and hitherto unrecognized process in the ecology of salmon.

Salmonids have usually been considered to be strictly planktivorous, and the importance of benthic food resources has only recently been recognized (*3*, *11*). Harpacticoid copepods are of particular importance to chum fry (*12*). These fish spend the first critical weeks of their sea life in river mouths and along beaches feeding on harpacticoids and other small benthos. The food of the harpacticoids is the bacterial flora associated with organic detritus. The estuary receives pulsed inputs of detritus from several sources: (i) *Zostera* spp. meadows from the seaward areas, (ii) algae from the intertidal areas, (iii) *Carex* spp. marsh from the landward areas, and (iv) downstream transport from the upland areas of the watershed.

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2. The numbers of salmon spawning in the Nanaimo River range are as follows: *Oncorhynchus keta*, 22,000 to 70,000; *O. kisutch*, 800 to 6,000; and *O. tshawytscha*, 850 to 1,950 [K. V. Aro, *Fish. Res. Board Can. Manuscr. Rep. 1178* (1972)].
3. *An Environmental Assessment of Nanaimo Port Alternatives* (Lands Directorate, Canadian Department of Environment, Ottawa, 1974); J. Sibert, *Fish. Res. Board Can. Tech. Rep. 537* (1975); unpublished data.
4. The amount of food required to increase weight by a rate of 4 percent per day is 9 percent of the body weight per day [R. J. LeBrasseur, *J. Fish. Res. Board Can.* **26**, 1631 (1969)]. The daily food requirement of the estuarine population equals number of fish × mean weight of fish × 0.090.
5. Analysis of the stomach contents of approximately 50 chum fry collected from March through June 1975 agreed with the results of more extensive samples (approximately 500) collected in previous years (*3*). Individual fry contained as many as 1000 harpacticoid copepods; the species present, in order of abundance, were *Harpacticus uniremis*, *Huntemania jadensis*, and *Tisbe furcata*.
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8. Primary productivity was estimated by injecting 500 μc of $Na_2H^{14}CO_3$ into 3.4 liters of water enclosed over the sediment surface by light and dark plexiglass chambers. After a 4-hour incubation period, sediment cores were taken and the ^{14}C activity determined in the nitric acid extract [C. Van Raalte, *Bot. Mar.* **17**, 186 (1974)]. Heterotrophic activity was estimated by adding 0.15 μc of [^{14}C]glucose (specific activity 310 μc/μmole) to 0.6 ml of sediment slurried in 30 ml of filtered estuarine water and incubated for 1 hour in the dark. This addition of glucose was less than 0.3 μg/liter, low enough to avoid stimulatory effects [J. Sibert and T. J. Brown, *J. Exp. Mar. Biol. Ecol.* **19**, 97 (1975)]. Chlorophyll, corrected for phaeopigments by acidification, was determined by the fluorometric technique of J. D. H. Strickland and T. R. Parsons [*Bull. Fish. Res. Board Can. 167* (1972)]. The ATP was determined by the techniques of O. Holm-Hansen and C. R. Booth [*Limnol. Oceanogr.* **11**, 510 (1966)] and D. M. Karl and P. A. LaRock [*J. Fish. Res. Board Can.* **32**, 599 (1975)]. The total organic carbon was determined in a carbon-hydrogen analyzer (Coleman model 33).
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9 November 1976; revised 22 December 1976

Galilean Satellites of Jupiter: 12.6-Centimeter Radar Observations

Abstract. *Observations of the Galilean satellites with the radar system at the Arecibo Observatory, Puerto Rico, show that their surfaces are highly diffuse scatterers of radio waves of length 12.6 centimeters; spectra of the radar echoes are asymmetric and broad. The geometric radar albedos for the outer three satellites—0.42 ± 0.10, 0.20 ± 0.05, and 0.09 ± 0.02 for Europa, Ganymede, and Callisto, respectively—show about the same relative decreases as do the optical albedos, although the latter presumably bear only on material much nearer the surface. Radii of 1420 ± 30, 2640 ± 80, and 2360 ± 70 kilometers for Europa, Ganymede, and Callisto were determined from the radar data and are in good agreement with the corresponding optically derived values. Io, observed successfully only once, appears to have an albedo comparable to Ganymede's, but no radius was estimated for it.*

Interest in the Galilean satellites of Jupiter has heightened in recent years as the result of an avalanche of new observations. The Pioneer 10 and Pioneer 11 spacecraft have photographed all four of these planet-sized satellites at resolutions substantially better than those obtainable in earth-based observations and have provided important information on the magnetic and plasma environment of Jupiter within which these satellites move (*1*). Ground-based observations have shown that large portions of the surfaces of Europa and Ganymede appear to be covered with water frost, whereas Callisto's surface seems to be covered mostly with darker material, perhaps siliceous in composition (*2*). Most surprising of all are the findings of substantial amounts of hydrogen, potassium, sodium, and sulfur in the environment of Io, the innermost of the Galilean satellites (*3*). The flow of new information has been so rapid that no review article is recent enough to encompass it all.

