CHAPTER 10
TISSUE RESPONSE TO IMPLANTS
• To implant a material surgeon has to injure tissue.

• Success of entire operation depends on kind & degree of tissue response.

• Biocompatibility entails mechanical, chemical, pharmacological & surface compatibility.

• Tissue response toward injury vary widely according to site, species, contamination, etc.
10.1 NORMAL WOUND HEALING PROCESS

10.1.1 Inflammation

- **Inflammatory reaction**: immediate response to any injury.
  - Constriction of capillaries (stops blood leakage) dilation.
  - Increase activities in **endothelial cells** lining capillaries.
  - Capillaries covered by adjacent **leukocytes**, **erythrocytes**, & **platelets**.
Concurrently with vasodilation, leakage of plasma from capillaries occurs. This fluid combined with migrating leukocytes & dead tissues constitute exudate. Once enough cells (Table 10-1) accumulated by lysis, exudate becomes pus. Pus can occur in nonbacterial inflammation.

Local lymphatics also damaged. Leakage of fluids from capillaries provide fibrinogen & other formed elements of blood clotting system quickly plug damaged lymphatics, thus localizing inflammatory reaction.
• All reactions - vasodilation of capillaries, leakage of fluid into extravascular space, & plugging of lymphatic => classic inflammatory signs: redness, swelling, & heat which can lead to local pain.

• Extensive tissue injury or wound contains either irritants or bacteria, inflammation extensive tissue destruction.
• **Collagenase**, proteolytic enzyme carries out tissue destruction. Released from **granulocytes** which in turn are lysed by the **lower pH** at the wound site. pH can drop to below 5.2 at the injured site from normal values, 7.4-7.6.

• If no drainage for necrotic debris, lysed granulocytes, formed blood elements, etc., then the site becomes a severely destructive inflammation necrotic abscess.
• **Chronic inflammatory process:** without the healing process occurring within 3-5 days

• Marked by presence of **mononuclear cells** called **macrophages** coalesce to form multinucleated giant cells [Fig 10-1].
• **Macrophages**: phagocytic & remove foreign material or bacteria.

• Sometimes mononuclear cells evolve into **histiocytes** which regenerate collagen. Regenerated collagen unite wound or wall-off unremovable foreign materials by **encapsulation**.

• **Lymphocytes** occur as clumps or foci. Primary source of **immunogenic agents** which become active if **foreign proteins** not removed by body's primary defense. An **autoimmune** reaction suggested as a foreign body reaction of nonproteinous materials like silica.
**Table 10-1. Definitions of Cells**

<table>
<thead>
<tr>
<th>Types of cells</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chrondroblast</td>
<td>An immature collagen (cartilage)-producing cell.</td>
</tr>
<tr>
<td>Endothelial</td>
<td>A cell lining the cavities of the heart and the blood and lymph vessels.</td>
</tr>
<tr>
<td>Erythrocyte</td>
<td>A formed element of the blood containing hemoglobin (red blood cell).</td>
</tr>
<tr>
<td>Fibroblast</td>
<td>A common fixed cell of connective tissue that elaborates the precursors of the extracellular fibrous and amorphous components.</td>
</tr>
<tr>
<td>Giant cell</td>
<td>A large cell derived from a macrophage in the presence of a foreign body.</td>
</tr>
<tr>
<td>Foreign body giant cell</td>
<td>A large cell having many nuclei.</td>
</tr>
<tr>
<td>Multinucleated giant cell</td>
<td></td>
</tr>
<tr>
<td>Granulocyte</td>
<td>Any blood cell containing specific granules; included are neutrophils, basophils, and eosinophils.</td>
</tr>
<tr>
<td>Leukocytes</td>
<td>A colorless blood corpuscle capable of ameboid movement, protects body from microorganisms and can be of five types: lymphocytes, monocytes, neutrophils, eosinophils, and basophils.</td>
</tr>
<tr>
<td>Macrophage</td>
<td>Large phagocytic mononuclear cell. Free macrophage is an ameboid phagocyte and present at the site of inflammation.</td>
</tr>
<tr>
<td>Mesenchymal</td>
<td>Undifferentiated cell having similar role as fibroblasts but often smaller and can develop into new cell types by certain stimuli.</td>
</tr>
<tr>
<td>Mononuclear</td>
<td>Any cell having one nucleus.</td>
</tr>
<tr>
<td>Osteoblast</td>
<td>An immature bone-producing cell.</td>
</tr>
<tr>
<td>Phagocyte</td>
<td>Any cell that destroys microorganisms or harmful cells.</td>
</tr>
<tr>
<td>Platelet</td>
<td>A small circular or oval disk-shaped cell (3-Am diameter) precursor of a blood clot.</td>
</tr>
</tbody>
</table>
10.1.2. Cellular Response to Repair

