An OB/GYN View of Early Medical Termination and *Clostridium sordellii* Infection

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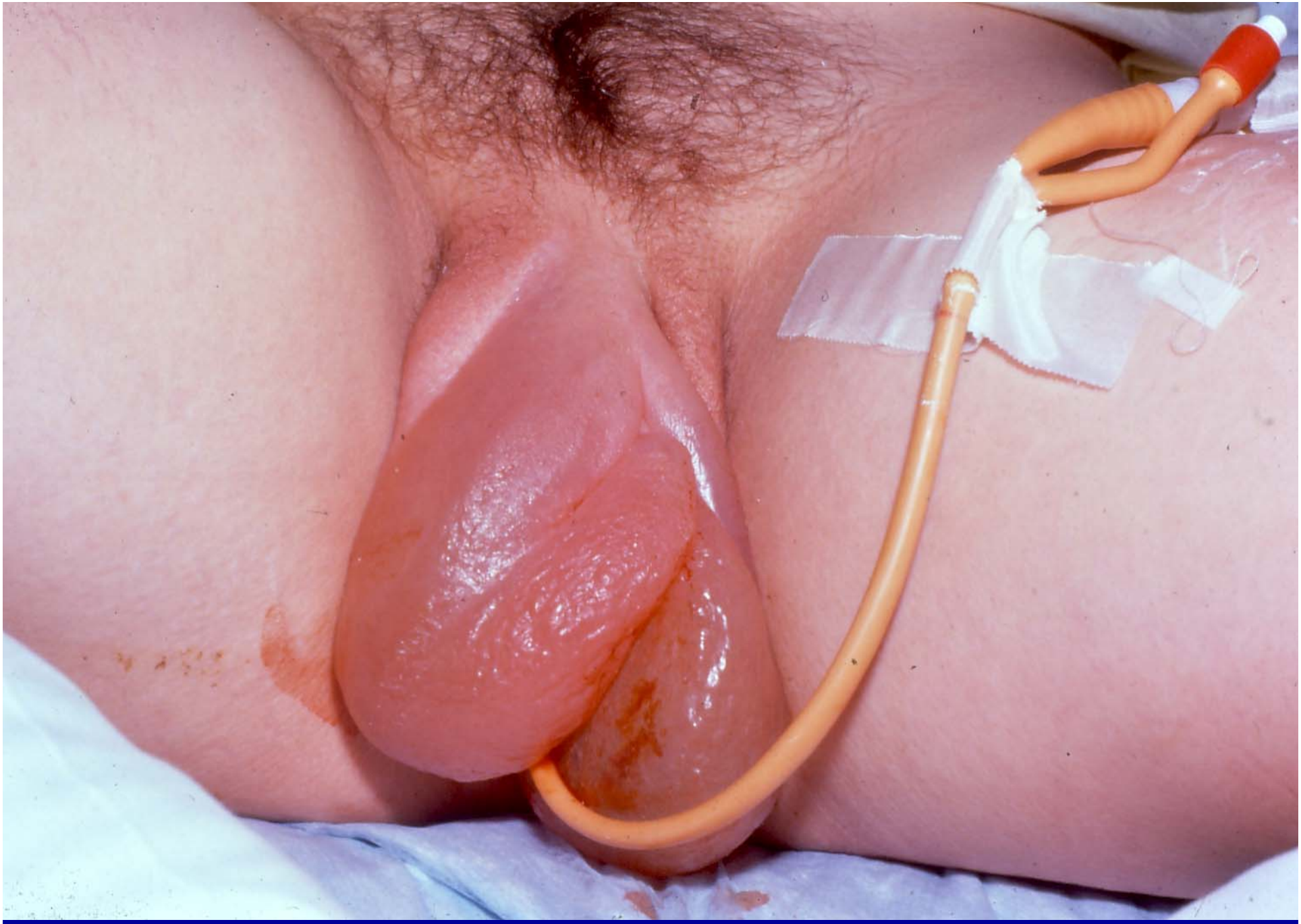
