■特集／バイオマテリアル■

バイオマテリアルの生体組織反応

Biological Tissue Response by Biomaterials

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はじめに

近年，各種疾患の診断，治療面での進歩に伴い金属や高分子材料，あるいはセラミックスなど種々のバイオマテリアルを用いた医療用具が開発，使用されてきており，それら医用材料の材質の安全性に関する問題がクローズ・アップされる様になってきた．これら医用材料の安全性に関しては，現在化学的試験と生物学的試験の2つが行われており[1]，化学的試験としては材質及び溶出物質に関する試験が，また生物学的試験は溶出液を用いての急性毒性，皮内反応，発熱性，溶血性，変異原性試験や培養細胞を用いての細胞毒性試験，及びそれらを動物の体内に埋め込む埋没（移植）試験等がある．臨床面においてこれらの医用材料がとくに長期間用いられる場合には，それら材料と生体組織との親和性がきわめて重要であり，これには材料を体内に埋込んだ時におこる周囲組織の生体反応や，人工血管や人工腎透析装置などにみられる様にそれが血液と接する場合の溶血や血栓形成などが一番問題となる．バイオマテリアルの生体組織との反応としては種々の事が考えられるが，そのいくつかは他章にも述べられているので，ここでは主としてバイオマテリアルの体内埋込みの際におこる生体組織反応，ことに長期間埋没による腫瘍発生を中心に述べる事にする．

医用材料と生体組織との反応

一般に医用材料と生体組織との反応を考える時，その組織反応の原因となるものとしては化学的なものと物理的なものに大別される．前記各種生物学的試験のうち，溶出液を用いる試験は前者による反応をみるものであり，生体内への埋没（あるいは移植）試験はこれら両方を検討するものであろう．化学的な原因としては，材料からの各種溶出物質，たとえば重金属材料から溶出するイオン，高分子材料などから溶出する各種低分子物質（材料中の各種添加物や不純物に由来するもので，残留モノマー，低分子ポリマー，可塑剤，酸化防止剤，触媒，乳化剤，凝固剤，着色剤，溶剤等が含まれる），材料中に存在する反応基や材料の分解物，材料に吸着した蛋白質のような異種成分などが考えられる．また物理的原因としては，材料の物理的性状，すなわち形態，大きさ，硬さ，表面の性状や生体内における可動性などが関係すると考えられる．材料を生体内に埋没した場合，これら化学的及び物理的原因で生体内にはあらゆる病理学的現象，すなわち変性，萎縮，壊死や潰瘍形成などの退行性病変，うつ血，充血，出血，浮腫や血栓形成などの循環障害，肉芽，肥大，再生や器質化などの進行性病変，炎症や腫瘍などがおこりうる．一般にこれらの反応形態は埋没期間により異なり，短期間の埋没あるいは埋没初期においては主として退行性病変，循環障害や炎症などが，長期間の埋没では進行性病変や腫瘍が主体となる．

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医用材料の体内埋没による初期の組織反応

ラットやマウスを用いた動物実験の結果では，材料が体内に埋没された最初の１～２週目位までに認められる生体の組織反応としては，埋没された材料を中心に組織球やリンパ球が浸潤し，材料の表面に一層の細胞層を形成すると共に，毛細管の新生や線維芽細胞の出現，あるいは異物巨細胞の出現をみる．ついで急性期の変化に引続き，浸潤した細胞，新生毛細管や線維芽細胞などを中心とする肉芽は次第に繊維性の被膜を形成する様になる．これら急性期の反応は埋没される材料の性状により大きく影響され．たとえば乳房形成の目的でバックに充填された液状シリコンを外科的に挿入された場合，もしその液状シリコンが組織内にもれると異型性の強い異物巨細胞の出現を伴う高度の異物反応がみられる事はよく知られている．また場合によっては好中球などの高度の細胞浸潤，出血，浮腫などを伴ったり，局所の強い循環障害と共に同部が変性，壊死や潰瘍に陥入る場合もある．この様な埋没された材料を中心とする生体組織の反応は，ラットの場合には１～３カ月でしだいに弱まっていく．すなわち，埋没された材料周辺の細胞浸潤が消退すると共に，被膜の細胞成分もしだいに減少し，ヘマトキシリン－エオジン染色で均一に染まる硝子化の傾向に向かう（図１）．材料が埋没された時のこの様な組織反応は異物である材料に対しての生体の防衛反応として解釈され，病理組織学的には初期の材料を中心とする炎症反応，引き続く異物としての材料排除機転の反応に要約される．この様な埋没による急性期の組織反応（材料の小片をウサギの脊柱傍筋肉内に移植，72時間後に反応を観察する，プラスチックなどの安全性に関する生物学的試験としての移植試験）と，材料上で直接細胞を培養して細胞毒性を検索する試験や，溶出液を用いての急性毒性試験や皮内反応試験などの結果とはかなり良い相関を示すと言われている．また歯科領域で用いられている金属材料の場合，生体内で腐触し，溶出した金属イオンのため，局所のアレルギー反応や潰瘍をおこすほか，吸収されて体内諸臓器にそれが蓄積する事が知られている．一方，材料が高分子材料の場合，一般に疎水性の材料よりも親水性の材料の方が生体組織に対する適合性が良いと言われている．これは親水性であれば酸素や無機イオン，生体の代謝産物などが透過しやすく，生体組織の代謝が障害される事が少ないためと考えられている．この様なバイオマテリアルの生体組織反応は，材料の材質や物理的性状，埋没期間のほか，動物の種によっても大きく異なる．たとえばニワトリやモルモットでは異物に対する反応はきわめて弱く，結果的にはごく薄い被膜が形成されるにすぎない．これに対し，ラットやマウスでは反応は強い．またひとでは異物反応は長く持続し，瘢痕様の被膜形成は１～２年後にはじめて認められると言う．

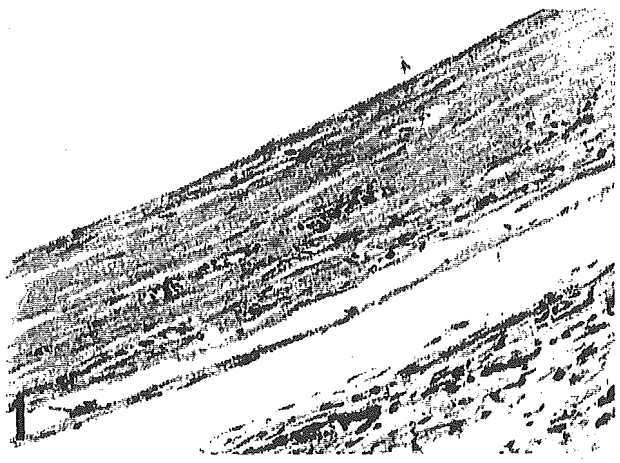

図１　ポリマー埋没後３カ月のラットにみられた被膜．被膜は硝子化せる線維性結合織よりなり，異常所見は認められない．

医用材料の長期間埋没による生体組織の反応―癌化

以前より，ひとにおいては摘出されずに体内に放置された銃弾や砲弾の破片，人工血管，骨折治療で骨の固定などに用いられた釘や銀線などの異物が原因で，10～数10年という長い潜伏期の後に腫瘍が発生したとの報告が散発的ながら報告されている．また人工的に挿入された異物ばかりでなく，胆石と胆嚢癌，膀胱結石や寄生虫と膀胱癌，あるいはアスベストによる肺癌及び肋膜中皮腫の例などにみる様に，異物がひとの腫瘍の原因となりうる幾つかの例が指摘されている．この様な異物による局所の腫瘍発生は一般に異物発癌 solid-state または foreign-body tumorigenesis（or carcinogenesis）と呼ばれている．しかしながら異物発癌に関する実験的研究

の歴史はそれほど古くなく，1941年，ラットの皮下にベークライトの円板を埋没して局所に肉腫の発生する事を報告したTurnerの論文が最初である[2]．彼はその論文の中で，埋没後20カ月で8例中3例のラットに皮下肉腫の発生をみたと述べている．その後，Oppenheimerら[3][4]，Druckreyおよび Schmähl，Nothdurftなど多くの人が実験的異物発癌を報告しており，近年においてはBrand[5][6]一派の詳細な報告が，また我が国においては今井及び増原[7]，筆者ら[8]の報告がある．なお異物発癌に関しては石川及び高山の総説[9]があるので参照されたい．