Attempts to study the Galilean satellites by radar began in 1970 with an unsuccessful effort (*4*) to detect echoes of 3.8-cm signals directed at Callisto. In 1974, an

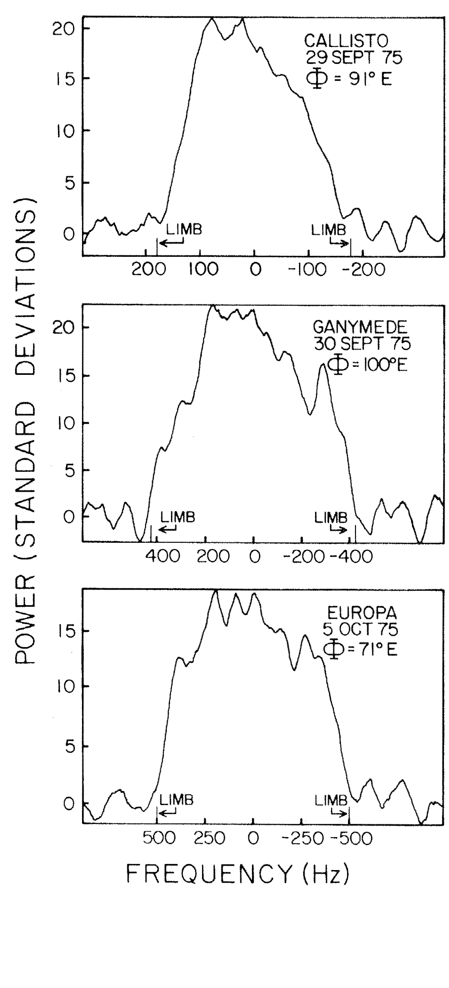

Fig. 1. Radar scattering spectra taken in same-sense polarization (see text) for Europa, Ganymede, and Callisto. Positions of the limbs are shown under the assumption of synchronous rotation and an optically derived value for the radius (see Table 1). The spectra have been smoothed by using a resolution of 10 percent of the calculated limb-to-limb bandwidth. The ordinate is given in units of the standard deviation of the noise accompanying the signal. Variations in the spectra of amplitudes of the order of 3 standard deviations are probably not significant.

attempt (5) to observe Ganymede by using a more sensitive radar system, operating at a wavelength of 12.6 cm, was apparently successful, but the echoes were quite weak. With this background in mind, we awaited with interest the opportunity in 1975 to apply the newly improved 12.6-cm Arecibo radar system (6), which has a sensitivity five times greater than any other system, to the study of the Galilean satellites.

A total of 12 nights was available for observation, distributed randomly between 28 September and 1 November 1975. An additional night was assigned for observing Io on 16 January 1976. Each night's observations consisted of a linearly polarized, continuous-wave transmission lasting approximately 1 hour (the round-trip signal flight time to Jupiter), followed by reception of the echo for the same duration. The limited antenna tracking time, a total of about 2½ hours, precluded more than one such alternation per night. Observation of only one satellite was attempted on a particular night. A choice between two modes of linear polarization for reception was available: aligned with (that is, the same as) the sense of the transmission, or orthogonal to that sense.

Because of the narrow antenna beamwidth (6), it was necessary to interrupt each observation, during both transmission and reception, at about 15-minute intervals, to monitor and correct as necessary the antenna tracking; the natural radio emission of Jupiter was used for this purpose. The interruptions during the receiving interval were timed to correspond to those in the transmitting interval by adding the round-trip signal flight time. During reception, the receiver was continuously tuned according to a precalculated ephemeris to compensate for the changing Doppler shift of the echo. Spectra of the radar echoes were obtained by Fourier transformation of a 205-msec-long series of samples of the signal waveform, each sample taken at an interval of 200 μsec. The squared magnitudes of successive transformations were summed to improve the signal-to-noise ratio. The basic frequency resolution was about 5 hertz, although convolutional broadening of this resolution has been applied before plotting in order to smooth the results.