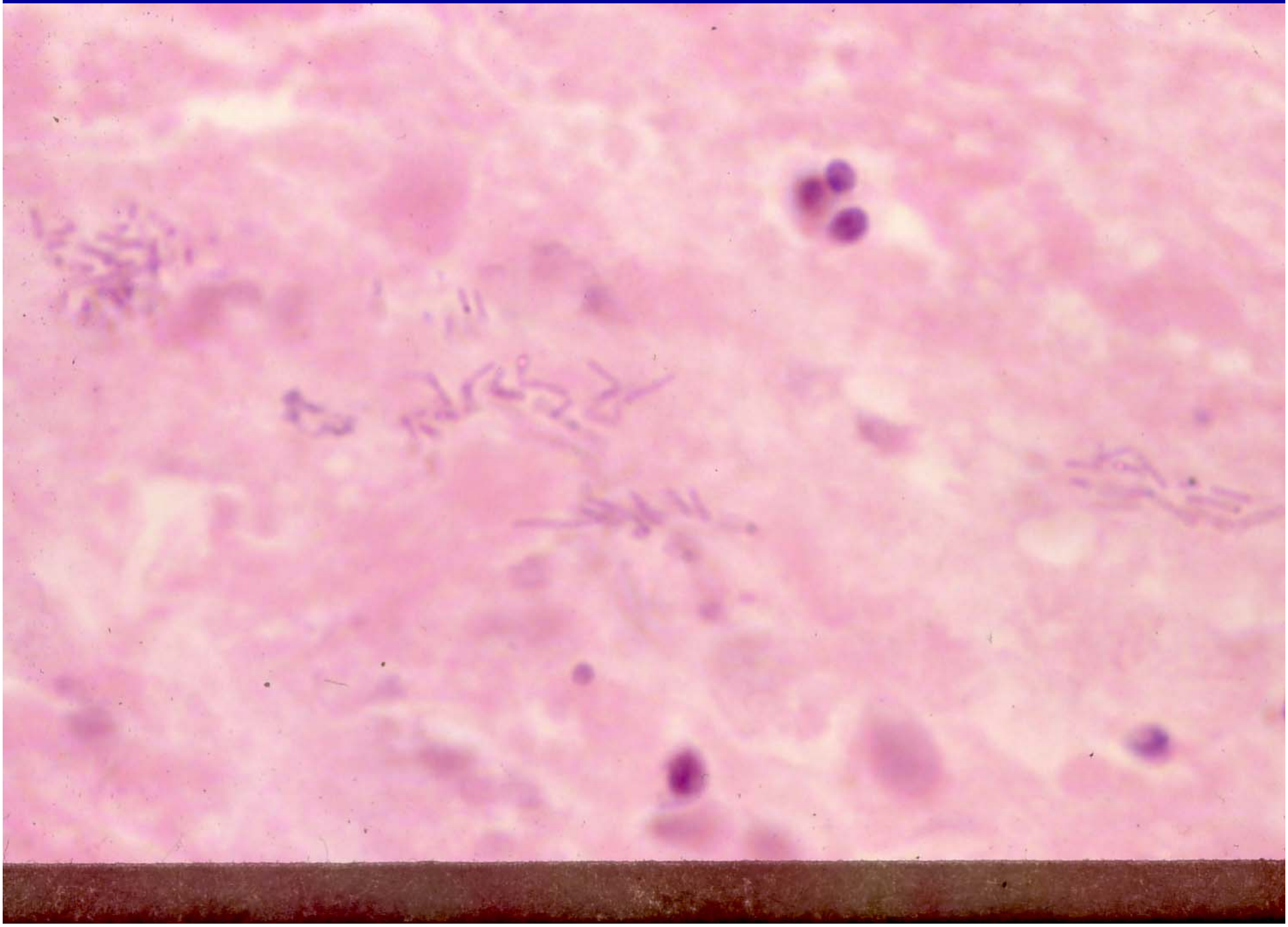
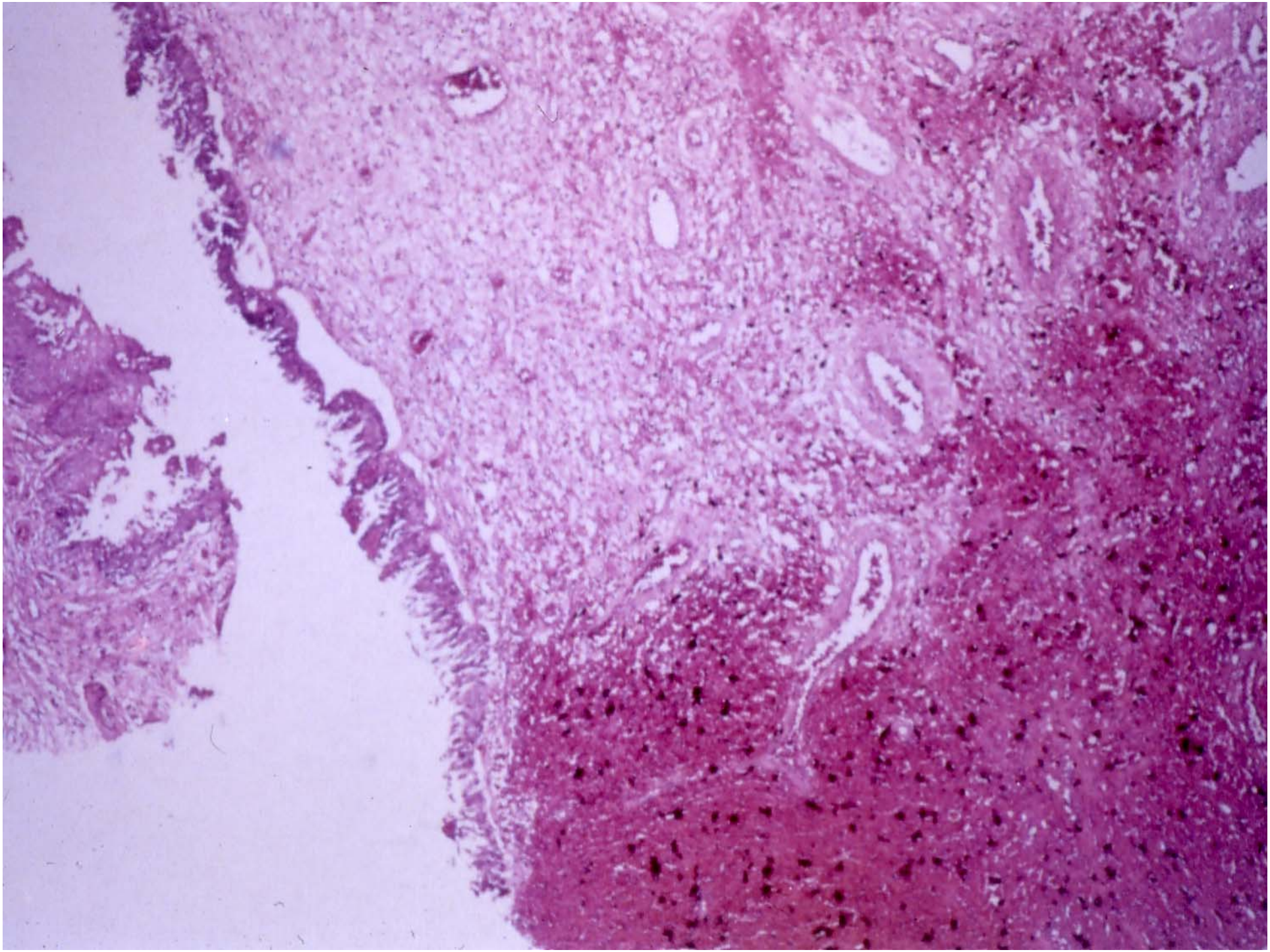
