H shifts when studying electroosmosis.