- **Mesenchymal cells** evolve into migratory **fibroblasts** injured site, while necrotic debris, blood clots, etc. are removed by **granulocytes** & **macrophages**.

- Inflammatory exudate contains **fibrinogen** which is converted into **fibrin** by enzymes => **scaffolds** injured site.

- Fibroblasts use fibrin scaffolds as a framework onto which collagen is deposited. **New capillaries** are formed following the migration of fibroblasts; then the fibrin scaffolds are removed by the **fibrinolytic enzymes** activated by endothelial cells. Endothelial cells together with the fibroblasts liberate collagenase which limits the collagen content of the wound.
• After 2-4 wks of fibroblastic activities wound undergoes remodeling by decreasing glycoprotein & polysaccharide content of scar tissue & lowering number of synthesizing fibroblasts. New balance of collagen synthesis & dissolution reached & maturation phase of wound begins.

- Schematic diagram of sequential events of cellular response of soft tissues[Fig 10-2].
• Tensile strength of wound is not proportional to amount of collagen deposited in injured site [Fig 10-3]. Latent period for the collagen molecules to polymerize.

• Collagen restructuring process requires > 6 months to complete although wound strength never reaches original value.
• Wound strength can be affected by many variables, **nutrition**, **temp**, **presence of other wounds**, & **oxygen tension**. Other factors such as **drugs**, **hormones**, **irradiation**, & **electrical stimulation**.
• Bone Repair
  – Healing of bone fractures is regenerative rather than simple repair as seen in other tissues except liver. However, the extent of regeneration is limited in humans. The cellular events following fracture of bone[Fig 10-4].
• When bone is fractured form a blood clot around fracture site.

• **Fibroblasts & osteogenic cells** in periosteum migrate & proliferate toward injured site. Lay down a fibrous collagen matrix for **callus**

  • **Osteoblasts** evolve from osteogenic cells near bone surface to calcify callus into trabeculae, forming a spongy bone.

• **Osteogenic cells** migrate away from established blood supply become **chondroblasts** lay down cartilage. 2-4 wks periosteal callus, three parts [Fig 10-5].
• Simultaneously with external callus formation similar repair process occurs in marrow cavity. Abundant supply of blood, cavity turns into callus rather quickly & becomes fibrous or spongy bone.

• New trabeculae develop in fracture site by appositional growth & spongy bone turns into compact bone. Begins after about 4 wks.
• Other interesting observations made on healing of bone fractures in relation to synthesis of polysaccharides on collagen.

• Amount of collagen & polysaccharides closely related to cellular events following fracture.

• Amount of collagen starts to increase onset of remodeling process. After about 1 wk.
- **Electrical potential** (biopotential) measured in long bone before & after fracture[Fig 10-6]. High **electronegativity** in vicinity of fracture marks increased **cellular activity**. Maximum negative potential in epiphysis in normal bone since this zone is site of greatest activity (growth plate is in epiphysis).

Example 10.1

- The healing process of wounds in the skin have often been investigated since such healing is relevant to every surgery. In one study electrical stimulation was used to accelerate the healing of wounds in rabbit skin as shown in the following figure. The mean current flow was 21 mA and the mean current density was 8.4 mA/cm². After 7 days, the load to fracture of the skin (removed from the dead animals) on the control samples was 797 g and on the stimulated experimental side it was 1,224 g on the average [J.J. Konikoff, *Annals Biomed. Eng.*, 4, 1, 1976].

