A MURINE TUMOR PRODUCING A MATRIX OF BASEMENT MEMBRANE

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Solid tumors produce extracellular matrices. This is particularly apparent in the case of chondrosarcomas where tumor cells exist in lacunar spaces surrounded by extracellular matrix. The matrix produced by normal cartilage cells contains cartilage-specific proteoglycan (1, 2), hyaluronic acid (3, 4), cartilage (type II) collagen (5, 6), and associated proteins (7). In forming the matrix, proteoglycan monomers bind to hyaluronic acid through a portion of their protein core and form large aggregates (4, 7). Such aggregates are stabilized by smaller link proteins which interact with both the hyaluronic acid strand and the proteoglycan (7). In vitro studies indicate that the proteoglycan aggregates interact with collagen fibrils (8–10). Both the collagen (5, 6) and the proteoglycan (1, 2) in cartilage are thought to be unique to this tissue and required for the distinctive structure and function of normal cartilage matrix.

Earlier, we had studied a well-differentiated chondrosarcoma maintained for many years by serial passage in rats (11). About half the protein in this tumor is collagen. Chemical studies established that this collagen is homologous to the collagen isolated from chick, human, and calf hyaline cartilages, and is presumed to be the corresponding rat protein. The major glycosaminoglycan in the tumor has also been isolated and identified as chondroitin-4-sulfate (12, 13). These findings confirm the histological definition of the rat tumor as a differentiated chondrosarcoma.

Recently we have studied a transplantable murine tumor which also has been classified as a chondrosarcoma, on the basis of morphological criteria. The tumor appeared spontaneously in the ST/EH mouse strain and has been maintained for many years by serial passage in other strains of mice (14). This murine tumor was found to contain pleomorphic cells usually arranged in clusters and surrounded by an extracellular matrix which stained homogeneously with hematoxylin and eosin, and with the periodic acid-Schiff reagent. Only a slight reaction of the tumor extracellular matrix was noted with alcian blue, a stain specific for sulfated glycosaminoglycans (15). On the basis of these findings the tumor was identified as a poorly differentiated chondrosarcoma. The ultrastructural and chemical characterizations of the tumor reported here

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indicate, however, that this murine tumor matrix is not cartilaginous, but of the basement membrane type.

Materials and Methods

Tumor Passage and Maintenance. Tumors were maintained in BALB and C57BL/6J mouse strains by subcutaneous implantation of minced tumor tissue. Large solid tumors (4-5 g) that formed under the skin in approximately 4 wk were used in this study.

Morphological and Histochemical Studies. Tumor tissue was excised and fixed in either Bouin's solution, if the material was to be embedded in paraffin, or fixed in paraformaldehyde-glutaraldehyde (16) followed by postfixation in 1% OsO_4, if the material was to be embedded in plastic (17). Staining of paraffin-sectioned material for histochemical studies was done by established procedures (18). The procedures included stains which show preferential binding to collagen such as Van Gieson's stain, Mallory's aniline blue, and Masson's trichrome stain. In addition, we used stains showing preferential association for sulfated glycosaminoglycans including alcian blue and toluidine blue. Sections were also routinely stained with hematoxylin and eosin, periodic acid-Schiff reagent, and Weigert's resorcin fuchsin reagent for elastin.

Tissue specimens to be embedded in plastic were stained en bloc with aqueous solutions of uranyl acetate (1.5%). Thin sections (500 A) were cut on a Porter-Blum MT-2 ultramicrotome, floated onto copper grids, and stained with uranyl acetate (1% in 50% ethanol) followed by lead citrate (0.2% in 0.1 N NaOH). Sections were examined in an AEI EM 6B electron microscope (AEI Scientific Apparatus, Inc., Elmsford, N. Y.) or in a Philips 300 electron microscope (Philips Electronic Instruments, Inc., Mount Vernon, N. Y.).

Analysis of the Tumor Collagen

AMINO ACID ANALYSIS. Segments of whole tumor tissue were rinsed briefly in 20% (3.4 M) NaCl-0.05 M Tris-HCl, pH 7.4, to remove soluble (noncollagenous) proteins, desalted by dialysis, and then hydrolyzed under N_2 in distilled HCl at 108°C for 24 h, and the amino acid content determined by automated procedures (19).

In some studies, tumor tissue was first homogenized in 0.5 M acetic acid and then incubated with pepsin (5% of the dry weight of the tumor) for 6 h at 15°C (20, 21), conditions known to degrade most proteins but to leave the helical portion of collagens intact. Insoluble material was removed by centrifugation at 15,000 rpm for 30 min at 4°C, and the supernatant fluid was adjusted to pH 8 by the addition of solid Tris-HCl, and then concentrated NaOH, dropwise, to inactivate the pepsin. Collagenous protein was precipitated from the supernatant fluid by addition of NaCl to 20% (wt/vol). The collagenous precipitate was washed with several volumes of 20% NaCl (3.4 M)-0.05 M Tris-HCl, pH 7.4. The precipitate was then resuspended in cold distilled H_2O, desalted by dialysis, and then redissolved by dialysis against 0.5 M acetic acid, and lyophilized. Weighed portions of the lyophilized material were analyzed for amino acid content. From the amino acid analyses, the collagen content of each of the fractions was calculated as described by Green and Goldberg (22).