埋没により腫瘍を発生させるバイオマテリアルの頻

多くのフィルム状異物をラット皮下に埋没させてその発癌性を検討したOppenheimerら[4]の研究によれば，セロファン，ダクロン，イバロン，Kel-F，ナイロン，プリオフィルム，ポリエチレン，ポリエチレンメタクリレート，ポリスチレン，ポリビニルクロライド，サラン，シラスチック，テフロンなど実験したすべてのフィルムが発癌性を示し，その化学構造と発癌性との間にはっきりとした関係はないと報告している(表1)．そのほか多数の金属，水晶やガラス等についても発癌性の報告がある．これら発癌性を示す多くの異物のうち，バイオマテリアルとしてもっとも重要なものは合成有機ポリマーである．一般にポリマーはモノマーに比べ毒性が低く，体内でもほとんど代謝されないと考えられ，長期間体内に埋没するバイオマテリアルとして汎用されている．しかし動物への埋没ではほとんどのポリマーが発癌性を示すことが分かっている．実験的に発癌性を示した合成有機ポリマーとしては，ポリビニルピロリドン(PVP)，ポリスチレン，ポリエチレン，ポリエチレンメタクリレート，メチルメタクリレート，ポリシアノアクリレート，ダクロン，テフロン，シリコン，メチルポリシロキサンなどはその代表例であろう．そのほか人工腎臓の透析膜に用いられるセロファンにも発癌性がある．また歯科領域はもちろん，骨固定，人工関節，手術縫合など外科領域においても広く用いられている種々金属のうち，金，銀や白金などの重金属，鉄，タンタルやヴィタリウムなどもその箔状物を皮下に埋没することにより局所に肉腫を作ることが分かっている．しかしベリリウム，ニッケルやコバルトなどは粉末状のもので発癌性を示し，異物発癌よりはむしろ化学発癌であると考えられている．

表1 各種材料のラット皮下埋没による腫瘍発生率及び潜伏期

材料	腫瘍発生率(%)	潜伏期(日)
Cellophane A	35.7	495—779
B	45.4	322—665
C	46.1	390—706
Dacron	19.5	330—693
Ivalon sponge	8.8	567—857
Kel-F	23.3	259—581
Nylon	27.0	441—651
Pliofilm	15.0	359—708
Polyethylene	12.5	392—722
Polyethylene methacrylate	20.0	581—658
Polystylene	25.9	359—556
Polyvinyl chloride	38.6	189—727
Saran	11.9	390—847
Silastic	40.0	300—609
Teflon	23.5	439—748

異物発癌を修飾する各種因子

(イ)異物の性状

前述のごとく，異物発癌の発生には物理的要因と化学的要因の2つが考えられるが，主なる要因は物理的原因であることが指摘されている．同じ材料でもその形態により発癌性が異なり，ある一定以上の大きさの板状やフィルム状のものでは腫瘍を発生させるが，細片状や粉末状のものでは腫瘍は発生しない．また化学的にはまったく不活性であると考えられるガラスの様なものでも発癌することなどがその理由である．物理的要因と考えられる因子には材料の物理的性状，すなわち形態，大きさ，硬さや表面の性状などが大きく関係しているといわれている．直径1 cm以上の平板状あるいはフィルム状の材料はいかなる材料でも発癌するが，0.4 mm以下の細片あるいは粉末状のものでは発癌せず，また同じ平板状あるいはフィルム状でも多孔性になると腫瘍の発生は低下し，Brandらによるとミリポアフィルターを用いての実験では孔の穴が0.22 μmより大きいと腫瘍は発生しないという．Batesは表面の粗なものと平滑なものを用いて異物発癌を比較し，平滑な

表2　各種材料の形態による腫瘍発生率の違い

材料	連続シート	穴あきシート	スポンジ	線維	粉末
Cellophane	33	18	0	0	0
Fluorocarbons	23	19	—	—	0
Glass	18	—	—	0	—
Nylon	42	7	—	0	0
Polyesters	20	5	—	0	—
Polyethylene	15	15	—	3	0
Polystyrene (10mm)	9	—	—	—	—
(15mm)	28	—	0	—	—
(20mm)	35	—	—	—	—
Polyurethane	33	—	0	—	—
Polyvinyl alcohol	—	—	9	—	—
Polyvinyl chloride	24	0	—	—	—
Silicone elastomer	41	—	—	—	—
Silver metal	32	—	—	—	0
Stainless steel	13	—	—	—	—
Tantalum	11	—	—	—	0
Tin	10	—	0	—	—
Vitallium	14	—	—	—	0

表3　各種ポリマーのラット皮下埋没による皮下腫瘍の発生率及び潜伏期間

材料	性	皮下腫瘍発生率 担癌動物数/有効匹数	(%)	平均潜伏期間(週)
PVP-1	雄	3/17	(18)	76 (62—92)
	雌	5/20	(25)	79 (62—96)
PVP-2	雄	5/15	(33)	66 (59—71)
	雌	6/20	(30)	81 (63—98)
PVP-3	雄	8/18	(44)*	72 (57—87)
	雌	13/20	(65)**	82 (46—104)
HEMA	雄	7/19	(37)*	67 (40—95)
	雌	8/20	(45)*	67 (32—98)
Silicone	雄	1/14	(7)	82
	雌	2/19	(11)	84 (73—94)
Control	雄	0/10	—	—
	雌	0/12	—	—

* Silicone群の雄または雌と比較して有意差あり（χ^2テスト，$p<0.05$）

** Silicone群雌（χ^2テスト，$p<0.01$）及びPVP-1群雌（$p<0.05$）と比較して有意差あり

ものの方が粗なものに比べ早期に腫瘍が発生するという．これら材料の形態による発癌性の違いをHalpernの論文[10]よりまとめ表2に示した．

(2)動物の種，系および性差

動物における異物発癌には種差があることが指摘されているが，化学物質による発癌の様に系統差あるいは性差ははっきりしない．種差に関しては，一般にラットやマウスは感受性が高いのに反し，ニワトリやモルモットでは腫瘍は発生しない．これに対し，犬やハムスターはマウスやラットほど強くはないが発癌するとの報告がある．またガラスはラットに腫瘍を作りうるがマウスでは発癌の報告がなく，テフロンではその逆にマウスの方が感受性が強いことがいわれている．一方，性差については，筆者らのラットを用いての実験でははっきりしなかったが，Brandらによるとマウスを用いての実験では，雄に比べ雌の方が潜伏期が短かったが，逆に異型性は雄の方が強かったという．

(3)そのほかの因子

前述のごとく異物発癌においては物理的要因が主であることは確かであるが，プラスチックの様な合成高機ポリマーの場合には化学的要因をまったく無視することはできないのが現状である．表1や表2に示した様に，材料の化学構造には関係なくいずれにおいても発癌性が認められるが，その発癌率には強弱の差が認められる．筆者らも種々の異なるシート状のバイオマテリアル(厚さ 0.3～0.5 mm)を 10×20 mm の大きさに切り，Wistar ラットの皮下に埋没，2年間観察しその発癌性を比較検討した．その発生頻度は表3に示すごとくかなり違っており，腫瘍発生までの潜伏期にも違いがみられた[8]．この発生率の差の原因についてはよく分からない．この実験に用いたのと同じ検体の溶出液を用いての細菌あるいは培養細胞での突然変異試験はいずれも陰性であった．しかしながら，HEMA は生体内で変質しやすいこと，PVP には種々の可塑剤などが使われており，その種類や量は製品により異なること，一方 silicone は生体内でより安定であることなどが指摘されており，同じ大きさ，形のものを同じ系統，ロットのラットに埋没し，同じ飼育条件で行った実験でもあり，異物から遊離した少量の化学物質の関与を否定できない．現に塩化ビニル樹脂（PVC）には可塑剤としてフタル酸エステルが使われており，血液バッグ中に貯蔵した血液中には僅かながらフタル酸エステルが溶出することが知られている．さらにフタル酸エステルの一種であるDEHP〔ジー（2－エチルヘキシル）フタレート〕を餌に混じ，マウス及びラットに2年間投与すると肝細胞癌が発生する