(a) Calculate the percent increase of strength by stimulation.

(b) The width of the testing sample was 1.6 cm. Assuming 1.8 mm thickness of skin, calculate the tensile stress for both control and experimental sample.

(c) Compared with the strength of normal skin (about 8 MPa) what percentages of the control and experimental skin wound strengths were recovered?

(d) Compare the results of (c) with the result of Figure 10-3.
Answer

\[
\frac{1,224 \div 797}{797} = 0.536 \text{ (53.6%)}
\]

(a)

(b)

\[
\text{Stress} = \frac{797 \times 10^{-3} \times 9.8 \text{ N}}{1.8 \times 16 \times 10^{-6} \text{ m}^2} = 0.27 \text{ MPa}
\]

\[
\text{Stress} = \frac{1,224 \times 10^{-3} \times 9.8 \text{ N}}{1.8 \times 16 \times 10^{-6} \text{ m}^2} = 0.42 \text{ MPa}
\]

(c)

\[
\frac{0.27}{8} = 0.034 \text{ (or 3.4%)} , \quad \frac{0.42}{8} = 0.052 \text{ (or 5.2%)}
\]

(d) About same recovery.
10.2 BODY RESPONSE TO IMPLANTS

- Response of the body to implants **host site & species, degree of trauma** imposed during implantation, & all variables associated with a normal wound-healing process. Implant's **chemical composition & micro- & macrostructure**. Local (cellular) & systemic response.
10.2.1. Cellular Response to Implants

- Body's reaction to foreign materials expel them.
- Extruded or walled-off if it cannot be removed.
- Particulate or fluid ingested by giant cells (macrophages) & removed.
- Typical tissue response: polymorphonuclear leukocytes appear near implant followed by macrophages called foreign body giant cells. If implant is chemically & physically inert only thin layer of collagenous tissue encapsulates implant.
• If implants chemically or physically irritating to surrounding tissue inflammation. Inflammation (both acute & chronic type) delay normal healing => process resulting in granular tissues.

• Some implants cause necrosis of tissues by chemical, mechanical, & thermal trauma.

• Tissue response to various implants due to wide variations in experimental protocol[Table 10-2]
Table 10-2. Effect of implantation on the Properties of Various Suture Materials'

<table>
<thead>
<tr>
<th>Material</th>
<th>Wound tensile strength</th>
<th>Suture tensile strength</th>
<th>Tissue reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorbable plain catgut</td>
<td>Impaired</td>
<td>Zero by 3-6 days</td>
<td>Very severe</td>
</tr>
<tr>
<td>Chromic catgut</td>
<td>Impaired</td>
<td>Variable</td>
<td>Moderate (much less severe than plain catgut, but more than nonabsorbable materials)</td>
</tr>
<tr>
<td>Nonabsorbable silk</td>
<td>No effect</td>
<td>Well maintained</td>
<td>Slight</td>
</tr>
<tr>
<td>Nylon multifilament</td>
<td>No effect</td>
<td>Very low at 6 months</td>
<td>Moderately severe and prolonged</td>
</tr>
<tr>
<td>monofilament</td>
<td>No effect</td>
<td>Well maintained</td>
<td>Slight</td>
</tr>
<tr>
<td>Polyethylene terephthalate</td>
<td>No effect</td>
<td>Well maintained</td>
<td>Very slight</td>
</tr>
<tr>
<td>PTFE (Teflon®)</td>
<td>No effect</td>
<td>Well maintained</td>
<td>Almost none</td>
</tr>
</tbody>
</table>

4/25/05——Ch 10 Tissue Response
a: From Ref. 7.
• Degree of tissue response: both physical & chemical nature of implants.