EXTRACTION OF COLLAGEN FROM THE TUMOR BY NONENZYMATIC MEANS. Mice were made lathyritic by being fed a diet containing β-aminopropionitrile (BAPN)[1] fumarate (4 g BAPN/kg ground Purina rat chow, Ralston Purina Co., St. Louis, Mo.) for 10–14 days before sacrifice. Tumors were excised, dissected free of capsular material, homogenized in 0.5 M acetic acid containing phenylmethanesulfonyl fluoride (10^{-3} M), and extracted in the cold overnight. The suspension was centrifuged at 12,000 rpm for 20 min to isolate the extracted material. The pelleted residue was re-extracted overnight under the same conditions, and the two extracts were pooled and brought to neutrality by dialysis against 1 M NaCl-0.05 M Tris-HCl, pH 7.4 (Fig. 1). The retentate was centrifuged at 12,000 rpm for 30 min to remove insoluble material (neutral salt precipitate fraction) and NaCl was then added to the supernatant fluid to a final concentration of 3.4 M (20%) to precipitate collagenous proteins. Precipitated collagenous material was removed by centrifugation at 12,000 rpm for 30 min. The material which remained soluble (20% salt supernate) was desalted by dialysis and lyophilized before amino acid analysis. The collagenous 20% salt precipitate was washed repeatedly with 3.4 M NaCl-0.05 M Tris-HCl, pH 7.4, and then suspended in, and dialyzed against 1.7 M NaCl (10% wt/vol)-0.05 M Tris-HCl, pH 7.4. Material which remained

[1] *Abbreviations used in this paper:* BAPN, β-aminopropionitrile; SDS, sodium dodecyl sulfate.

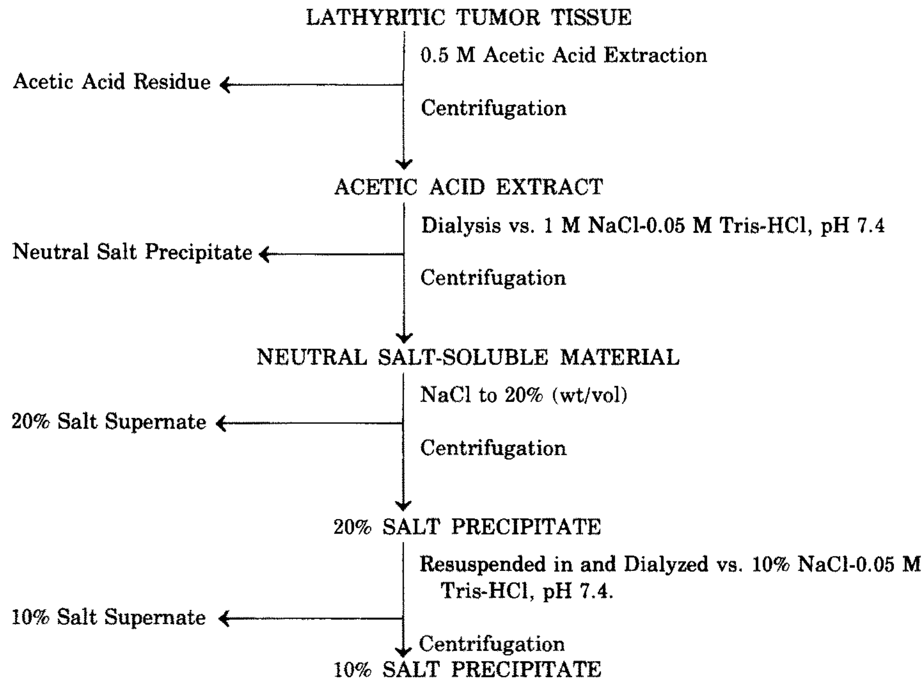

FIG. 1. Flow diagram of nonenzymatic isolation of basement membrane collagen from murine tumor.

insoluble was collected by centrifugation at 12,000 rpm for 30 min, resuspended in H_2O, desalted by dialysis, then dialyzed against 0.1% acetic acid and either lyophilized or stored at 4°C until further use. This fraction was designated the 10% salt precipitate. The material resolubilized in 10% neutral salt buffer, designated the 10% salt supernatant fraction, was also desalted by dialysis, lyophilized, and stored as were the other fractions obtained during the isolation procedure.

In general, we have been able to extract 25–50% of the collagenous protein in the tumor by this procedure. The yield seems to be greatest from severely lathyritic animals. The major portion of the extracted collagenous protein (greater than 75%) is subsequently found in the fraction which remained insoluble in 10% NaCl-0.05 M Tris-HCl, pH 7.4 (10% salt precipitate fraction).

BIOSYNTHESIS OF TUMOR COLLAGEN. Segments of tumor tissue were incubed at 37°C in the Dulbecco-Vogt modification of Eagle's medium, lacking proline, glycine, and lysine, but containing BAPN-HCl (Calbiochem, San Diego, Calif.) and ascorbic acid (both at 100 μg/ml), as well as penicillin (50 U/ml) and streptomycin (50 μg/ml) (Gibco Diagnostics, The Mogul Corp., Chagrin Falls, Ohio). 20 min later the medium was removed and replaced with fresh medium containing 200 μCi [^{3}H]glycine, 20 μCi [U-^{14}C]glycine, 20 μCi [U-^{14}C]proline, and 20 μCi [U-^{14}C]lysine (New England Nuclear, Boston, Mass.). The incubation was continued for 2, 8, or 24 h. In some studies, labeled medium was removed after 2 h and replaced with unlabeled medium for the remainder of the incubation period. The medium was decanted after the incubation, and collagenous protein was extracted from the labeled tissue as described above for unlabeled tissue.

ELECTROPHORETIC PROCEDURES. Aliquots of lyophilized fractions of unlabeled or labeled proteins extracted from the tumor were dissolved in a solution of 4 M urea, 0.04% sodium dodecyl sulfate (SDS), 0.02 M sodium phosphate, pH 7.2, and electrophoresed in SDS on 5% acrylamide gels according to the method of Furthmayr and Timpl (23). Before electrophoresis, some samples were reduced by the addition of β-mercaptoethanol (25 μl/ml). Where indicated, samples were coelectrophoresed with type I collagen as a standard. Unlabeled samples were electrophoresed at 8.5 mA/gel for 5 h; labeled samples were electrophoresed at 8.5 mA/gel for 8 h to effect a wider

separation between the polypeptide bands, needed for subsequent slicing of the gel. Gels were fixed and stained with 1% Coomassie Blue in 10% acetic acid for 30 min and then destained overnight in 7% acetic acid containing 5% methanol. Gels containing labeled proteins were cut into 1-mm segments on a gel slicer (Bio-Rad Laboratories, Richmond, Calif.). Each gel slice was then swollen in NCS tissue solubilizer (Amersham/Searle Corp., Arlington Heights, Ill.) at 37°C, overnight, then brought to neutrality with glacial acetic acid, and counted in a scintillation cocktail of Spectrofluor (Amersham/Searle Corp.) in toluene (30 ml/pint).