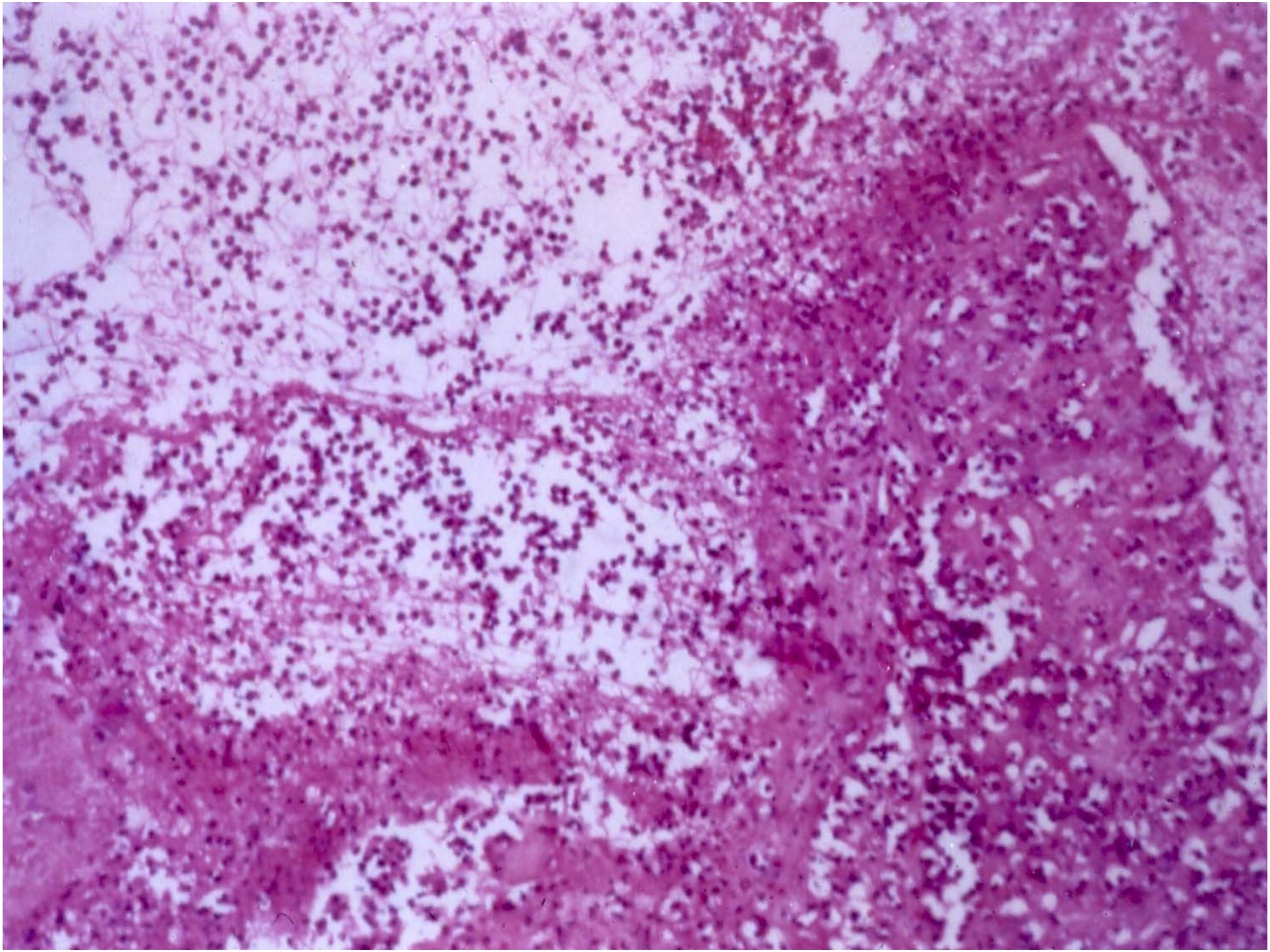
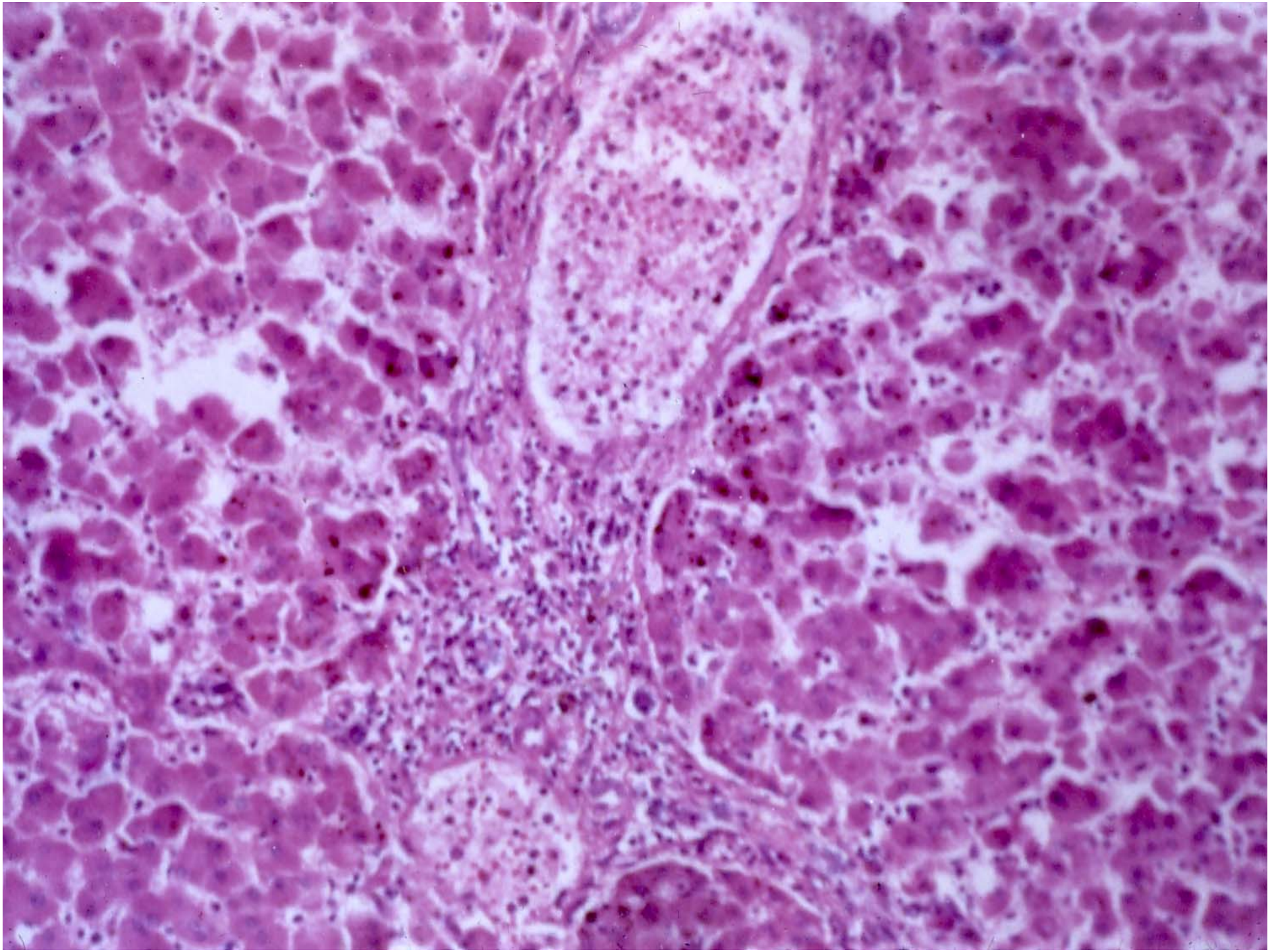