• **Most pure metals** evoke severe tissue reaction. High-energy state or high free energy, to lower their free energy by oxidation or corrosion. Ti & its alloys minimal tissue reaction due to tenacious **oxide layer**, resists further diffusion of metal ions and gas (O_2). Oxide layer makes them ceramiclike materials; very inert. Corrosion-resistant metal alloys, CoCr, 316L s.s., & Ti- alloys, similar effects on tissues.
• **Most ceramics** TiO$_2$, Al$_2$O$_3$, ZrO$_2$, BaTiO$_3$, multiphase ceramics of CaO-Al$_2$O$_3$, CaO-ZrO$_2$, & CaO-TiO$_2$. **minimal tissue reactions** thin layer of encapsulation[Fig 10-7].
• Some glasses (e.g., 45% SiO$_2$, 24.5% Na$_2$O 24.5% CaO, & 6.0% P$_2$O$_5$) direct bonding to bone by dissolution of silica-rich gel film at interface [Fig 10-8].

Figure 10-8. Electron micrograph of the interface between 45S5 Bioglass-ceramic (C) and bone (B). The arrows indicate region of gel formation, undecalcified. ×10,000. Courtesy of L. L. Hench.
Pure polymers inert toward tissues if there are no additives present (e.g., antioxidants, fillers, antidiscoloring agents, plasticizers). Monomers, adverse tissue reaction.

Degree of polymerization tissue reaction.

100% polymerization is impossible to achieve range of different-size polymer molecules leached out of polymer.
• **Particulate form** of a very inert polymeric material cause severe tissue reaction. e.g. ptfe (Teflon®), [Fig 10-9].

**IMPLANT : TISSUE**

**MINIMAL RESPONSE**

Thin Layer of Fibrous Tissue

Silicone rubber, Polyolefins, PTFE (Teflon)

PMMA, most ceramics, Ti- & Co-based alloys

**CHEMICALY INDUCED RESPONSE**

Acute, Mild Inflammatory Response

Absorbable sutures, Some thermosetting resins

Chronic, Severe Inflammatory Response

Degradable materials, Thermoplastics with toxic additives, Corrosion metal particles

**PHYSICALLY INDUCED RESPONSE**

Inflammatory Response to Particulates

PTFE, PMMA, Nylon, Metals

Tissue Growth into Porous Materials

Polymers, Ceramics, Metals, Composites

**NECROTIC RESPONSE**

Layer of Necrotic Debris

Bone cement, Surgical adhesives

Example 10-2

• Describe major differences between normal wound healing and the tissue responses to "inert" and "irritant" materials. What factors besides the choice of material can affect the local tissue response to an implant?
Answer

• The tissue response to an "inert" material is very much like normal wound healing. No foreign body giant cells appear and a thin fibrous capsule is formed. The tissue in this capsule differs very little from normal scar tissue. In response to irritant materials, foreign body giant cells appear and an inflammatory response is evoked. There is an abundance of leukocytes, macrophages, and granulocytes. Granular tissue will be formed, serving the functions of phagocytosis and organization, and appears only under circumstances of irritation or infection. Healing is slow and a thick capsule forms. If the material is chemically reactive or mechanically irritating, necrosis of surrounding tissue may result. It has been suggested that the size and shape of an implant should be important factors to consider for what type of tissue reaction it could elicit.
10.2.2 Systemic Effects by Implants

- **Systemic effect**: Polymethylmethacrylate bone cement (dough state) applied in femoral shaft shown to lower blood pressure significantly.

Biodegradable implants such as absorbable sutures & surgical adhesives, & large number of wear & corrosion particles released by metallic implants.
• Corrosion-resistant metal alloys not completely stable structurally & some elements released in the body. Elevated ion levels in various organs interfere with normal physiological functions.

• Divalent metal ions inhibit activities of various biochemical substances such as enzymes & hormones.
• **Polymers** contain additives that cause cellular & systemic reactions to a greater degree than pure polymer itself. Silastic® rubber contains silica powder, to enhance mechanical properties. Silica powder itself is reactive when implanted in a confined area, no problems have been apparent with its use. However, late complication may occur if a large amount of silica is released into tissue & retained in various organs.
Various organs have different affinities for different metallic elements [Table 10-3].