In some instances the gels were fixed and then stained with the periodic acid-Schiff reagent according to the method of Fairbanks et al. (24).

COLLAGENASE DIGESTIONS. Labeled and unlabeled proteins extracted from the tumor were incubated with purified bacterial collagenase (Advanced Biofactures Corp., Lynbrook, N. Y.) under conditions outlined by Peterkofsky and Diegelman (25). The preparation of enzyme used was judged to be free of contaminating proteolytic enzymes since no digestion of [^{14}C]tryptophan-labeled proteins was observed in control experiments (26).

Results

Morphological and Histochemical Characterization of the Tumor. After implantation of minced tissue subcutaneously into mice, the tumor grows rapidly, forming large masses (~5 g) within 3-4 wk. A fibrous capsule surrounds the tumor but is readily separable from the tumor mass, although fine strands of capsular material containing blood vessels penetrate the tumor. Histological examination of tumor tissue revealed that the tumor retained its previously reported appearance (14, 15) of clusters of cells separated by considerable quantities of extracellular matrix (Fig. 2). Examination of sections treated with reagents known to stain collagen or sulfated glycosaminoglycans, confirmed previous studies (15) that the tumor matrix stains with collagen-specific stains, but does not react with stains specific for sulfated glycosaminoglycans.

Ultrastructural studies revealed that the tumor cells contained well-developed Golgi complexes and an extensive rough endoplasmic reticulum often distended with flocculent deposits (Figs. 3 and 4), features consistent with active protein synthesis. Although tumor cells are often clustered in rosette-like patterns typical of glandular epithelia, the tumor cells remain separated from one another by 150–200-A spaces and lack the junctional specializations characteristic of adjacent epithelial cells (27). Unlike normal chondrocytes which reside within lacunae, tumor cells were not found in distinct lacunar spaces; rather, extracellular matrix abuts directly on the clusters of tumor cells (Figs. 3 and 4). Indeed, coated vesicles open to the extracellular matrix can be seen along the cell membranes of tumor cells (Fig. 4). No substructure was noted in the extracellular tumor matrix. Neither the thin unbanded collagen fibrils nor dense granules of stained proteoglycan aggregates characteristic of cartilaginous matrices (28–30) were present (Fig. 5). Rather the ultrastructural appearance of the matrix resembles a basement membrane (31–33).

Chemical Studies. The amino acid composition of tumor tissues was determined using automated procedures (19). These analyses revealed that a maximum of 10% of the protein in the tumor is collagenous, as estimated from the content of hydroxyproline residues and the known occurrence of hydroxyproline in purified collagens (22) (Table I).

As a preliminary approach to the isolation and characterization of the collagen in this tumor, we digested whole tumor tissue with pepsin at 15°C for 6 h.

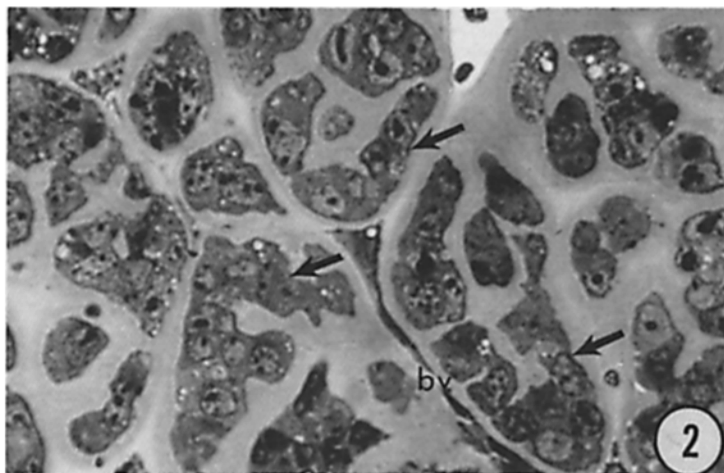

FIG. 2. A light micrograph illustrating the histological appearance of the tumor. Clusters of tumor cells (arrows) are surrounded by large quantities of extracellular matrix material. Thin septi presumed to originate from the tumor capsule, penetrate the tumor matrix and may contain small blood vessels (*bv*). × 120.

This procedure has been employed earlier to solubilize cross-linked collagen (34), to remove noncollagenous peptides from procollagen (21), and to solubilize the collagenous portion of basement membranes (35, 36). The composition of the protein solubilized by limited enzymatic digestion and then partially purified by salt precipitation was distinctive (Table I) and closely resembled that of basement membrane (type IV) collagen, but differed significantly from the compositions of types I, II, and III collagens. Particularly indicative of basement membrane collagen are the levels of hydroxylysine, alanine, valine, and arginine in the tumor protein, values which compare favorably with those found in enzyme digests of authentic basement membranes (37). Considerably less 3-hydroxyproline was found in the tumor protein than occurs in basement membrane isolated from lens or kidney. However, this difference may not indicate a basic difference between the proteins since 3-hydroxyproline occurs as a result of a post-translational modification during collagen synthesis (38).

Because of the similarity in amino acid composition of the protease-solubilized protein from the tumor with that of basement membrane collagen similarly prepared from lens capsules or kidney glomeruli, we wished to determine the nature of the polypeptide content in this tumor preparation. Although the α-polypeptide chains of types I-III collagens have a molecular weight of approximately 95,000 (39), the α-chains present in pepsin digests, as well as in SDS-mercaptoethanol extracts, of lens or kidney basement membranes have been reported to be of higher molecular weights (35, 40–44).