ことが1980年米国NCIから報告されている．また塩ビモノマーの癌原性はよく知られている．

異物による発癌の機序

これら異物発癌の発生機序に関し，以前より異物周囲の被膜の関与が指摘されている．異物発癌の発生しない動物種においては被膜の形成がきわめて悪く，反対にマウスやラットでは厚い線維性被膜が形成される．この被膜の厚さと腫瘍発生との関連においては，被膜が薄ければ被膜内にある細胞は被膜外にある血管から酸素や栄養素を容易に供給され，また代謝産物を除去しうるが，被膜が厚くなるにつれ血行障害のため被膜内の細胞は正常な代謝を行うことができず，突然変異などにより癌化する確率が高くなると説明されている．しかし，最近Karpら[11]はこの様な血行障害による酸素欠乏状態は癌化の必要条件ではないと述べている．Brandら[5]はマウスを使った実験で異物発癌の成立過程をいくつかの段階にわけて説明している．すなわち，異物発癌の芽ともいうべきpreneoplastic cellsは異物を挿入した最初の頃（4～8週頃）までは異物周囲の肉芽組織中にある（Stage 1)．挿入後2カ月位すると被膜が形成されるがpreneoplastic cellsはクローンとなって被膜や周囲の結合織の中にあり，まだ異物の表面にはない（Stage 2)．つづいて次の段階にはこのpreneoplastic cellsは異物の表面に移動し（Stage 3)，やがて異物上に定着し自律性をもって増殖する様になる（Stage 4)．このpreneoplastic cellsは異物上に付着し，異物がないと腫瘍になれないが被膜とは無関係で，異物を除いた被膜を移植しても腫瘍は発生しないと述べている．しかしこの説ですべての異物発癌の機序を説明することは困難で，今後さらに検討する必要があろう．前述の筆者らの異物発癌の実験においても被膜と異物発癌の関係を検索したが，この実験においては移植3カ月目の時点ではほとんどの被膜は硝子化せる線維性組織よりなり，なんら異常所見は認めなかった(図1)．その後，局所に腫瘍の発生を認めず，ほかの原因で死亡したラットの局所を詳細に検討した結果，いくつかの被膜において異物に接した被膜内面にpreneoplastic changeと考えられる異型細胞の出現，増生を認めた(図2，3)．この実験では移植3カ月目を除いては

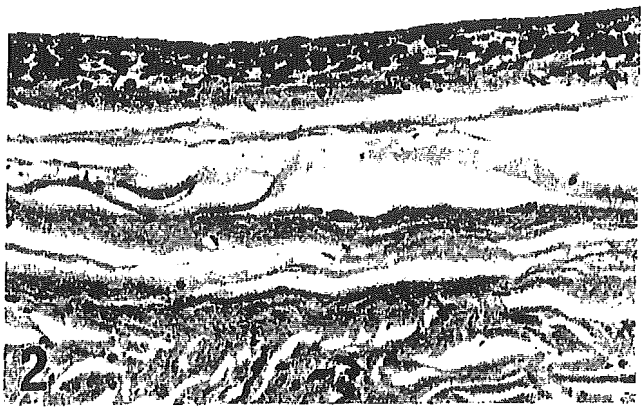

図2 PVP埋没後75週目の雄ラット被膜．薄い被膜の内面には異型細胞の増生がみられる．

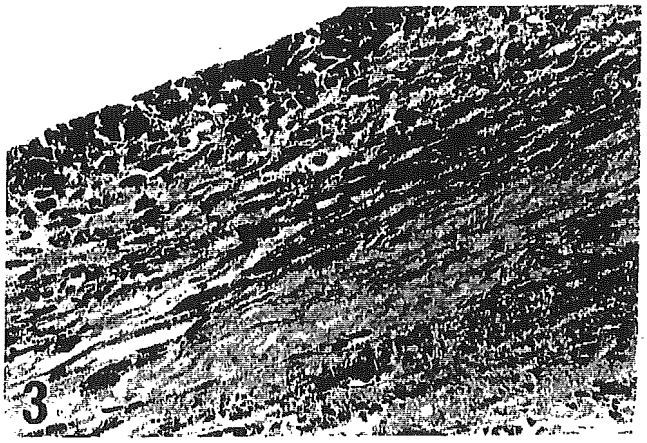

図3 PVP埋没89週目の雌ラットにみられた被膜内面の異型細胞増殖．

経時的屠殺を行っていないため異型細胞の出現する過程を詳細に追究することはできなかったが，この結果からも被膜が異物発癌の成立になんらかの重要な働きをしており，腫瘍は被膜内層より発生することが示唆された．

異物により発生する腫瘍の病理

Oppenheimerら[4]は異物の皮下埋没により局所に発生した腫瘍は組織学的にはほとんど線維肉腫(fibrosarcoma）であると報告している．またCarter[12]によれば，自然発生，誘発を問わずラット軟部織腫瘍の大部分は線維肉腫であるが，ひとのそれに比べれば組織像が多彩であると述べている．一方，近年ひとの軟部織腫瘍に関する研究の結果，もっとも多い腫瘍は線維肉腫ではなく悪性線維性組織球腫(malignant fibrous histiocytoma）であることが指摘されてきている．その後，発癌物質の皮下投与によ

ラットに誘発した軟部織腫瘍の多くはひとのそれと同じく悪性線維性組織球腫が主体を占めることが筆者ら[13]により報告されている．このラットにみられた悪性線維性組織球腫は，組織学的には人のそれ

表4　各種ポリマーのラット皮下埋没により得られた皮下腫瘍の組織学的分類

悪性線維性組織球腫	
線維芽細胞型	28(4)*
組織球型	15(2)*
多型細胞型	10
そのほかの腫瘍	
線維腫	1
線維肉腫	5(1)*
計	59(7)*

( )* 数字は遠隔転移をみた腫瘍

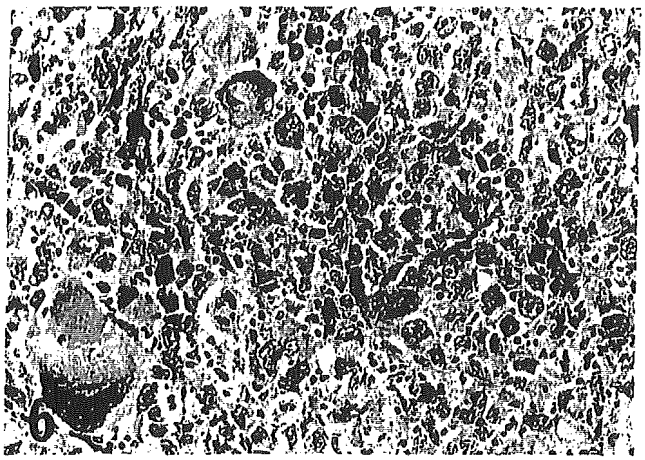

図6　PVP　埋没64週目雄ラットの悪性線維性組織球腫(pleomorphic type)．びまん性の組織球様細胞の増生とともに異型性の強い巨細胞の出現をみる．

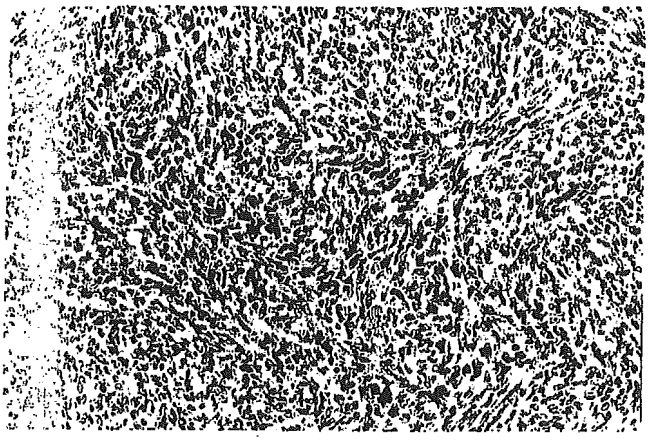

図4　PVP　埋没88週目雌ラットの悪性線維性組織球腫(fibroblastic type)．Storiform pattern を示す紡錘型細胞の増生がみられる．