Table 10-3 Concentrations of Metals in Tissue and Organs of the Rabbit after implantation of Various Metals

<table>
<thead>
<tr>
<th>Elements</th>
<th>Surrounding muscle 6wk</th>
<th>Liver 16wk</th>
<th>Liver 6wk</th>
<th>Kidney 16wk</th>
<th>Spleen 6wk</th>
<th>Lung 16wk</th>
<th>Lung 6wk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>16wk</td>
<td>6wk</td>
<td>16wk</td>
<td>6wk</td>
<td>16wk</td>
<td>6wk</td>
<td>16wk</td>
</tr>
<tr>
<td>Vitalium® (61.9%Co, 28-34% Cr, 4.73% Mo, 12% Ni, 0.61% Fe)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chromium</td>
<td>30,25,0</td>
<td>0,0,15</td>
<td>0,5,5</td>
<td>5,5,10</td>
<td>0,5,5</td>
<td>5,5,10</td>
<td>0,5</td>
</tr>
<tr>
<td>Cobalt</td>
<td>25,45</td>
<td>0,30,0</td>
<td>5,10</td>
<td>5,5,10</td>
<td>0,0,105</td>
<td>5,10,70</td>
<td>5,5,20</td>
</tr>
<tr>
<td>Nickel</td>
<td>20,35</td>
<td>10</td>
<td>5,10</td>
<td>5,5,80</td>
<td>5,110</td>
<td>5,5,30</td>
<td>5,205</td>
</tr>
<tr>
<td>Titanium</td>
<td>5,5,10</td>
<td>0,10,0</td>
<td>0,0,5</td>
<td>0,0,10</td>
<td>0,0,10</td>
<td>0,0,10</td>
<td>0,0,5</td>
</tr>
<tr>
<td>Molybdenum 0.5,5</td>
<td>0,5,0</td>
<td>0,15,80</td>
<td>10,10,70</td>
<td>0,20,50</td>
<td>20,20,75</td>
<td>0,0,10</td>
<td>0,0,5</td>
</tr>
<tr>
<td>Iron</td>
<td>0,40,90</td>
<td>0,80,0</td>
<td>0,200,600</td>
<td>100,160,80</td>
<td>0,90,180</td>
<td>110,120,90</td>
<td>0,250,270</td>
</tr>
</tbody>
</table>

316 stainless steel (17.8% Cr, 13.4F, Ni, 2.37, Mo, 0.23F, Cu. 66.27% Fe)

| Chromium | 145,65 | 115,295 | 5,10 | 5,5 | 5,5 | 5,5 | 5,5 | 5,20 |
| Cobalt | 5,5 | 0,10 | 5,20 | 0,15 | 5,10 | 5,115 | 5,5 | 15,600 |
| Nickel | 15,50 | 200,70 | 20,20 | 0,95 | 0,10 | 10,10 | 5,10 | 1000,65 |
| Titanium | 20,10 | 25,10 | 0,10 | 5,5 | 0,5 | 5,65 | 0,5 | 10,50 |
| Molybdenum | 5,10 | 10,35 | 10,75 | 50,65 | 10,85 | 75,80 | 5,10 | 15,150 |
| Iron | 80,70 | 90,190 | 220,590 | 500,520 | 110,210 | 180,200 | 180,420 | 580,220 |

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*Values are in ppm dry ash, given to the nearest 5 ppm.*
Example 10-3
A biodegradable suture will have a strength 1 MPa after 6 weeks of implantation. The strength of the implanted suture decreases according to
\[ \sigma = \sigma_o + b \ln t/a \]
as determined by curve fitting to experimental data, where \( \sigma_o \) is the original strength, \( b = -2 \) MPa, \( t = \) time in weeks,
and \( a \) is a characteristic time, 1 week.
Determine the original strength of the suture.
**Answer**

\[ \sigma_o = \square - b \ln t/a = 1 + 2 \ln 6/1 = 4.58 \text{ MPa}. \]
10.3 BLOOD COMPATIBILITY