The polypeptide composition of the collagenous protein prepared by pepsin digestion of the tumor was determined by electrophoresis in a SDS-acrylamide gel system (23). In the absence of a reducing agent, relatively little of the protein in the sample penetrated into the gel (Fig. 6). Of the four major polypeptides which entered the gel, none co-migrated with α-, β-, or γ-components of type I

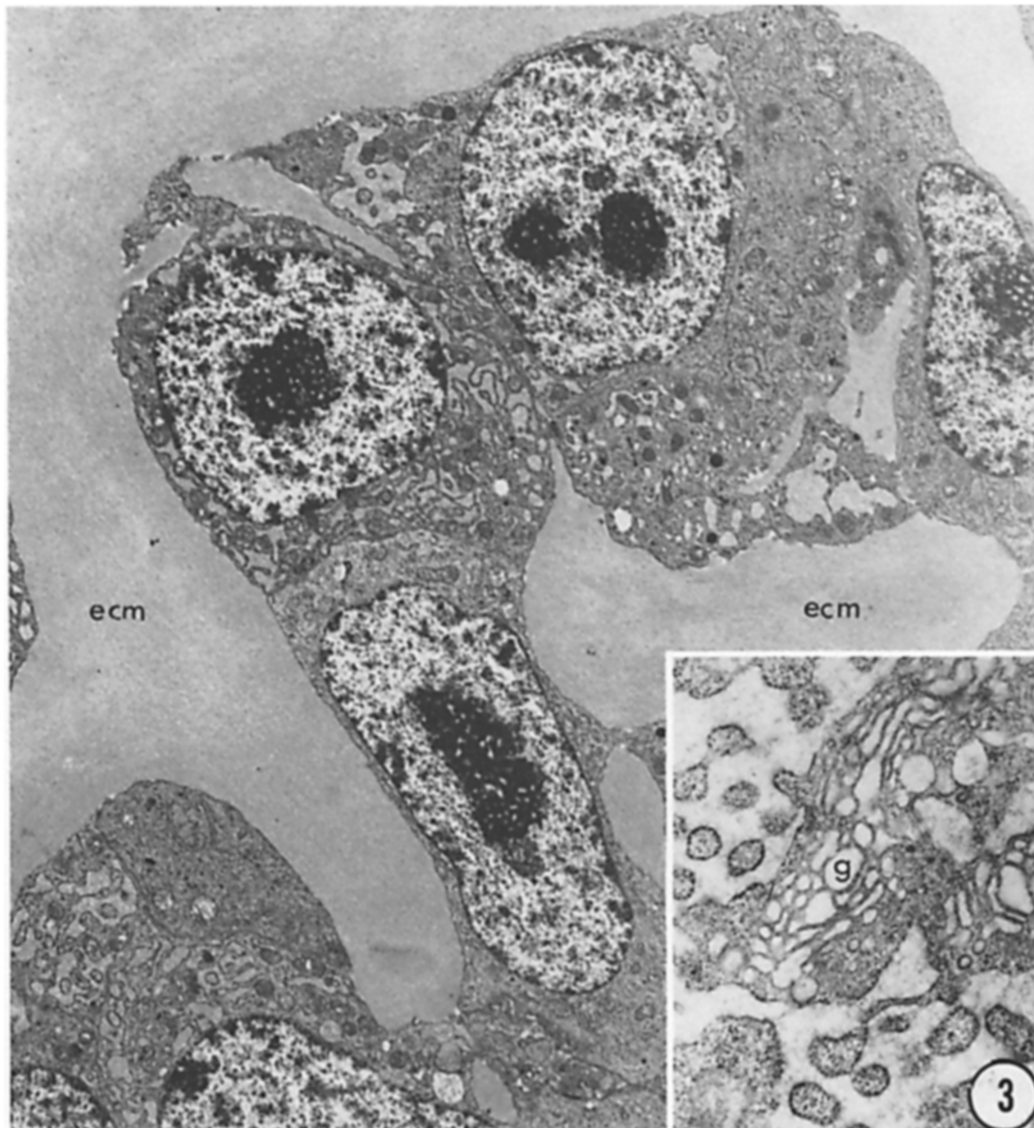

FIG. 3. A low magnification electron micrograph showing clusters of mononucleate tumor cells surrounded by amorphous extracellular matrix (*ecm*). The inset shows at higher magnification the prominent Golgi complexes (*g*) as well as vacuoles and cisternae filled with flocculent material, characteristic of these cells. × 7,200; inset; × 20,000.

collagen. In the presence of the reducing agent β-mercaptoethanol, multiple polypeptides with apparent molecular weights ranging from 10,000 to 300,000 were observed after electrophoresis on SDS gels (Fig. 6). A relatively minor polypeptide migrated in the region of the β-components and another in the region of the α-chains in the gel. Because of the heterogeneity of the enzymatically prepared tumor collagen peptides, as well as the results from nonenzyme-