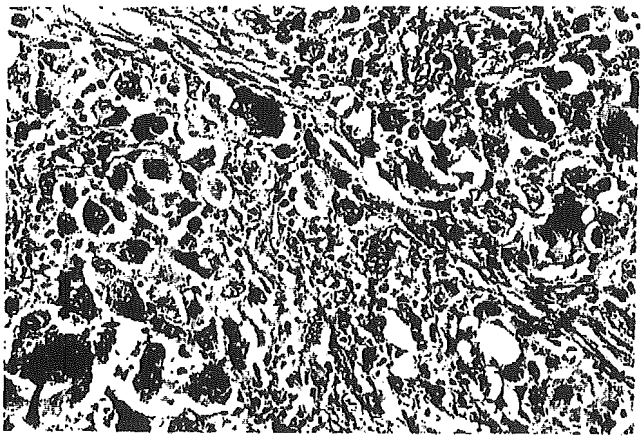

図7　PVP　埋没71週目雄ラットの悪性線維性組織球腫(pleomorphic type)．増生せる線維性組織の中に異型性を示す巨細胞を散在性に増生している．

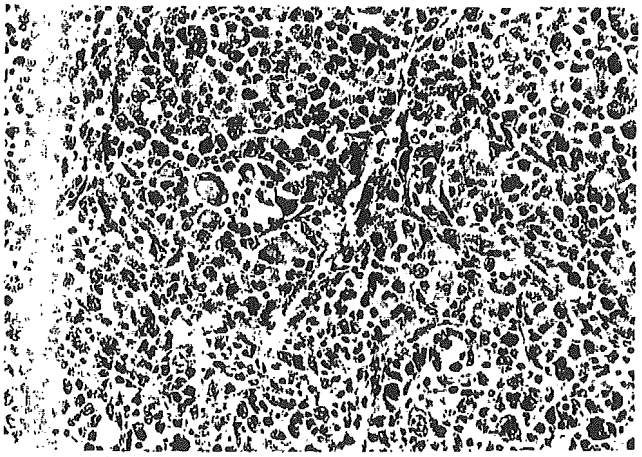

図5　HEMA　埋没81週目雄ラットの悪性線維性組織球腫(histiocytic type)．腫瘍はびまん性ないしシート状に増殖する組織球様細胞よりなる．

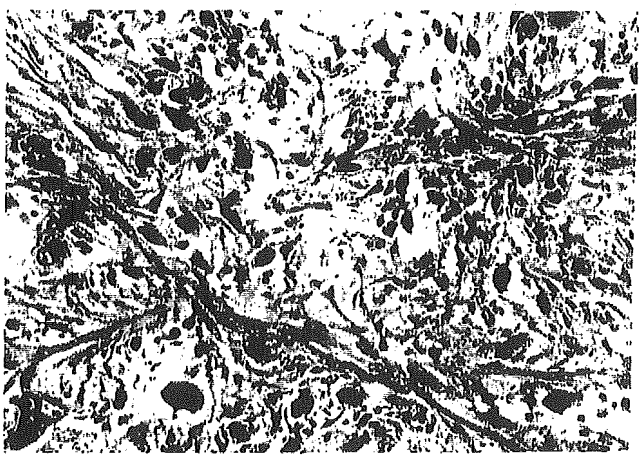

図8　HEMA 埋没54週目雌ラットにみられた myxomatous な変化の強い悪性線維性組織球腫

にきわめて類似し，いわゆる storiform pattern を示す紡錘型細胞を主体とする fibroblastic type，線維成分に乏しく組織球様細胞が索状あるいはびまん性に増殖し，中に黄色腫様蜂巣や Epulis 型巨細胞を混じる histiocytic type，異型性の強い巨細胞を主体とする pleomorphic type の3つに大きく分けられる．またいずれの type にもところどころ myxomatous な部分がみられた．さきの筆者らのバイオマテリアルを埋没し発生した皮下腫瘍の組織学的及び電子顕微鏡学的検索の結果を表4に示したが，化学物質投与による場合と同じく悪性線維性組織球腫が大部分であり，その中では fibroblastic type がもっとも多く（図4），ついで histiocytic type（図5），pleomorphic type（図6，7）であった．なかには myxomatous な変化の強い腫瘍も認められた(図8)．このことはひとにおけると同様にラットの軟部織腫瘍も悪性線維性組織球腫が主体であることを示している．

おわりに

ひとにおいてバイオマテリアルが長期間用いられた場合，それら材料と生体組織との親和性はきわめて重要である．この様なバイオマテリアルが体内に埋没された場合の生体組織反応は，異物である材料に対しての生体の防衛反応であり，病理学的には異物を中心とする炎症反応及び異物排除機転としての反応に要約される．長期間の埋没では，ある大きさ以上の，あるいは形のシート状材料は材料の化学構造のいかんを問わずラットやマウスの局所に悪性線維性組織球腫を主体とする肉腫を発生させ，その主なる原因は物理的要因であると考えられている．しかしながら，この様な動物的実験での異物発癌の結果より，そのもののひとにおける癌原性 risk を評価するに際しては，まだはっきりしたコンセンサスが得られていないのが現状であり[14]，この問題に関する検討は急務の事と考える．

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Short Communication

Malignant Fibrous Histiocytomas Induced in Rats by Polymers*

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Summary. Five polymeric materials (3 polyvinyl chlorides, 1 polyhydroxyethyl methacrylate, and 1 dimethyl polysiloxane) were implanted into subcutaneous (SC) tissues of rats. Subcutaneous tumors developed in all experimental groups. The incidences of the tumors differed however, although the experimental conditions were the same for all these materials. This result indicates that chemical characteristics of the materials may influence the incidence of SC tumors. From the histological and electron-microscopic findings many of these tumors were diagnosed as malignant fibrous histiocytomas.

Key words: Malignant fibrous histiocytoma – Polymers – Foreign-body tumorigenesis – Rats

Introduction

Turner (1941) first reported foreign-body tumorigenesis and there have been many subsequent reports on this subject (Bischoff and Bryson 1964; Brand et al. 1975a, b; Carter et al. 1971; Druckrey and Schmähl 1952; Hueper 1964; Nothdurft 1955; Nothdurft and Mohr, 1958; Oppenheimer et al. 1948, 1955; Stinson 1964).

These workers reported that the size and/or shape of implanted materials was the most important factor in the induction of tumors. Concerning the histology of foreign-body tumors, Oppenheimer et al. (1955) reported earlier that almost all were fibrosarcomas. Thereafter, Johnson et al. (1973) suggested that these tumors arose from pluripotential mesenchymal cells.

In the present study, we compared the tumorigenic activities of five polymeric materials following implantation into the subcutaneous (SC) tissue of rats.

Materials and Methods

Five kinds of sterilized sheet materials 0.3–0.5 mm in thickness, being three plasticized polyvinyl chlorides (PVC), one polyhydroxyethyl methacrylate (HEMA), and one dimethyl polysiloxane (silicone), were cut into 10 × 20 mm pieces.

Slc: Wistar rats (Shizuoka Laboratory Animal Center, Hamamatsu) aged 11 weeks were divided into five experimental groups each consisting of 20 males and 20 females and one control group consisting of 12 males and 12 females: Group 1 received PVC-1; group 2, PVC-2; group 3, PVC-3; group 4, HEMA and group 5, silicone; group 6 was the control group. The test materials were implanted in the SC tissues (the interscapular region) of animals in each experimental group. All animals were maintained on CE-2 basal diet (CLEA Japan Inc., Tokyo) and tap water.

All animals were allowed to live for 2 years, when all survivors were sacrificed. Dead or moribund animals were autopsied and examined for the development of tumors in the SC tissue and other organs. Tumors and all organs were fixed in buffered 10% formalin and the sections were routinely stained with hematoxylin and eosin. Some of the SC tumors were stained with PAS, PTAH, Azan, van-Gieson, and silver stains, in addition to hematoxylin and eosin. Several tumor samples were also examined by electron microscopy.