• Blood coagulation: most important factor for blood compatibility, implants should not damage proteins, enzymes, or formed elements of blood (red blood cells, white blood cells, & platelets). Including hemolysis (red blood cell rupture) & initiation of platelet release.
• **Clot**: formed inside blood vessels, *thrombus* or an *embolus* [Fig 10-10].
10.3.1. Factors Affecting Blood Compatibility

- **Surface roughness**: rougher surface more area is exposed to blood. Sometimes, thrombogenic materials with rough surfaces are used to promote clotting in porous interfaces to prevent initial leaking of blood & later tissue ingrowth through pores of vascular implants.

- **Surface wettability**: its hydrophilic (wettable) or hydrophobic (nonwettable) character, initially thought to be important factor. Wettability parameter, contact angle with liquids, does not correlate consistently with blood clotting time.\(^{(15)}\)
• **Chemical nature** of material surface interfacing with blood is related to electrical nature of surface since type of **functional groups** of polymer (no intrinsic surface charge exists for metals & ceramics although some ceramics & polymers can be made piezoelectric) determines the type and magnitude of the surface charge.

• Surface of **intima** is negatively charged largely due to mucopolysaccharides, especially **chondroitin sulfate & heparin sulfate**.
10.3.2. Nonthrombogenic Surfaces

(1) Heparinized or biologic surfaces,
(2) Surfaces with anionic radicals for negative electric charges,
(3) Inert surfaces, and
(4) Solution-perfused surfaces. Early attempts to obtain nonthrombogenic surfaces [Fig 10-11].

• **Heparin**: polysaccharide with **negative charges** due to sulfate groups as shown:
• Initially, heparin attached to a graphite surface that had been treated with the quaternary salt benzalkonium chloride (GBH process). Later, by exposing the polymer surface to a quaternary salt, tridodecylmethylammonium chloride (TDMAC). Further simplified by making a TDMAC & heparin solution in which implant immersed.
• **Heparinized** materials: significant increase in thromboresistance compared with untreated controls.

• **Leaching of heparin** into medium is a drawback, improvement by **cross-linking** heparin with glutaraldehyde & covalently bonding it directly onto surface.

• **Biological molecules**: albumin, gelatin (denatured collagen), & heparin coat on implants. Albumin alone thromboresistant & decrease platelet adhesion.
• **Negatively charged surfaces** with anionic radicals (acrylic acid derivatives) produced by copolymerization or grafting. Negatively charged electrets (polarized molecules) on the surface of a polymer (e.g., Teflon®) to enhance thromboresistance.

• **Hydrogels, polyhydroxyethylmethacrylate (polyHEMA) & acrylamide** inert materials. These coatings washed away when exposed to bloodstream.

• **Perfusion of water (solution)** through interstices of fabric which is interfaced with blood. Advantage of minimizing damage to formed elements since no material will directly contact blood.
Example 10-4.

- In the circulation of the blood, the formed elements are being destroyed by the blood pump and tube wall contacts. A bioengineer measured the rate of hemolysis (red blood cell lysis) as 0.1 g/” pumped. If the normal cardiac output for a dog is 0.1 ”/kg/min, what is the hemolysis rate? If the animal weights 20 kg and the critical amount of hemolysis is 0.1 g/kg of body weight, how long can the bioengineer circulate the blood before reaching a critical condition? Assume a negligible amount of new blood formation.
Answer

• Hemolysis rate = 0.1 g/l x 0.1 l/kg/min x 20 kg = 2 mg/min.

Critical hemolysis = 0.1 g/kg x 20 kg = 2 g
10.4 Carcinogenicity

• Sheets or films of many polymers produced cancer when implanted in animals, especially rats.