Figure 1 shows selected spectra obtained with the same polarization sense as the transmission for the satellites Europa, Ganymede, and Callisto. Orthogonal-sense spectra look similar but noisier. Figure 2 shows the single successful (same-sense) result for Io. Two earlier attempts to observe Io were unsuccessful. These failures may have been due to substantial errors in our ephemeris, which

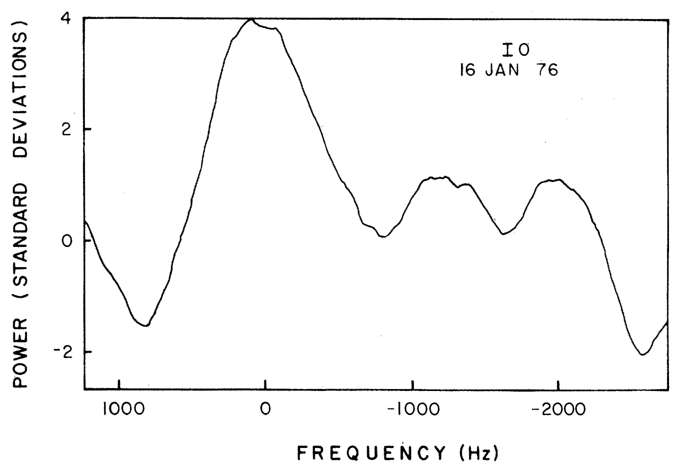

Fig. 2. Spectrum for Io for the same polarization conditions as in Fig. 1. The spectrum has been smoothed with a filter comparable to the expected spectral shape in order to enhance detectability. The limb-to-limb bandwidth for Io for the assumptions given in Fig. 1 is 2375 hertz. The orbital phase for this observation was 274°.

Table 1. Radar results from 12.6-cm observations of the Galilean satellites. Mean time of reception is given in coordinated universal time (UTC). Orbital phase was measured in the direction of orbital motion from superior geocentric conjunction with Jupiter. Received polarization was either in the same sense (S) as or in the orthogonal sense (O) to the linearly polarized transmission. Absence of a stated standard error in a radius value implies that the radius was fixed at that value for the analysis. Column 7 gives values of the exponent of the scattering law, $\sigma(\theta) \sim \cos^n\theta$. The geometric albedo includes contributions from both senses of polarization of the received signals. The weighted means and accompanying uncertainties of the radius values have been rounded to the nearest multiple of 10 km.

Target	Date	Mean time of reception (UTC)	Orbital phase (degrees)	Polarization	Radius (km)	N	Radar cross section (πa^2)	Geometric albedo
Io	16 January 1976	22 : 40	274	S			0.3 ± 0.1	
Europa	5 October 1975	05 : 26	71	S	1413 ± 26 1550	0.6 ± 0.2 1.1 ± 0.2	1.18 ± 0.30	
	27 October 1975	03 : 50	137	O	1454 ± 83 1550	1.1 ± 0.5 1.4 ± 0.4	0.49 ± 0.12	
Weighted mean for Europa					1420 ± 30	0.7 ± 0.2		0.42 ± 0.10
Ganymede	30 September 1975	06 : 00	100	S	2625 ± 89 2635	1.4 ± 0.3 1.4 ± 0.2	0.44 ± 0.11	
	1 October 1975	05 : 50	150	S	2692 ± 192 2635	2.1 ± 0.6 1.9 ± 0.3	0.53 ± 0.13	
	1 November 1975	03 : 45	269	O	4048 ± 1290 2635	4.7 ± 3.0 1.5 ± 0.3	0.30 ± 0.08	
Weighted mean for Ganymede					2640 ± 80	1.6 ± 0.3		0.20 ± 0.05
Callisto	28 September 1975	06 : 10	70	S	2362 ± 84 2500	1.3 ± 0.4 1.8 ± 0.4	0.27 ± 0.07	
	29 September 1975	05 : 45	91	S	2325 ± 115 2500	1.5 ± 0.4 2.1 ± 0.3	0.28 ± 0.07	
	28 October 1975	03 : 45	0	S	3224 ± 638 2500	3.5 ± 2.0 1.5 ± 0.3	0.25 ± 0.06	
	30 October 1975	03 : 45	43	O	2612 ± 1158 2500	4.2 ± 5.0 3.7 ± 1.0	0.11 ± 0.03	
Weighted mean for Callisto					2360 ± 70	1.5 ± 0.3		0.09 ± 0.02