Results and Discussion

The incidences of SC tumors and mean survival times of rats in each group are shown in Table 1. Subcutaneous tumors were detected in all groups except the control group. The incidences of the tumors, however, were different in the various groups, although the materials were all tested in the same experimental conditions. The incidence was highest in group 3, followed in declining order by group 4, group 2, group 1, and group 5. The incidences of the tumors in males of group 3 and both sexes of group 4 were significantly higher than those in each sex of group 5 (χ^2 test, $P < 0.05$), and the incidence in females in group 3 was higher than in group 5 females ($P < 0.01$) and group 1 females ($P < 0.05$). The reason for the differences in the tumor incidence is obscure. But HEMA is reported to be degraded in SC tissue, and it seems rather active and unstable in the animal body (Kojima et al. 1974). Medical-grade PVC materials are known to contain many plasticizers, and the types and amounts of plasticizers in PVC differ with the manufacturer (Watanabe et al. 1978). On the other hand, silicone has been said to be stable. These chemical characteristics of the materials may influence the incidence of SC tumors, although the chromosomal aberration tests in mammalian cells and mutation tests in micro-or-

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Table 1. Incidences of subcutaneous tumors and mean survival times of rats after implantation of polymeric materials

Group	Material	Sex	No. of rats		Mean survival time (weeks)
			Tumor-bearing/ effective	With subcutaneous tumors (%)	
1	PVC-1	M	11/17	3 (18)	79 (62– 93)
		F	10/20	5 (25)	85 (39–104)
2	PVC-2	M	12/15	5 (33)	76 (59– 98)
		F	13/20	6 (30)	89 (63–104)
3	PVC-3	M	13/18	8 (44)[a]	75 (47– 95)
		F	16/20	13 (65)[b]	84 (46–104)
4	HEMA	M	14/19	7 (37)[a]	72 (33– 98)
		F	16/20	9 (45)[a]	78 (32–101)
5	Silicone	M	11/14	1 (7)	89 (67– 98)
		F	8/19	2 (11)	89 (48–104)
6	Control	M	8/10	0	81 (68– 91)
		F	5/12	0	95 (57–104)

[a] Significant difference in each sex from group 5 (χ^2 test, $P>0.05$)
[b] Significant difference from females in group 5 ($P<0.01$) and in group 1 ($P<0.05$)

ganisms with extracts from the same materials as were used in this study were all negative.

In addition to SC tumors, many other tumors were observed in all groups, including the control group. Histological findings in other tumors were similar to those in spontaneous tumors observed in this strain of rats, as reported previously (Maekawa et al. 1983).

All SC tumors were detected in the back, where samples were implanted, and almost all implanted samples were involved in the tumors. Histologically, these tumors were variegated, but there were no histological differences within the groups. Almost all were mesenchymal tumors with spindle cells arranged in a storiform pattern, with sheets of histiocyte-like cells, or pleomorphic giant cells. Many mitotic figures were observed. No cross striation was detected in any tumors. Nine samples of a total of 59 SC tumors were examined by electron microscopy, which revealed a mixture of fibroblast-like cells, histiocyte-like cells, and undifferentiated cells in these tumors. From these histological and electron microscopic findings, 53 of 59 tumors were diagnosed as malignant fibrous histiocytomas. Johnson et al. (1973) suggested that SC tumors induced by the implantation of foreign bodies arose more often from the nonfibroblastic pluripotential mesenchymal cells than from fibroblasts, although Oppenheimer et al. (1955) reported earlier that almost all of these tumors were fibrosarcomas and Carter (1973) also reported that fibrosarcomas are the most frequent soft-tissue tumors in rats. On the other hand, it has been suggested during the last two decades that malignant fibrous histiocytomas are the most common type of soft-tissue tumors in humans. Many of the tumors observed in this study had the same histological and electron microscopic characteristics as malignant fibrous histiocytomas in humans and also as malignant fibrous histiocytomas induced in rats by SC injection of chemical carcinogens (Maekawa et al. 1982). These findings indicate that malignant fibrous histiocytomas are the most common SC tumors not only in humans but also in rats.

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FOREIGN-BODY TUMORIGENESIS IN RATS BY VARIOUS KINDS OF PLASTICS - INDUCTION OF MALIGNANT FIBROUS HISTIOCYTOMAS

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SUMMARY

Five kinds of plastics (3 polyvinyl chlorides, 1 polyhydroxyethyl metacrylate and 1 dimethyl polysiloxane) were implanted into subcutaneous tissues of Wistar rats of both sexes. Subcutaneous tumors developed in all experimental groups. The incidences of the tumors, however, differed from each other, although these materials were tested on the same experimental condition. This result indicates that chemical characters of the materials may influence the incidence of subcutaneous tumors. Histologically, most of these subcutaneous tumors were mesenchymal tumors with spindle cells arranged in a storiform pattern, with sheets of histiocyte-like cells or pleomorphic giant cells. Electron microscopy showed mixture of fibroblastic cells, histiocytic cells and undifferentiated cells in these tumors. From these histological and electron microscopical findings, many of the tumors were diagnosed as malignant fibrous histiocytomas.

Key words : Foreign-body tumorigenesis, Plastics, Malignant fibrous histiocytoma, Rat

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Turner (1941) first reported foreign-body tumorigenesis and there are many subsequent reports on this subject (Bischoff and Bryson 1964; Brand et al. 1975a, 1975b, 1977; Carter et al. 1971; Druckrey and Schmähl 1952; Hueper 1964; Nothdurft 1955, 1958; Oppenheimer et al. 1948, 1955; Stinson 1964). These workers reported that the size and/or shape of implanted materials was the most important factor in induction of tumors. On the other hand, concerning the histology of fereign-body tumors, Oppenheimer et al. (1955) reported earlier that almost all were fibrosarcomas. Thereafter, Johnson et al. (1973) suggested that these tumors arose from pluripotential mesenchymal cells.

In the present study, we compared the tumorigenic activities of five kinds of plastics (3 polyvinyl chlorides, 1 polyhydroxyethyl methacrylate and 1 dimethyl polysiloxane), that are used clinically, by implanting them into the subcutaneous tissue of rats and investigated the morphological findings of the induced subcutaneous tumors by light and electron microscopy. A short report on this subject is to be published (Maekawa et al. 1984).

MATERIALS AND METHODS

Five kinds of sterilized sheet materials of 0.3-0.5 mm in thickness; 3 plasticized polyvinyl chlorides (PVC), 1 polyhydroxyethyl methacrylate (HEMA), and 1 dimethyl polysiloxane (silicone), were provided by Dr. T. Ooba, Department of Medical Supplies, National Institute of Hygienic Sciences, Tokyo. These sheet materials were cut into 10×20 mm pieces with scissors before use.

Eleven weeks old Slc: Wistar rats, purchased from Shizuoka Laboratory Animal Center (Hamamatsu), were divided into five experimental groups each consisting of 25 males and 25 females, and 1 control group consisting of 15 males and 15 females: Group 1, PVC-1; Group 2, PVC-2; Group 3, PVC-3; Group 4, HEMA; Group 5, silicone and Group 6, control. The test materials were implanted in the subcutaneous tissues (the interscapular region) of animals in each experimental group. All animals were maintained on CE-2 basal diet (CLEA Japan Inc., Tokyo) and tap water.

For observation of early histological changes in the subcutaneous tissues in which materials were implanted, 5 males and 5 females in each experimental group, and 3 males and 3 females in the control group were sacrificed at week 13 after implantation. The remaining animals were allowed to live for 2 years, and then all survivors were sacrificed. Dead or moribund animals were autopsied completely, and examined for development of tumors in the subcutaneous tissue and other organs. Tumors and all organs were fixed in buffered 10% formalin and the sections were routinely stained with hematoxylin and eosin. Some of subcutaneous tumors were stained with PAS, PTAH, Azan, van-Gieson and silver stains, in addition to hematoxylin and eosin. Several samples of the tumors were also examined by electron microscopy.