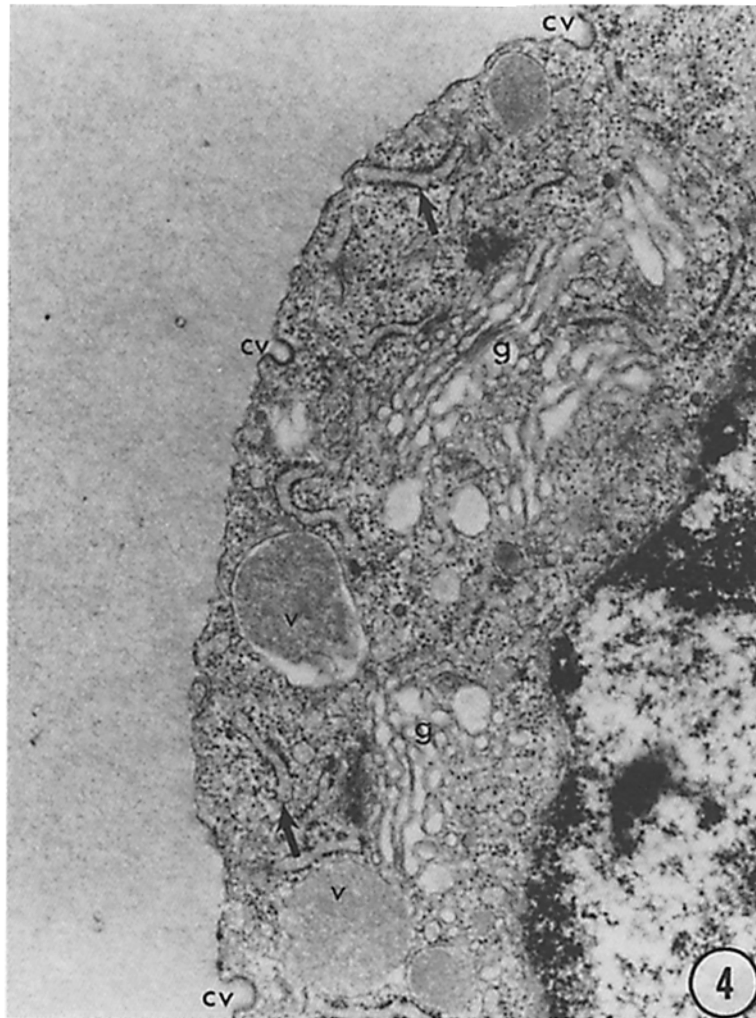

FIG. 4. The tumor cell cytoplasm shown in this electron micrograph has prominent Golgi complexes (*g*) and vesicles (*v*) filled with amorphous material which in some vesicles closely resembles the extracellular matrix material, and random cisternae of rough endoplasmic reticulum (arrows) also filled with amorphous material. Coated vesicles (*cv*) open to the extracellular matrix. No substructure is apparent in the extracellular matrix which abuts directly on the tumor cell membrane. × 18,000.

treated preparations, to be discussed below, we believe that pepsin produces many cleavages in this collagenous protein.

Nonenzymatic Extraction of the Tumor Collagen. Preliminary experiments established that collagenous protein was extracted from the tumor with 0.5 M acetic acid, but not with neutral salt solution (1 M NaCl, 0.05 M Tris-HCl, pH 7.4). The yield of collagenous protein has been greater from tumors grown in lathyritic mice, but we have no direct evidence that lysine-derived cross-links

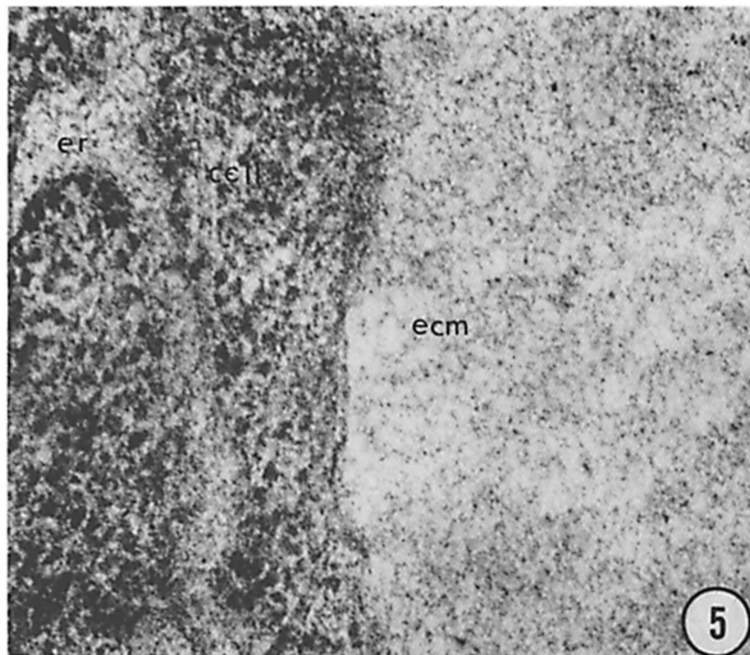

FIG. 5. The amorphous nature of the tumor extracellular matrix (*ecm*) is illustrated in this higher magnification electron micrograph. No collagen fibrils or proteoglycan matrix granules are present in the tumor matrix. *er*: endoplasmic reticulum × 80,000.

occur in this protein. Routinely, 25–50% of the collagenous protein was extracted, from tumors grown in lathyritic animals, in acetic acid.

To purify collagenous protein from the acid extract, we first dialyzed it against neutral salt buffer. While a precipitate did form during dialysis, most (>80%) of the collagenous protein remained soluble, as judged by the low content of hydroxyproline residues in the neutral salt precipitate fraction of most preparations. After centrifugation of the neutralized extract, NaCl was added to 20% (wt/vol) to the supernatant fluid, to precipitate collagenous protein. The 20% salt precipitate was then resuspended in 10% neutral salt buffer. The material which remained insoluble in this buffer was designated the 10% salt precipitate and was found to contain the majority of the extracted collagenous protein (~ 80%), as established by amino acid analyses. Although some collagenous protein (5%) was solubilized in the 10% neutral salt buffer, it was tentatively identified as type I collagen on the basis of composition and the presence of $\alpha1$ and $\alpha2$ chains in the protein.