was based on Sampson's theory (*7*). Further observations will be needed to distinguish this possibility from the possibility of significant variation in the radar cross section with aspect, since the orbital phase angles on the unsuccessful nights differed considerably from the value for the successful observation. The anticipated maximum limb-to-limb frequency spreads of these spectra, based on optically derived values of radius (*1*) and synchronous rotation (*8*), are shown in Figs. 1 and 2. It is immediately obvious from the broad frequency spread of the echoes that the radar scattering is unusually diffuse, very different from that seen for the inner planets and the moon (*9*). The spectra of both Ganymede and Callisto are significantly skewed, as though one side of each planet as viewed from the earth was more reflective than the other. Unfortunately, observing opportunities for these objects at substantially different orbital phases were not available in 1975, so that further investigation of the source of this asymmetry was not possible.

Because of the highly diffuse character of the scattering, it seemed appropriate to approximate it by using an angular law of the form $\cos^n\theta$, where θ is the angle of incidence, as well as of scattering, measured with respect to the local vertical to a resolved surface element. Lambert scattering corresponds to the value $n = 2$; the value $n = 1$ yields a uniformly bright disk; larger values correspond to limb darkening. Applying the definition of radar cross section (*10*) and transforming into the frequency domain under the assumption of a rigid, spherical, rotating target (*10*), we obtain

$$\sigma(f) = \frac{4\Pi^{1/2}a^2\alpha}{f_0}\,\frac{\Gamma\left(\frac{n+3}{2}\right)}{\Gamma\left(\frac{n+2}{2}\right)}\left[1-\left(\frac{f}{f_0}\right)^2\right]^{n/2} \qquad (1)$$

for the cross section per unit frequency interval and

$$\sigma = \alpha 4\pi a^2 \qquad (2)$$

for the radar cross section for the entire target, where a is the target's radius, α is its geometric albedo (*11*), f_0 is one-half the maximum limb-to-limb Doppler shift resulting from the planet's rotation, Γ is the usual gamma function, and f is the Doppler frequency measured from the center of the (assumed symmetric) spectrum.

Using Eq. 1 and a standard weighted-least-squares technique, we analyzed each night's unconvolved observations of the outer three satellites (Io's spectrum was too weak to provide useful results) to estimate as many as six parameters: α, f_0 (*12*), n, the frequency corresponding to echoes from the subradar point (*13*), and the slope and height of the noise baseline. In addition to these estimates, a separate estimate of σ was obtained from each spectrum by simple integration of the observed spectral power after subtraction of the noise baseline. Because of the asymmetry displayed by several of the spectra, the latter procedure is felt to provide a more reliable value of the total cross section. Ephemeris errors affect these estimates only indirectly and only insofar as the asymmetry of a spectrum is responsible for an error in attributing the independently estimated center frequency to echoes from the subradar point; were the ephemeris adequate, the independent estimate would not be needed.

Table 1 lists the results obtained from each night's observations for two separate analyses: one in which f_0 was estimated, and one in which it was fixed at a calculated value (*12*), based on the assumption that the rotation of each satellite is synchronous with its orbital period (*8*) and on the optically determined value of its radius (*1*). When f_0 was estimated, synchronous rotation was still assumed and the result quoted as the corresponding estimated value of the radius. The errors given are the formal standard deviations obtained from the solution, after uniform scaling of the error estimates of the input data to yield a root-weighted-mean-square value of unity for the postfit residuals. This procedure partially compensates for model-dependent errors, which cannot be negligible since some of the spectra have noticeable asymmetries.

The radii determined here for Europa and Callisto appear to be somewhat less than those obtained from optical observations, but in all cases by less than twice the square root of the sum of the squares of the combined optical (*1*) and radar standard deviations. It is encouraging that for Ganymede, where a favorable occultation has been used to obtain the quoted optical value for the radius, the agreement is excellent. The more reliable values for n seem to fall between 1.3 and 2.1 for Ganymede and Callisto, while lying nearer unity for Europa.

All three satellites display echoes with a high degree of depolarization, a result consistent with their extremely diffuse scattering laws. The radar cross sections increase from a value for Callisto which is not markedly different from that seen by radar for Venus (*9, 14*), to a value for Europa which is matched only by Saturn's rings (*15*) and which would be expected for a rough and predominantly icy surface. No explanation is apparent for the marked disagreement between the cross section of $0.5\pi a^2$ found here for Ganymede and the fourfold lower value of $0.12\pi a^2$ reported earlier by Goldstein and Morris (*5*) for observations at the same radar wavelength and over a wide variety of orbital phases. (Both of these cross sections reflect only the contributions of the same-sense polarization echoes.)