RESULTS

At week 13, all implanted materials were covered with thin capsules. Histologically, these capsules were composed of hyalinizated connective tissues. Slight infiltration of small round cells and macrophages, and proliferation of capillary vessels and fibroblast-like cells were observed, but no atypical cell growth was detected in any rats. The maximum thickness of these capsules in each group was calculated and it was about 0.21-0.30 mm, and there was no difference on the mean thickness among the experimental groups.

The incidences of total and subcutaneous tumors and mean survival times in each group are shown in Table 1. The first tumor was seen in a rat autopsied in week 32

Table 1 Incidences of Subcutaneous Tumors in Rats After Implantation of Various Kinds of Plastics

Group	Material	Sex	No. of rats				
			initial	effective	tumor-bearing (%)	with subcutaneous tumors (%)	Mean survival time (w)
1	PVC-1	M	20	17	11(65)	3(18)	79(62- 93)
		F	20	20	10(50)	5(25)	85(39-104)
2	PVC-2	M	20	15	12(80)	5(33)	76(59- 98)
		F	20	20	13(65)	6(30)	89(63-104)
3	PVC-3	M	20	18	13(72)	8(44)**	75(47- 95)
		F	20	20	16(80)*	13(65)***	84(46-104)
4	HEMA	M	20	19	14(74)	7(37)**	72(33- 98)
		F	20	20	16(80)*	9(45)**	78(32-101)
5	silicone	M	20	14	11(79)	1(7)	89(67- 98)
		F	20	19	8(42)	2(11)	89(48-104)
6	control	M	12	10	8(80)	0	81(68- 91)
		F	12	12	5(42)	0	95(57-104)

* Significant difference from females in Group 6 (X^2 test, $p<0.05$).
** Significant difference from each sex in Group 5 ($p<0.05$).
*** Significant difference from females in Group 5 ($p<0.01$) and in Group 1 ($p<0.05$).

after implantation. All rats surviving beyond this time were counted in effective numbers, except several rats in which autolysis was too advanced to allow histological examination. In males, there was no significant difference in the incidences of total tumors between each experimental and control group. On the other hand, in females, the incidences of total tumors in all experimental groups except Group 5 were higher than that in the control group, and the incidences in Group 3 and 4 were significantly higher than that in the control group (χ^2 test, $p<0.05$). Subcutaneous tumors were detected in all groups except the control group, although the incidences differed in each

group. The incidence was highest in Group 3, followed in order by those in Group 4, Group 2, Group 1 and Group 5. The incidences in males of Group 3 and both sexes of Group 4 were significantly higher than those in each sex of Group 5(χ^2 test, $p<0.05$), and the incidence in females of Group 3 was higher than those in females of Group 5 ($p<0.01$) and Group 1 ($p<0.05$).

All subcutaneous tumors were detected in the back where samples were implanted. Almost all implanted samples were involved in the tumors. Samples were sometimes found folded or rolled in the tumors and many films of the materials other than silicone were brittle. Tumors were round or oval in shape and white or brown in color. The tumors differed in size: the largest weighing 150 g measured $87\times67\times68$ mm, and the smallest was the size of a soy-bean. Histologically, these subcutaneous tumors were variegated, but there was no histological differences in the groups. Almost all were mesenchymal tumors with spindle cells arranged in a storiform pattern, with sheets of histiocyte-like cells, or pleomorphic giant cells (Photos 1-3). In some cases, matrix of the tumors showed myxoid or osteoid-like and in 2 cases ossification was marked. Many mitotic figures were observed. No cross striation was detected in any tumors. Nine samples of a total of 59 subcutaneous tumors were examined by electron microscopy. Electron microscopy showed a mixture of fibroblast-like cells, histiocyte-like cells and undifferentiated cells in these tumors. Fibroblast-like cells were mostly elongated or polygonal in shape and had smooth or slightly lobulated nuclei. Their cytoplasm contained abundant rough-surfaced endoplasmic reticulum and also other cytoplasmic organellae in various developmental stages. On the other hand, histiocyte -like cells varied in shape and had round or reniform nuclei. The cytoplasm contained abundant ribosomes and moderate amounts of smooth or rough surfaced endoplasmic reticulum. Dilated endoplasmic reticulum and intracytoplasmic actin-like filaments were observed in some cases. From these histological and electron microscopical findings, 53 out of 59 subcutaneous tumors were diagnosed as malignant fibrous histiocytomas, as shown in Table 2. As other subcutaneous tumors, 5 fibrosarcomas and 1 fibroma were detected. Only 7 of 59 tumors metastasized to the remote organs such as the lung, and 6 of these 7 tumors were malignant fibrous histiocytomas.

In addition to subcutaneous tumors, many other tumors were observed in all groups including the control group. Tumors of the testis/uterus and leukemias were the most common and were detected in all groups. Tumors were also detected in various organs, but at low incidences. In any experimental groups, no significant increase in the incidence of these tumors was observed. Histological findings of these tumors were quite similar to those of spontaneous tumors observed in this strain of rats, as reported previously (Maekawa et al. 1983).

In the back of rats without subcutaneous tumors, implanted samples were enclosed in capsules. The thickness of these capsules were the same or slightly more than that of capsules at week 13. In some cases, however, atypical cell proliferation was detected in the inner layer of the capsules (Photo 4) and some of these lesions were diagnosed as

pre-neoplastic or early neoplastic changes. These lesions were observed in all experimental groups, although the incidences were low and there was no difference in each group.

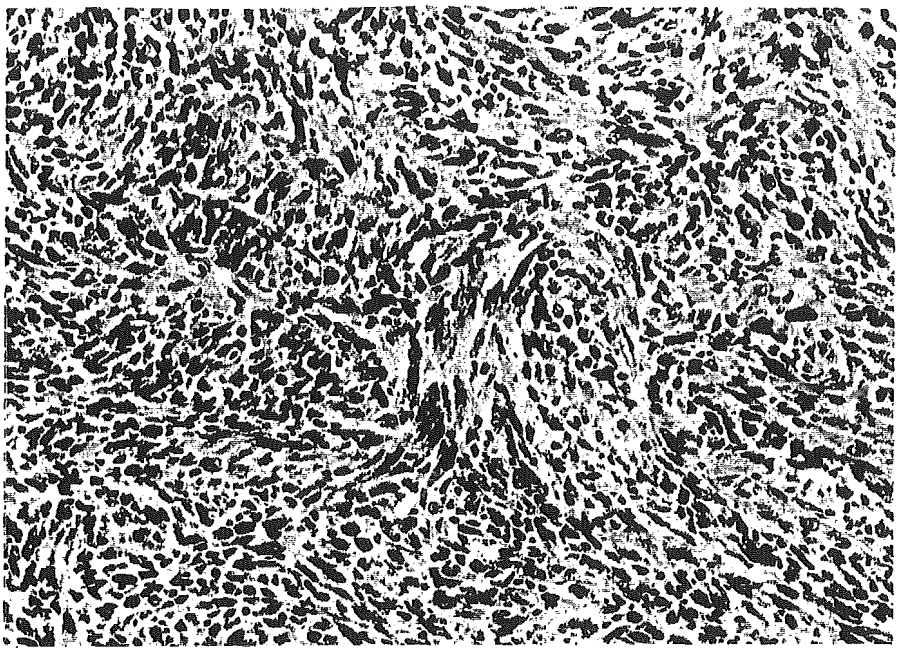

Photo. 1. Malignant fibrous histiocytoma (fibroblastic type) with prominent storiform pattern, observed in a female rat in PVC-1 group killed at the 86th week. Hematoxylin and Eosin. (×100)