The collagenous protein which remained insoluble in 10% neutral salt buffer was quite distinct. Amino acid analysis (Table I) revealed that the ratio of hydroxylysine to hydroxyproline approached that found in the pepsin-treated material; however, the content of hydroxyproline is reduced when compared to the hydroxyproline content of the pepsin-treated material. It is likely that the protein in the 10% salt precipitate fraction is not pure since components with a

TABLE I
Amino Acid Composition of the Tumor Collagen

Amino acid	Residues/1000			
	Whole mouse tumor*	Pepsin-treated mouse tumor‡	10% NaCl ppt of acid-extracted mouse tumor	Bovine lens capsule§
3-Hydroxyproline	–	2.3	2.4	15
4-Hydroxyproline	11	111	37	85
Aspartic acid	90	53	88	55
Threonine	47	27	49	29
Serine	51	32	58	42
Glutamic acid	112	92	113	92.5
Proline	61	55	57	68
Glycine	113	338	161	275
Alanine	65	35	59	42.8
Half-cystine	32	7	19	28
Valine	67	40	52	30
Methionine	16	5	16	8
Isoleucine	35	27	34	28.8
Leucine	96	49	71	58
Tyrosine	41	7	32	10
Phenylalanine	36	27	31	32
Hydroxylysine	6	47	15	35
Histidine	24	11	22	10.2
Lysine	51	9	37	13.2
Arginine	47	28	46	43

* Whole tumor segments were rinsed briefly in 20% NaCl at neutral pH, before hydrolysis for amino acid analysis. Under these conditions, collagen is not solubilized.

‡ Tumor tissue was digested with pepsin, as in Materials and Methods. The collagenous protein in the extracted material was isolated by precipitation with 20% NaCl at neutral pH.

§ From Kefalides, 1973 (reference 37).

somewhat greater proportion of glycine, hydroxyproline, and hydroxylysine are obtained after chromatography of this material on carboxymethyl cellulose (R. W. Orkin, unpublished results).

Electrophoresis of the 10% salt precipitate fraction under denaturing conditions and in the presence of a reducing agent, resolved three high molecular weight polypeptides which migrated between the region of α- and β-chain components of type I collagen used as a standard (Fig. 7). In the absence of the reducing agent, no distinct polypeptide bands were observed on Coomassie Blue-stained gels (Fig. 7), indicating that the peptides were linked into high molecular weight components by disulfide bonds. The three polypeptides resolved on SDS gels after reduction had apparent molecular weights of 185,000, 165,000, and 145,000. These three polypeptide bands also stained intensely with the periodic acid-Schiff reagent suggesting that they are glycoprotein in nature. Incubation with collagenase before electrophoresis destroyed these three polypeptide bands (Fig. 8). These findings indicate that these three polypeptides

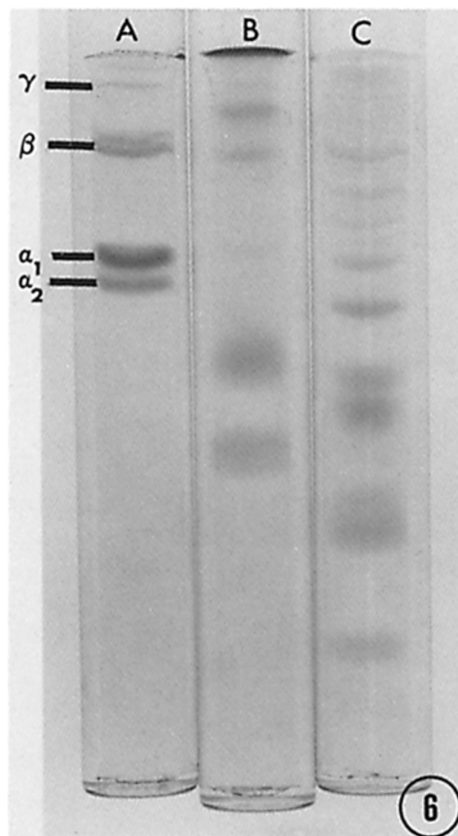

FIG. 6. SDS-5% acrylamide gels showing the pattern of Coomassie Blue-staining peptides obtained after pepsin digestion of the tumor tissue. Note the heterogeneity of the pepsin-treated material. (A) Type I collagen isolated from rat skin; (B) pepsin-extracted tumor material, electrophoresed without prior reduction; (C) pepsin-extracted tumor material, electrophoresed after reduction with β-mercaptoethanol.

comprise the collagenous component of the tumor matrix and are distinct from the α-chains found in types I–III collagens.

To establish a possible biosynthetic relationship among the three high molecular weight polypeptide chains, we incubated segments of tumor tissue in medium containing radioactive amino acid precursors of collagen and isolated the major collagenous fraction by nonenzymatic means, as indicated above. After electrophoresis on SDS gels, without reduction, a single radioactively labeled peptide penetrated the gels with an apparent molecular weight of 245,000 (Fig. 9 A), although no distinct Coomassie Blue-staining polypeptide band could be observed in this region of the gel. Upon reduction, there appeared to be a quantitive conversion of the radioactivity to the 185,000 molecular weight component (Fig. 9 B). Pulse-chase experiments were carried out in an attempt to demonstrate conversion of the 185,000 molecular weight chain to the two lower molecular weight polypeptides.