Curiously, the radar cross sections we obtained for Europa, Ganymede, and Callisto show nearly the same relative values as do the optical cross sections (*1*), despite the presumably far deeper penetration into the surface by the radio waves. Perhaps the surface material is reasonably homogeneous to a depth of at least 1 m. Also, the extremely diffuse scattering of radio waves from these surfaces, compared to that from the terrestrial planets, may indicate that erosive smoothing processes on the Galilean satellites are negligible or that substantial internal reflection is occurring within the surface material. The composition of the surface material cannot be determined uniquely from the radar data; optical spectra (*2*), on the other hand, show water frost to be a dominant component, perhaps covering, on average, 75 percent of Europa's surface area, with the contribution decreasing to about 10 percent for Callisto (*2*).

Further observations with the Arecibo radar, whose sensitivity has recently been increased almost twofold, should provide much better aspect coverage of all four Galilean satellites and should yield better insight into the causes of the asymmetric reflectivities.

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6. The S-band radar facility of the Arecibo Observatory, Arecibo, Puerto Rico, had the following system characteristics at the time of these observations: operating frequency, 2.38 Ghz; antenna gain, 70 db; one-way antenna full beamwidth at half power, 2.7 arc min; average transmitter power, 400 kw (continuous wave); and receiving system noise temperature, 45°K when not observing Jupiter directly (Jupiter added about 30°K).
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8. Throughout, an assumption of synchronous rotation implies that the satellite's rotation axis is normal to its orbital plane. The tidal evolution which presumably led to the synchronous rotation and the equatorial orbital plane ensures that the normality condition will apply to far higher accuracy than required by our analysis.
9. G. H. Pettengill, in *Radar Astronomy*, J. V. Evans and T. Hagfors, Eds. (McGraw-Hill, New York, 1968), pp. 275–321.
10. See, for example, P. E. Green, Jr., in *ibid.*, pp. 1–77.
11. J. Hopkins, *Glossary of Astronomy and Astrophysics* (Univ. of Chicago Press, Chicago, 1976), p. 62.
12. The parameter f_0 (center-to-limb Doppler shift) is related to the satellite radius a, rotation period P, and radar wavelength λ by $f_0 = 4\pi a/\lambda P$ if the rotation axis is normal to the line of sight.
13. The Doppler frequency corresponding to the echoes from the subradar point is related to the orbital motion of the satellite and will be used in improving estimates of the orbital parameters of the Jovian system.
14. Before making this comparison, we corrected for the absorption effects of Venus' atmosphere and, more important, for the surface roughness of Callisto [see, for example, T. Hagfors, in *Radar Astronomy*, J. V. Evans and T. Hagfors, Eds. (McGraw-Hill, New York, 1968), pp. 187–218].
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16. We wish to acknowledge the generous assistance of the staff of the Arecibo Observatory. The Observatory is operated by the National Astronomy and Ionosphere Center with support from the National Science Foundation and the National Aeronautics and Space Administration. Supported in part by NASA grant NGR 22-009-672 (G.H.P. and J.F.C.) and NSF grant MPS 72-05104 (I.I.S.).

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Tumor Cell Collagenase and Its Inhibition by a Cartilage-Derived Protease Inhibitor

Abstract. *Human osteosarcoma and mammary carcinoma cells were cultured separately in a medium supplemented with fetal calf serum, until they were confluent. The medium was then replaced by serum-free medium supplemented with heparin. Both cell cultures secreted collagenase, and this activity was inhibited by a cartilage-derived protein of low molecular weight. Since cartilage is rarely invaded by neoplasms, the presence of this inhibitor may play an important role in the regulation of tumor invasion.*

The discovery of a collagenase present in normal tissues under physiological conditions (*1*) stimulated interest in this enzymatic activity in tumors (*2*). Neoplastic human tissues possess a collagenase which in most cases is similar, if not identical, to that isolated from normal human skin, and yield the specific peptide fragments TC^A and TC^B (*3*). Abramson *et al.* (*4*) extended these observations by correlating the clinical aggressiveness of epidermoid carcinomas of the head and neck with a high specific activity of tumor collagenase.