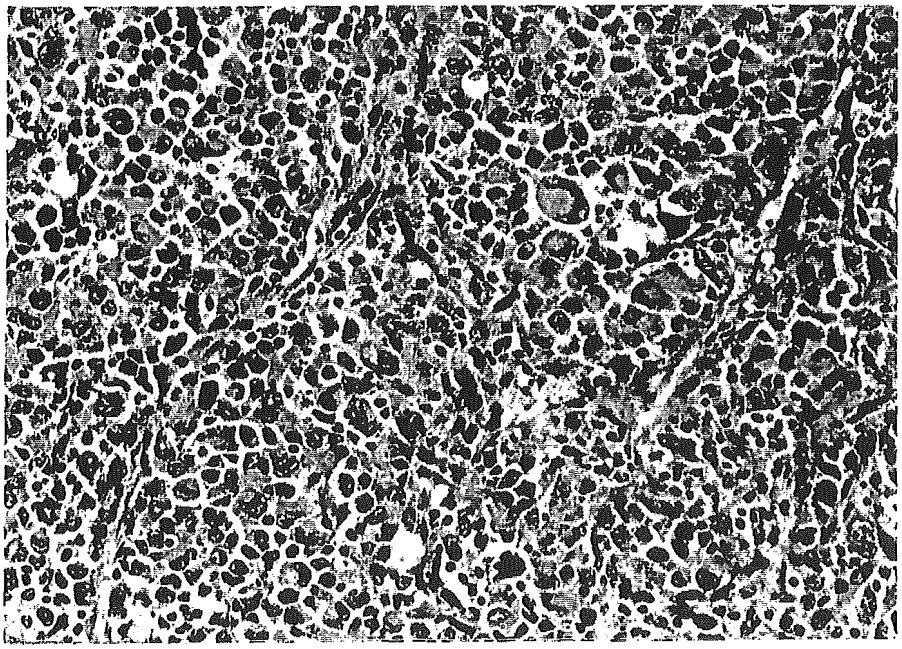

Photo. 2. Malignant fibrous histiocytoma (histiocytic type) of a male rat in HEMA group killed at the 40th week. Diffuse proliferation of histiocytic cells with glassy cytoplasm is prominent. H & E. (×100)

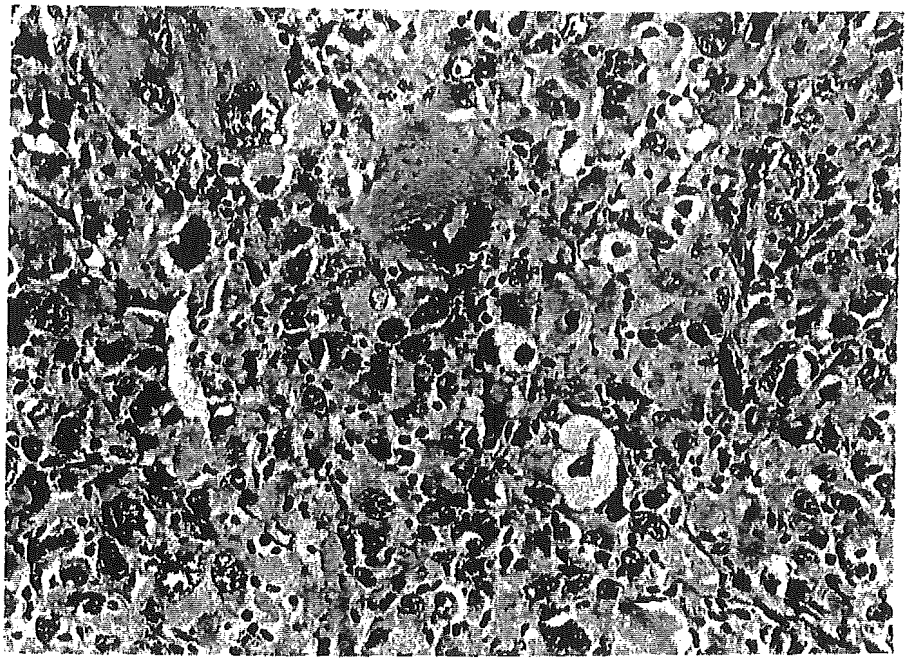

Photo. 3. Malignant fibrous histiocytoma (pleomorphic type) of a male rat in PVC-3 group killed at the 46th week. Pleomorphic giant cells with bizarre nuclei admixed with histiocyte-like cells are shown. H & E. (×200)

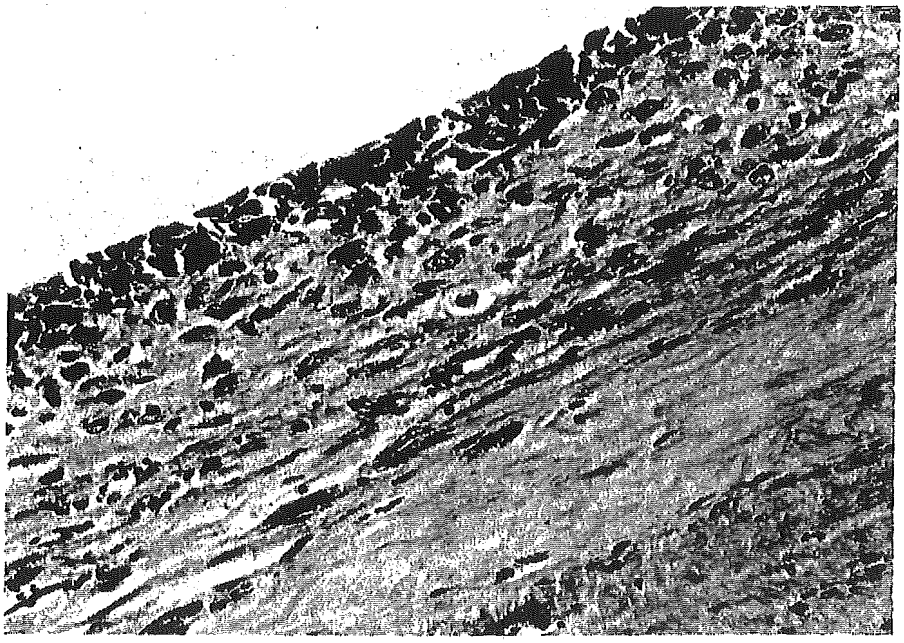

Photo. 4. A capsule of a female rat without subcutaneous tumors in PVC-2 group killed at the 89th week. Atypical cell proliferation is observed at the inner layer of the capsule. H & E. (×200)

Table 2 Histological Classification of Subcutaneous Tumors Induced by Various Kinds of Plastics

Malignant fibrous histiocytoma		
fibroblastic type	28	(4)*
histiocytic type	15	(2)*
pleomorphic type	10	
Other tumors		
Fibroma	1	
Fibrosarcoma	5	(1)*
Total	59	(7)*

* No. of rats with metastasis to remote organs

DISCUSSION

In this study, many tumors including subcutaneous tumors were observed. Except subcutaneous tumors, other tumors such as tumors of the testis, uterus, hematopoietic organs and endocrine organs are observed spontaneously at relatively high incidences in this strain of rats (Maekawa et al. 1983). All subcutaneous tumors, however, were detected in the back where samples were implanted and almost all implanted samples were detected in the tumors. Therefore, all subcutaneous tumors observed in this study were considered as induced tumors by implantation of polymers.

Earlier studies by Oppenheimer et al. (1948, 1955) and others (Bischoff and Bryson 1964; Brand et al. 1975a, 1975b, 1977; Carter et al. 1971; Druckrey and Schmähl 1952; Hueper 1964; Imai and Masuhara 1979; Nothdurft 1955, 1958; Stinson 1964; Turner 1941) showed that subcutaneous sarcomas could be induced in rats and mice by implantation of many kinds of foreign bodies, and the physical presence and nature of the foreign body, not its chemical reactivity, were responsible for tumor development. These workers also reported that the size and/or shape of implanted materials was important factors in induction of tumors, and all kinds of sheet materials of more 10 mm in diameter produced tumors. In this study, test materials as sheets of 10×20 mm induced tumors in all experimental groups. This result was consistent with the data of others. In this study, however, the incidences of the tumors were different in groups, although these materials were tested on the same experimental condition. The reason for the difference in the tumor-incidence is obscure. But medical grade PVC materials are known to contain many plasticizers, and the types and amounts of plasticizers in PVC differ with the manufacturer (Watanabe et al. 1978). HEMA is reported to be degraded in subcutaneous tissues and it seem rather active and unstable in the animal body (Kojima et al. 1974). On the other hand, silicone has been said to be stable. These

chemical characters of the materials may influence the incidence of subcutaneous tumors, although the chromosomal aberration tests in mammalian cells and the mutation tests in microorganisms with extract from the same materials used in this study were all negative.