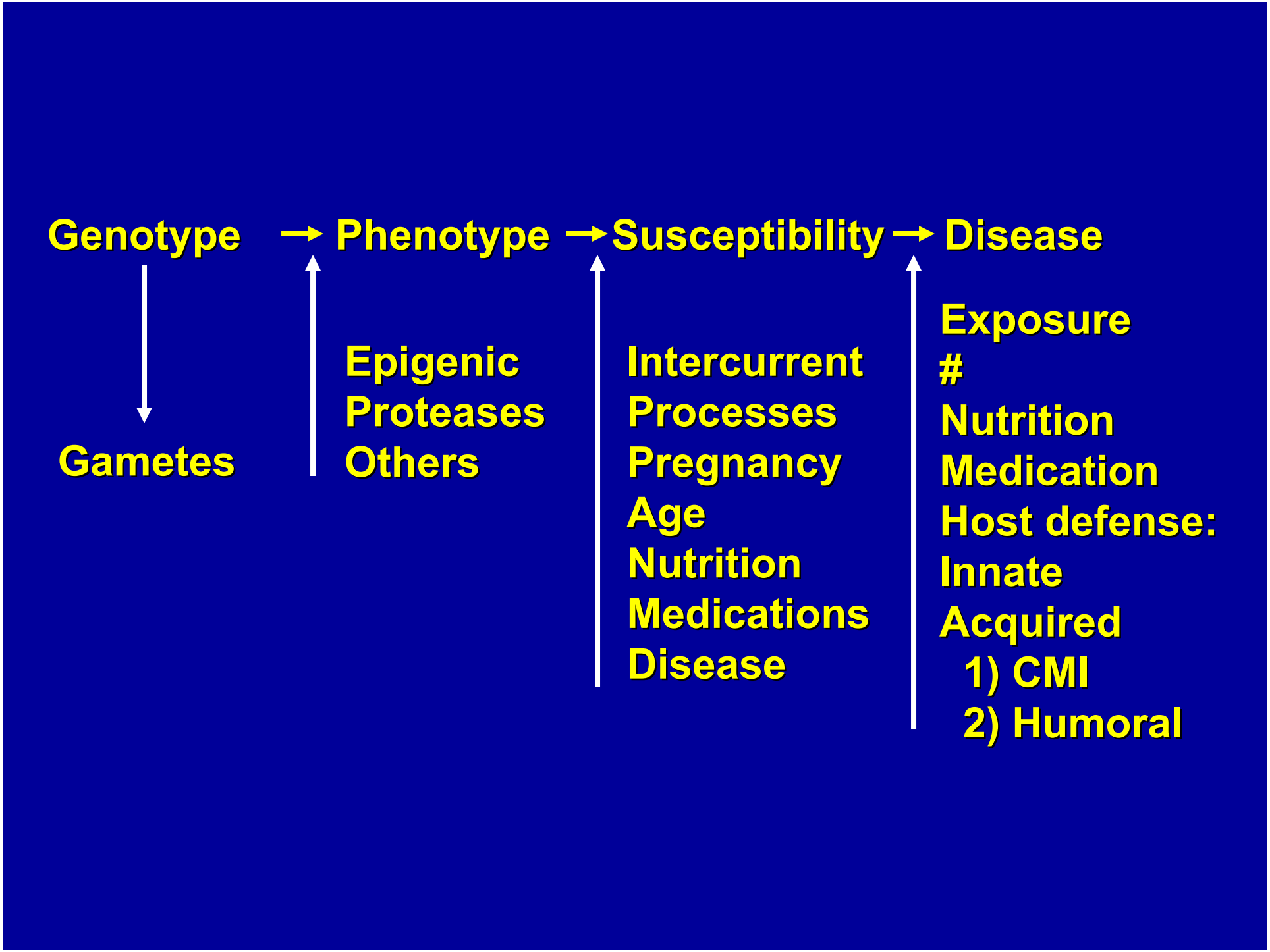


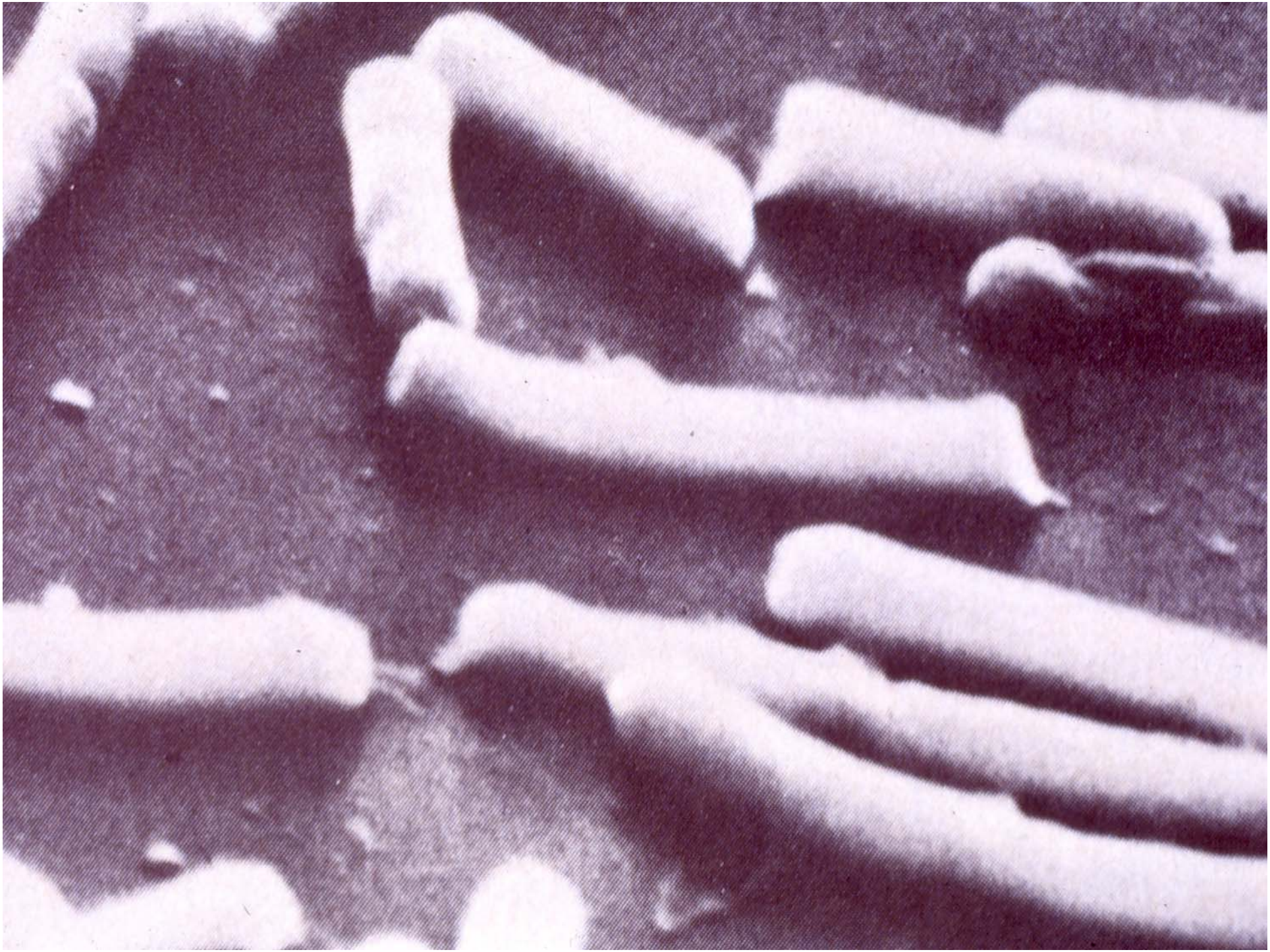


Estimates of "Reported" Mortality*

- @ 560,000 early medical terminations since 2000
  - 7 Reported deaths to 2006 (mid)
  - 0.00001254 reported deaths/procedure
- @ 1/80,000 deaths/procedure vs. @ 1/1,000,000 deaths/procedures early surgical termination*

- *0.1 per 100,000 @ ≤ 8 w (MF Green. NEJM 20006; 353: 2317)


Genotype
Phenotype
Susceptibility
Disease
Gametes
Epigenic
Proteases
Others
Intercurrent
Processes
Pregnancy
Age
Nutrition
Medications
Disease
Exposure
#
Nutrition
Medication
Host defense:
Innate
Acquired
1) CMI
2) Humoral



“Only a small fraction of bacteria present in most microbial ecosystems are amenable to propagation in the laboratory)”

D. Fredricks, et al.
NEJM 2005; 352: 1899-1911

Clostridium sordellii Virulence

- See "veterinary – *malignant edema, bloat, hemorrhage, sudden death in sheep*"
- Human: pregnancy assoc inf; wound inf, lethal toxic shock-like, edema
- Lethal toxin (TcsL) and hemorrhagic toxin (TcsH)
  - Large toxins (LCTs)
  - Ras super family (GTP-binging proteins*)
  - Phopholipase C, neuraminidase, hemolysin
- *Ras, Rac, Ral: blocks cell signaling ↓ cell activation, endothelium, myocardial cell functions

pH-Enhanced Cytopathic Effects of Clostridium sordelli Lethal Toxin

M Qa'dan, L Spyers, JD Ballard (Norman, OK) Infect Immunity 2001; 5487-5493

- C. sordellii lethal toxin (TcsL) is large clostridial toxin (LCT)
- Glycosylates Ras, Rac, Ral
- Study: explored process of TcsL in cell model suite of inhibitors; anti-sera, bafilomycin A1
- Optimal pH range for effect 4.0-5.0
  - Increased "intoxification" 5x
  - Lowers intoxicating dose 100x