In the area of bone neoplasms, the most common primary malignant tumor is the osteosarcoma. This tumor erodes and replaces bone tissue, whose major organic component is collagen. Cartilage, another collagenous tissue adjacent to many bones, is rarely or less readily invaded by osteosarcomas. This phenomenon is also observed in bony metastases from breast carcinomas. We present data demonstrating that human osteosarcoma and mammary carcinoma cells secrete collagenase in culture, and that this activity can be inhibited by a cationic protein of low molecular weight isolated from bovine hyaline cartilage (*5*).

For the present experiments we used human osteosarcoma cells (TE-85), a well-defined cell line of McAllister *et al.* (*6*), and human breast carcinoma cells (ALAB, lung metastasis), a cell line described by Reed and Gey (*7*).

Tissue culture medium, RPMI-1640 (Gibco), was supplemented with 10 percent fetal calf serum (FCS) (Reheis Chemical) which had been heat inactivated for 50 minutes at 56°C; 50 μg of gentamycin (Schering) per milliliter; and 5 μg of amphotericin-B (Squibb) per milliliter.

The bovine cartilage protease inhibitor was prepared as recently described (*5*). In brief, the cationic low molecular weight protein is obtained by ultrafiltration of an extract of bovine scapula cartilage followed by affinity chromatography on insoluble trypsin.

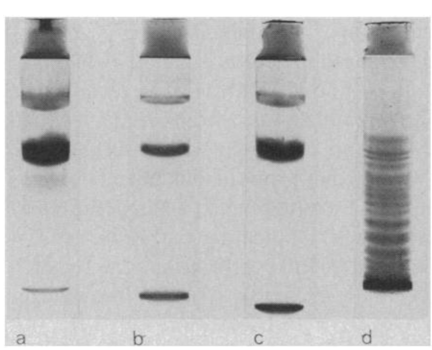

Fig. 1. Polyacrylamide gel electrophoresis. The effect of human mammary carcinoma collagenase on guinea pig collagen and its inhibition by a cartilage-derived protease inhibitor. (a) Collagen control. (b) Collagen plus trypsin. (c) Collagen plus mammary carcinoma collagenase and cartilage inhibitor. (d) Collagen plus mammary carcinoma collagenase.

Tumor cells (initial seeding 4×10^4 cells per milliliter) were cultured in Falcon tissue culture flasks (75 cm^2 growth area) each containing 15 ml of FCS-supplemented medium (37°C, in a humidified atmosphere of air and 5 percent CO_2). After the cells reached confluence (usually 5 days for the TE-85 cells and 4 days for the ALAB cells) the cell layers were extensively rinsed with FCS-free medium, according to the method of Werb and Burleigh (*8*). The flasks were then divided into two groups; both groups were cultured in FCS-free medium, but to one group sodium heparin (50 unit/ml; Sigma) was added. The cells were cultured at 37°C as described above, and the media were changed at 2-day intervals. The heparin-free cultures were maintained for up to 6 days, while the cells cultured with heparin were maintained for 14 days. The media were decanted, cleared from cellular debris by centrifugation, and those from each experiment were pooled. The media were adjusted so that they contained 50 m*M* tris and 5 m*M* $CaCl_2$, by means of 1*M* tris-HCl buffer, *p*H 7.6, and solid $CaCl_2$. They were then dialyzed against 100 m*M* tris-HCl buffer, *p*H 7.6, containing 5 m*M* $CaCl_2$ as described (*8*). Samples were then concentrated (50 : 1) with an Amicon PM-10 membrane and assayed for collagenase activity.

Collagenase and its inhibition by the cartilage inhibitor was measured by means of an assay for the release of ^{14}C-labeled glycine peptides from 100-μl portions of a solution of undenatured guinea pig skin collagen, as described for the inhibition of human collagenase with different inhibitors (*5*). Two hundred microliters of the concentrated, processed medium were used either directly or after prior incubation for 30 minutes at 22°C with 10 μg of the cartilage inhibitor. The reaction mixture was then incubated with the collagen at 37°C for 4½ hours. The reaction was terminated and the radioactivity measured in an automatic liquid scintillation counter (*5*).

The reaction products resulting from collagenolytic activity were separated by polyacrylamide gel electrophoresis according to the method of Nagai *et al.* (*9*).

In FCS-free media, neither of the tumor cell cultures could be maintained for more than 6 days because of severe necrosis and cell degeneration. When these media were examined for enzyme activity, only a negligible amount of collagenase could be found.