After a 2-h incubation period with isotopic precursors of collagen, tumor tissue

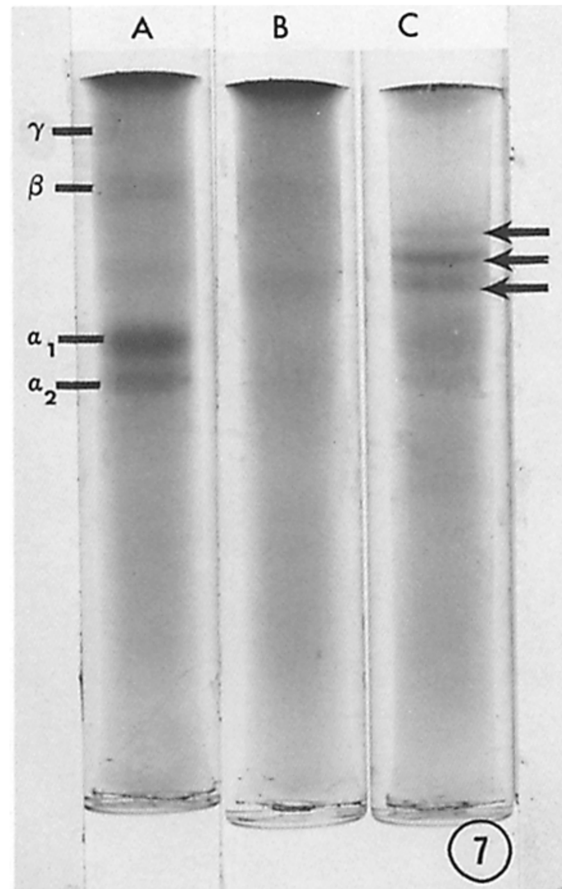

FIG. 7. SDS-5% acrylamide gels of (A) type I collagen standard (rat skin); (B) acid extracted, 10% salt precipitate fraction of tumor material, unreduced; (C) as in B, but reduced with β-mercaptoethanol before electrophoresis. Arrowheads indicate the three high molecular weight peptide bands.

was then incubated with unlabeled medium for an additional 8 or 24 h. In general, no quantitive transfer of label was observed during the chase period, although low levels of radioactivity were found associated with the 165,000 and 145,000 molecular weight polypeptides.

Discussion

The combined ultrastructural and biochemical studies presented here show that this tumor is not a type of cartilage. Previous studies, confirmed here, indicated that the tumor matrix lacked the high affinity for reagents that bind to sulfated proteoglycans (15). Our ultrastructural studies also failed to demonstrate the polygonal granules of proteoglycan aggregates characteristic of cartilage matrix (28–30). In addition, collagen fibrils were not evident in the amorphous tumor matrix. These morphological observations however do not prove that the tumor is noncartilaginous, or that it is not of cartilage origin, since one

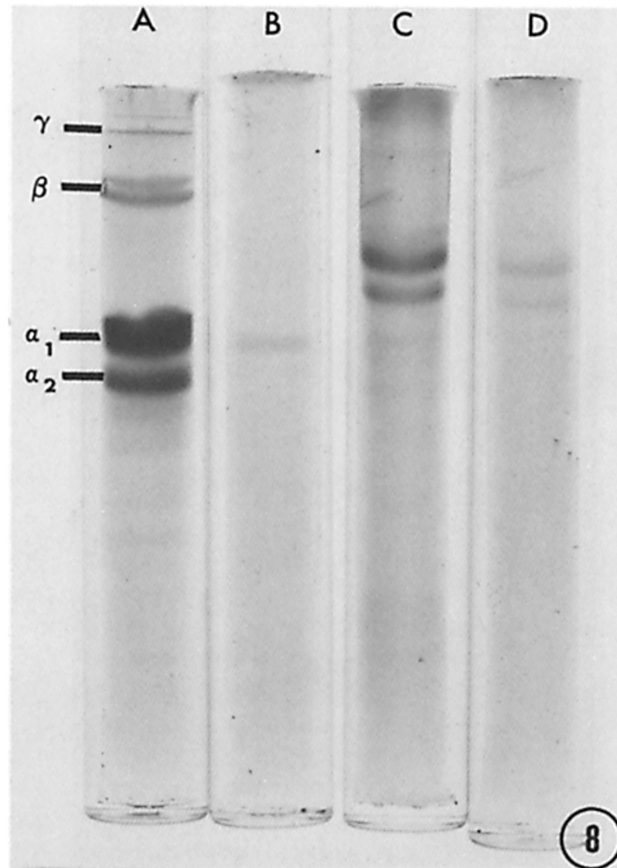

FIG. 8. SDS-5% acrylamide gels of the 10% salt precipitate fraction of acid-extracted tumor (basement membrane fraction) treated with purified bacterial collagenase. (A) Type I collagen; (B) As in A, but digested with collagenase before electrophoresis; (C) Tumor material, reduced with β-mercaptoethanol before electrophoresis; (D) As in C, but treated with collagenase before electrophoresis.

or more constituents of the matrix could be altered and lead to an apparently abnormal matrix appearance. Indeed, reduced synthesis (1) and the undersulfation (45, footnote 2) of proteoglycans have been found to occur in certain mutations affecting cartilage.

If the tumor were cartilaginous, biochemical studies would be anticipated to reveal the presence of at least some of the characteristic macromolecular components of cartilage. However, significant amounts of cartilage specific proteoglycans could not be extracted from this tumor (Dr. V. C. Hascall, personal communication). We undertook to identify the collagen in the tumor since normal cartilage (5, 6, 45), as well as an authentic chondrosarcoma of the rat (11) contain cartilage-specific type II collagen. Type I collagen may also be synthesized by areas of cartilage undergoing osteoarthritic degeneration (46),

[2] Orkin, R. W., B. R. Williams, R. E. Cranley, D. C. Poppke, and K. S. Brown. 1976. Defects in the cartilaginous growth plates of brachymorphic mice. Manuscript submitted for publication.