RU486 (Mifepristone) Development

- Philibert, Rousell-UCLAF
- 1980's >700 publication (f) anti-P, GC effects
- Uses (f) progesterone-dependent processes*
  - Termination early pregnancy[1]
  - Inhibition follicular development[2]
  - Cervical ripening[3], labor induction[3]
  - Breast cancer treatment (PR+)
  - Endometriosis
  - (f) GC dependent processes[4]

1. Grimes D. AJOG 1990; 162: 910-17
2. Shoup D. AJOG 1987; 157: 1421-26
3. Lefebvre Y. AJOG 1990; 162: 61-65
4. Beaufreve B. RU486 and Cushing's Syndrome. Lancet 1987; 2: 217

"RU486 is a prototype glucocorticoid antagonist … with strong anti-glucocorticoid activity in vitro and in vivo.

The unusually long half-life of this drug also poses problems with titrating its dose within a therapeutic range."

GP Chrousos
Ann N Y Acad Sci 1995; 761: 296-310

Plasma Concentrations and Receptor Binding of RU486 and Its Metabolites in Humans

- 5 female volunteers
  - Rapid absorption
  - T ½ ≈ 30 hr
  - Metabolites active/present < 1 hr

Day 1 Binding Affinities		
	PR (h endometrium	GCR
RU486	↑↑↑	↑↑↑ (x4 DEX)
Preg	↑↑	
ORG-2058 (synthetic preg)	↑↑↑↑	
RU486 metabolites	↑	↑↑ (more than DEX or CCRT)
DEX		↑↑
CORT		↑↑↑

RU486 metabolites bind GCR > PR, GRR > DEX, CORT
"Metabolites may contribute anti-P and more anti-GC effects"

RU486 (Mifepristone) Pharmacokinetics

- ✓ Rapid oral absorption (< 1h)
- ✓ Long half-life @ 30 h
- ✓ WB: similar serum [ ] various doses 100 mg-800 mg
- ✓ Excretion: methylation, hydroxylation (active metabolites)
- ✓ High affinity binding protein (AAG)
- ✓ Binding affinities ≈ hPR, hGCR

1. Heikinheimo O. J Steroid Biochem 1987; 87-97
2. Heikinheimo O. Hum Reprod 1987; 2: 379 (suggests "larger doses unnecessary" vs. Grimes 1990)

Termination of Early Pregnancy by Progesterone Antagonist RU486 (Mifepristone)

Couzimet B, Beaulieu E, et al. NEJM 1986; 18: (315): 1565-70

- 100 women with 10 d of expected menses
- 3 dosages: (400 mg in 4d, 600 mg in 4d, 800 mg in 2d)
- 85/100 evacuated uterus by d13
- 15/100 ↑ hCG →evacuation
- 18% prolonged uterine bleeding
- No correlation [RU486] with success
- RU486 "effective and safe" method for termination of early pregnancy
- ***"Use under close medical supervision"***

Prospective Randomized D-B, RCT Comparing Mifepristone (RU486) and Vaginal Misoprostol to Misoprostol Alone for Elective Termination in Early Pregnancy

John Jain, Daniel Mishell, et al (USC). Hum Repro 2002; 17: 1477-82

- RCT D-B, placebo-controlled @ 250 pts ≤ 56 d
- 200 mg mifepristone orally + placebo
- 800 mg vaginal misoprostol

	Mif + Miso	Misoprostol	P
"Success"	95.7%	88%	<.08

"M & M is superior"
"Mifepristone (RU486) alone may be acceptable when misoprostol is not available"