• Physical form of implant was important, & fibers & fabrics produced fewer tumors than sheets of same material, & powders produced almost no tumors. Other materials, by contrast, are carcinogenic by virtue of their chemical constitution.
10.4.1 Testing of Carcinogenicity

- **Chemical structure or function.**
- If material is similar structurally or pharmacologically to known carcinogenic agents, it evaluated further.
- **Known carcinogens:** aromatic amines, polynuclear aromatic hydrocarbons with multiple ring structures, alkylation agents including urethanes (ethylcarbamate, e.g., $\text{H}_2\text{NCOOC}_2\text{H}_5$), aflatoxins, halogenated hydrocarbons, including vinyl chloride monomer, chloroform, polychlorinated biphenyls (PCB's) & certain pesticides; metallic Ni, Cd, & Co.
In vitro tests.

• Exposing cultured cells to agent in question.
• Predicated on fact that carcinogenicity is correlated with mutagenicity.
• Following in vitro testing, cells examined for gene mutations, chromosomal aberrations, and/or deoxyribonucleic acid (DNA) damage & repair
• **Ames test**: exposing bacteria of *salmonella typhimurium* to suspect agent, & looking for reverse mutations. *In vitro* tests, advantages of quick & relatively inexpensive, but not sensitive to all carcinogenic agents [e.g. asbestos] & they do not reflect complexities in uptake, organ specificity, distribution, & excretion found in whole animals & in humans.

• **Long term animal bioassay**.
• **Rats & mice;** relatively low cost & short lifespan, waiting period is not excessive.

• **Four groups:** control (no exposure), maximum tolerated dose, & two intermediate doses. A minimum of **70** rodents per dose group per sex is used. The maximum tolerated dose, generates no overt toxicity, not reduce survival for reasons other than cancer. Following two years, animals which have died are examined, & those surviving are subjected to necropsy & histopathologic study. Control & dose groups compared statistically.
• **Relevance** to human, virtually all materials known to be carcinogenic in humans are also carcinogenic in animals.

• **Animal assays for solids.**
  • Cancer associated with implanted solids such as shrapnel, rifle bullets, & prosthetic implants: 'foreign body' carcinogenesis.
  • Suspect material implanted in flanks of rodents. In addition to examining for tumors, look for precancerous changes in cells around implant. Rationale is to increase sensitivity of test, since it is not feasible to increase 'dose' as done above.
Epidemiology.

• Most relevant since it deals directly with humans, but difficult. Latency period between exposure to a carcinogenic agent & development of disease is from 5 to 40 years in adult humans.

• Relatively insensitive unless a large fraction of population exposed, as in case of cigarette smoking.
10.4.2 Risk Assessment

• Many tests conducted at high dose levels, but human exposure is ordinarily at very low dose levels. Therefore, in carcinogenicity testing, the linearity hypothesis referred to in interpretation of results.

• Carcinogenic response is linear function of dose.
Example 10-5

- One wishes to identify materials which will cause cancer in one out of 100,000 human subjects, by experiments upon rats. Is it feasible to use the same dose rate humans would be exposed to? How many rats would one need under those conditions? Suggest an alternative experiment design based on linearity hypothesis.
Answer

• If the carcinogenic potential of the material is the same in humans as in rats, then, out of 100,000 rats, one would get cancer from the material, and perhaps 30,000 (30%) would get cancer by old age from other causes. A control group would contain an identical number of rats of which about 30% would also ultimately suffer cancer. There are two problems with this experiment: 200,000 rats is an excessive number representing an excessive expense, and it would be impossible to pick out the one extra cancer case from the 30,000 'naturally' occurring ones. A better approach would be to increase the dosage of the material by a factor of 10,000, so that 10% of the rats would suffer cancer from the exposure. A smaller number of rats could then be used. This approach is warrantable if the linearity hypothesis is valid.
• Other dose-response curves: 'threshold' or sub-linear response in which low-dose risk is less than that predicted by linear model, & supralinear model in which it is greater. Prediction of risk at low dose can vary by orders of magnitude depending on model. Standard procedure, use linear dose-response curve.

• Risk associated with implant materials, pure metallic Ni, Cd, & Co, known carcinogens when injected in solution into rat muscles. Ni, known industrial carcinogen, & Co, suspect one. Implants in animals often induce tumors, but epidemiological evidence for human implants suggests a rather small risk.
The End