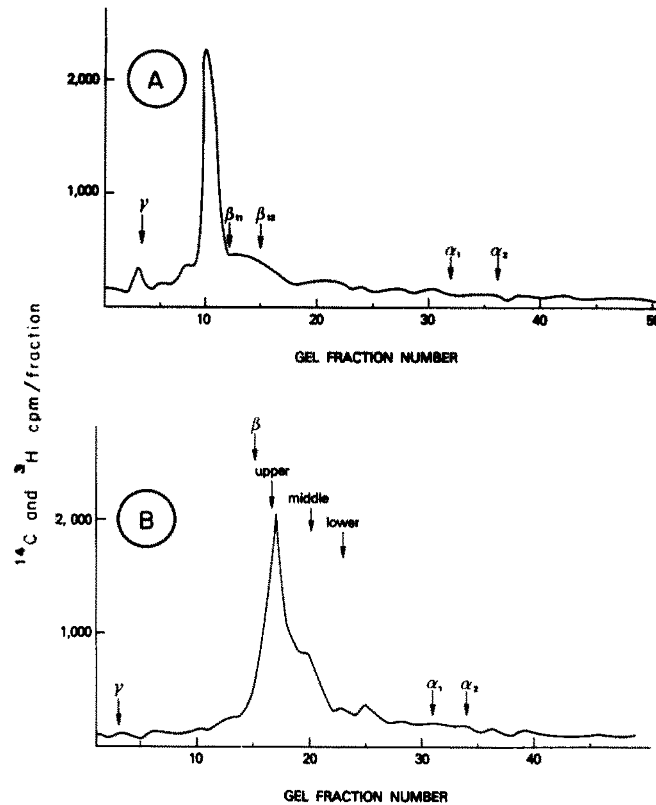

FIG. 9. Radioactivity profiles of peptides obtained from the acetic acid extracted 10% salt precipitate fraction of tumor tissue, electrophoresed on SDS-5% acrylamide gels before (A) and after (B) reduction with β-mercaptoethanol. Tumor tissue was incubated with isotopic precursors of collagen for 24 h, as described in the text. Identical radioactivity profiles were obtained after incubations of 2 and 8 h, as well as in experiments in which the labeled medium was removed after 2 h and the incubation continued in unlabeled medium for up to 24 h.

but type III and basement membrane (type IV) collagens have not been observed in cartilage. Here we could account for approximately 10% of the proteins in the tumor as collagen, but the ratio of hydroxylysine to hydroxyproline, collagen-specific amino acids, was considerably higher in the tumor protein than is found in types I, II, or III collagens (almost 10 times greater than in types I and III, and twice as great as that of type II).

Our first efforts to identify the collagen(s) in the tumor matrix involved digesting tumor tissue with pepsin at low temperatures. Since the helical regions of collagen are resistent to enzymatic attack, whereas most other proteins are degraded under these conditions, one can often solubilize and purify tissue collagens in this manner. We obtained material in such extracts that was precipitable by the same concentration of NaCl that precipitates other collagens. Amino acid analyses of this material closely resembled similar preparations from the basement membranes of kidney glomeruli (36) and lens capsule (37), and was distinctly different from the amino acid composition of other known

collagens. However, this preparation yielded a complex mixture of peptides of widely ranging sizes after reduction and electrophoresis on SDS gels, and no single major component was observed. In contrast, Kefalides (35, 37) has reported the presence of an α-like chain from pepsin digests of basement membrane of lens capsule, and has suggested that the collagenous component of this matrix is homologous in structure to the other collagens. Hudson and Spiro (47) however, isolated a complex mixture of peptides from similar preparations, and our electrophoretic patterns of the tumor material resemble their findings. Our results suggest that pepsin partially degrades the collagenous component of the tumor, but that the amino acid composition of this material closely resembles that of basement membrane (type IV) collagen.

High molecular weight collagenous components were extractable from the tumor, grown in lathyritic animals, with acetic acid. Type I collagen, probably of capsular origin, was a minor component of this extract (about 5%). Three polypeptide chains were obtained upon reduction of the basement membrane (10% salt precipitate) fraction which were considerably larger than α-chains (185,000, 165,000, and 145,000 vs. 94,500 for an α-chain). Since high molecular weight biosynthetic precursors which contain disulfide bonds in the terminal extension peptides have been demonstrated for types I–III collagens (48–50), we attempted to establish if these components were related as precursors and products. Preferential incorporation of label into the largest component was observed, with little transfer to the smaller chains. These results do not necessarily mean that the chains are unrelated since relatively little conversion of precursors of types I and III collagens occurs in cultured cells (21). These findings are consistent with those reported for the basement membrane collagen synthesized by embryonic parietal yolk sac cells (42–44). In this system, one high molecular weight collagenous component was isolated after molecular sieve column chromatography on agarose and SDS gel electrophoresis. This component eluted from the column at a slightly later position than β-components of type I collagen (42) and migrated in the region of β-chain components on SDS gels (44), making it likely that the basement membrane collagen synthesized by parietal yolk sac cells and that of the murine tumor are similar. As in the murine tumor, radioactivity in the collagenous material synthesized by parietal yolk sac cells was not converted to smaller molecular weight components (43, 44).

Neither the structure of basement membrane nor its prominent collagenous component, so-called type IV collagen, are well established. Although it has been suggested that this collagen is essentially similar in structure to types I–III collagens in that it is formed of α1-type chains (35), more recent studies (44) indicate that the collagenous component of basement membrane is more similar to procollagen, and that the precursor specific segments may prevent the arrangement of the protein into a fibrillar matrix. Our pulse-chase studies support these latter observations. In addition, we have noted that the collagenous protein isolated from the tumor is not as resistant to protease digestion as are the other collagens, a finding which suggests that there are basic differences in the structures of basement membrane collagen and types I–III collagens, which may allow the type IV collagen to form a homogenous deposit. Since large

quantities of basement membrane collagen can be prepared from this tumor, it should be possible to characterize it chemically.

Summary

We have studied a murine tumor previously classified as a poorly differentiated chondrosarcoma. Although the cells in this tumor are surrounded by large quantities of extracellular matrix material, the matrix fails to react with stains specific for the sulfated glycosaminoglycans present in normal cartilage. Here we show at the ultrastructural level that the tumor matrix is a homogeneous, nonfibrillar material, resembling basement membrane. Neither the proteoglycan matrix granules nor collagen fibrils characteristic of cartilage are present in the tumor matrix. Amino acid analyses of whole tumor tissue, enzyme-solubilized tumor components, and the protein extracted from lathyritic tumors confirmed that the tumor matrix is a basement membrane collagen. The collagenous protein extracted from the tumor by nonenzymatic means contains three unique polypeptides larger than the α-chain components of the other types of collagen. These studies indicate that the tumor is not a type of chondrosarcoma, but a basement membrane producing tumor.

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