Johnson et al. (1973) suggested that subcutaneous tumors induced by implantation of foreign-bodies arose more often from the non-fibroblastic pluripotential mesenchymal cells than fibroblasts, although Oppenheimer et al. (1955) reported earlier that almost all of these foreign-body sarcomas were fibrosarcomas, and Carter (1973) reported also that fibrosarcomas are the most frequent soft-tissue tumors in rats. On the other hand, it has been suggested during the last 2 decades that malignant fibrous histiocytomas are the most common type of soft-tissue tumors in humans. In our study, many subcutaneous tumors had the same characteristics to histological and electron microscopical findings of malignant fibrous histiocytomas in humans, and also those of the tumors induced by subcutaneous injections of chemical carcinogens in rats (Konishi et al. 1982; Maekawa et al. 1982; Takahashi et al. 1982). These findings indicate that malignant fibrous histiocytomas are the most common subcutaneous tumors not only in humans but also in rats.

Karp et al. (1973) reported that tissue anoxia was not so important in foreign-body tumorigenesis. Brand et al. (1975a) reported several stages in foreign-body tumorigenesis: preneoplastic cells accumulate in the tissue outside of the capsule at an early stage and then they migrate into the inner layer of the capsule, attach to the foreign-body and proliferate. Imai and Masuhara (1979) reported that the thickness of the capsule is very important in foreign-body tumorigenesis. In this study, histological observation of subcutaneous tissues in rats without subcutaneous tumors indicate that atypical cell growth or preneoplastic changes occurred first not in the tissue outside of the capsule, but in the inner layer of the capsules. This finding suggests that the capsule produced around the foreign-body has the most important role in foreign-body tumorigenesis, as reported by others.

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Bacteria-Induced Intestinal Cancer in Mice with Disrupted *Gpx1* and *Gpx2* Genes

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ABSTRACT

Two glutathione peroxidase (GPX) isozymes, GPX-1 and GPX-2 (GPX-GI), are the major enzymes that reduce hydroperoxides in intestinal epithelium. We have previously demonstrated that targeted disruption of both the *Gpx1* and *Gpx2* genes (GPX-DKO) results in a high incidence of ileocolitis in mice raised under conventional conditions, which include the harboring of *Helicobacter* species [non-specific-pathogen-free (non-SPF) conditions]. In this study, we have characterized GPX-DKO mice that have microflora-associated intestinal cancers, which are correlated with increased intestinal pathology/inflammation. We found that GPX-DKO mice raised under germ-free conditions have virtually no pathology or tumors. After colonizing germ-free mice with commensal microflora without any known pathogens (SPF), <9% of GPX-DKO mice develop tumors in the ileum or the colon. However, about one-fourth of GPX-DKO mice raised under non-SPF conditions from birth or transferred from SPF conditions at weaning have predominantly ileal tumors. Nearly 30% of tumors are cancerous; most are invasive adenocarcinomas and a few signet-ring cell carcinomas. On the basis of these results, we conclude that GPX-DKO mice are highly susceptible to bacteria-associated inflammation and cancer. The sensitivity exhibited in these mice suggests that peroxidative stress plays an important role in ileal and colonic pathology and inflammation, which can lead to tumorigenesis.

INTRODUCTION

Enteric microflora begin to colonize the gut at birth and affect development and maintenance of the mucosal immune response and epithelial cell functions (1). Recently, commensal bacteria have emerged as cofactors in the development of ileocolitis and intestinal malignancies. Human inflammatory bowel disease (IBD), which includes Crohn's disease and ulcerative colitis, increases cancer risk by 20–30-fold in patients with prolonged IBD histories (2). Many mouse models have been generated to facilitate studying the etiology, prevention, and treatment of IBD and gastrointestinal cancer associated with IBD. The essential role of enteric microflora in ileocolitis-associated cancer has been established in several of these mouse idiopathic IBD models with defects in the immune response. Interleukin (IL)-2 KO, IL-10 KO, T-cell receptor /p53 DKO, or tumor growth factor 1/Rag-2 DKO mice, when maintained under germ-free (GF) conditions, fail to develop severe inflammation, as well as small intestinal or colonic cancers that often occur when those animals are raised under conventional housing conditions (3–6). Because microflora can modulate epithelial cell signaling for immune reactions (7–9), this may explain the susceptibility of these immune-compromised mice to the development of IBD. Because GPX-DKO mice have an intact immune system at the outset of these studies, the role of microflora on the subsequent development of any pathological condition in these mice was unclear.

Glutathione peroxidases (GPX) are a family of four selenium-dependent antioxidant enzymes in mice (five in humans; Ref. 10) that reduce H_2O_2 and organic hydroperoxides by oxidizing glutathione. Taken together, the ubiquitous GPX-1 and epithelium-specific GPX-2 contribute nearly all glutathione-dependent H_2O_2-reducing activity in the intestinal epithelium (11). We have previously reported that GPX-DKO mice, with targeted disruption of *Gpx1* and *Gpx2* genes, exhibit ileocolitis between 2 and 7 weeks of age, which is accompanied by accumulation of lipid hydroperoxides, weight loss, and proctitis (12). Because some colitis models such as *Mdr1*-KO mice do not appear to develop cancer (13, 14), we set out to determine whether GPX-DKO mice would develop intestinal cancer. Because most mouse IBD and ileocolitis-associated cancer models are either immunodeficient or defective in membrane proteins that affect epithelial barrier integrity (15, 16), showing that intracellular GPX activity could prevent microflora-induced ileocolitis and cancer would strengthen the notion that peroxidative stress is one basis for the pathogenesis of inflammation-associated cancer. Although elevated reactive oxygen and nitrogen species are recognized as an integral part of the pathophysiology of IBD, there is little evidence to specify the precise role of hydroperoxides in IBD pathology (17, 18). Demonstrating that epithelial GPX activity could inhibit both IBD and IBD-associated cancers might set the stage for the prevention of IBD-related cancers with inhibitors of the major hydroperoxide-generating enzymes that reside in the mucosal epithelium or inflammatory cells.

Our original non-specific-pathogen-free (non-SPF) GPX-DKO mouse colony harbors several enterohepatic *Helicobacter* species such as *H. hepaticus*, which is widely spread in rodents (19). Although *H. hepaticus* was originally identified as causing hepatitis and hepatocellular tumors in A/JCr mice, its primary site of colonization is in the intestine. Colonization of *H. hepaticus* to SPF mice causes ileocolitis and colon cancer in immune-deficient animals such as nude mice, IL-10 KO, and T-cell deficient mice (20–22). However, *H. hepaticus* only induces mild or no colitis in SPF and immune competent wild-type C57BL/6 and *Mdr1*-KO mice (19, 23). In this article, we address whether specific microflora are essential for ileocolitis and its associated cancer in GPX-DKO mice by comparing the extent of ileocolitis and cancer incidences in mice harboring non-SPF, SPF, or no enteric microflora.

MATERIALS AND METHODS

Animals. GPX-DKO mice were generated by mating $Gpx1^{tm1Ysh/tm1Ysh}$ (*Gpx1*-KO) and $Gpx2^{tm2Coh/tm2Coh}$ (*Gpx2*-KO) mice. Both lines were on a mixed C57BL/6J and 129Sv/J or 129S3 mixed background, as we have reported previously (12). The original colony harbors *Helicobacter* species, including *H. hepaticus*, evaluated by PCR on fecal samples (Missouri University Research Animal Diagnostic Laboratory). A GF GPX-DKO colony was established by neonatal transfer at the Gnotobiotic Laboratory of the University of Wisconsin-Madison. Upon the closure of the University of Wisconsin-Madison gnotobiotic facility, the GF colony was transferred aseptically to the Gnotobiotic Laboratory kindly provided by Kathryn A. Eaton, DVM, Ph.D., at Ohio State University-Columbus for an additional 5 months. To establish a SPF colony, we have transferred GF mice from University of Wisconsin-

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