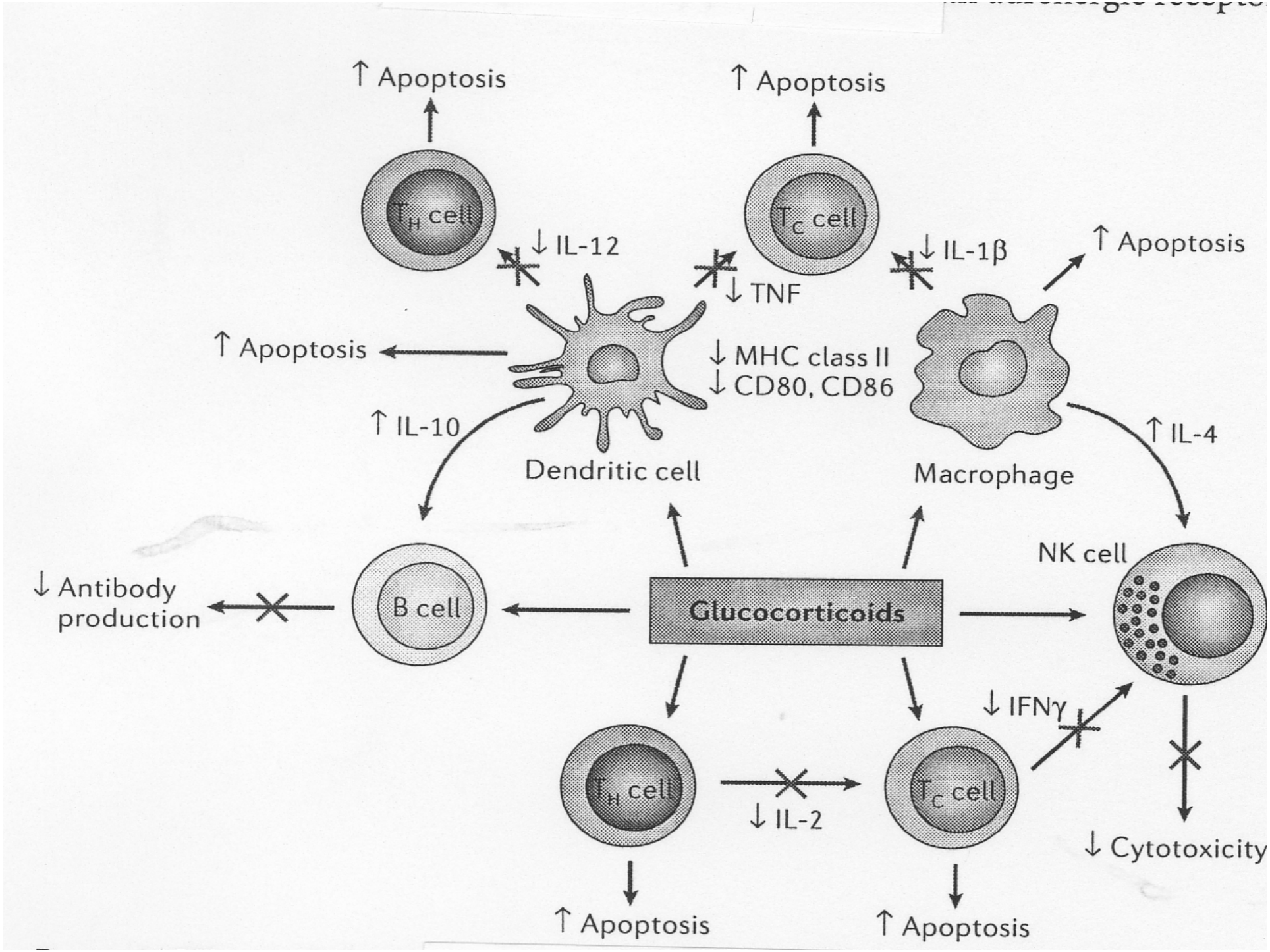
Q. Can medical early pregnancy termination be accomplished w/o mifepristone (RU486)?

- Yes.
  - Misoprostol as the primary agent for medical abortion (Borgatta L. Contraception 2004; 70: 121-126)
  - Retrospective: 2001-2, <8 wk, 34 pts with mifepristone, 2 doses 800 mg misoprostol vaginally q 24 h
  - #440

"Success"	"Aspiration"	Indicated"
90.8% (CI 88-94%)	9.2%	2.7%

  - $\bar{X}$ times passage: 8.5 h (S.D. 6.8-13h)
- "Misoprostol satisfactory agent for medical abortion in our setting"
- "Mifepristone given to 34 pts as well. ND outcomes
- "Other misoprostol alone 88%-96%"*

*Cousinet B. N Engl J med 1986; 315: 1565-70


↑ Apoptosis
↑ Apoptosis
T_H cell
T_C cell
↓ IL-12
↓ TNF
↓ IL-1β
↑ Apoptosis
↑ Apoptosis
↓ MHC class II
↓ CD80, CD86
↑ IL-10
↑ IL-4
Dendritic cell
Macrophage
NK cell
↓ Antibody production
B cell
Glucocorticoids
↓ IFNγ
T_H cell
↓ IL-2
T_C cell
↓ Cytotoxicity
↑ Apoptosis
↑ Apoptosis

Mifepristone (RU486) Adverse Effects in Animal Models**

Findings

I Castagliuolo. Am J Phys G I Liv 2001; 6: 539-545	RU486 enhanced C. d. toxin A intestinal secretion/inflammation
*E Sternberg. Proc Nat Acad Sci 1989; 86-2374	Lewis rats, ↑↑ severity of arthritis in response strep cell wall
*S Nadeau. J Neurosci 2003; 23: 5536-5544	↑↑ CNS damage from LPS, (f) TNFα
V Blais. Endo 2002; 143: 4820-4827	↑ Effects IL-1 on CNC
**JI Webster, EM Sternberg. Role of hypothalamic-pituitary-adrenal axis, glucocorticoids and glucocorticoid receptors in toxic sequelae of exposure to bacterial and viral products. J Endocrinology 2004; 181: 207-221	

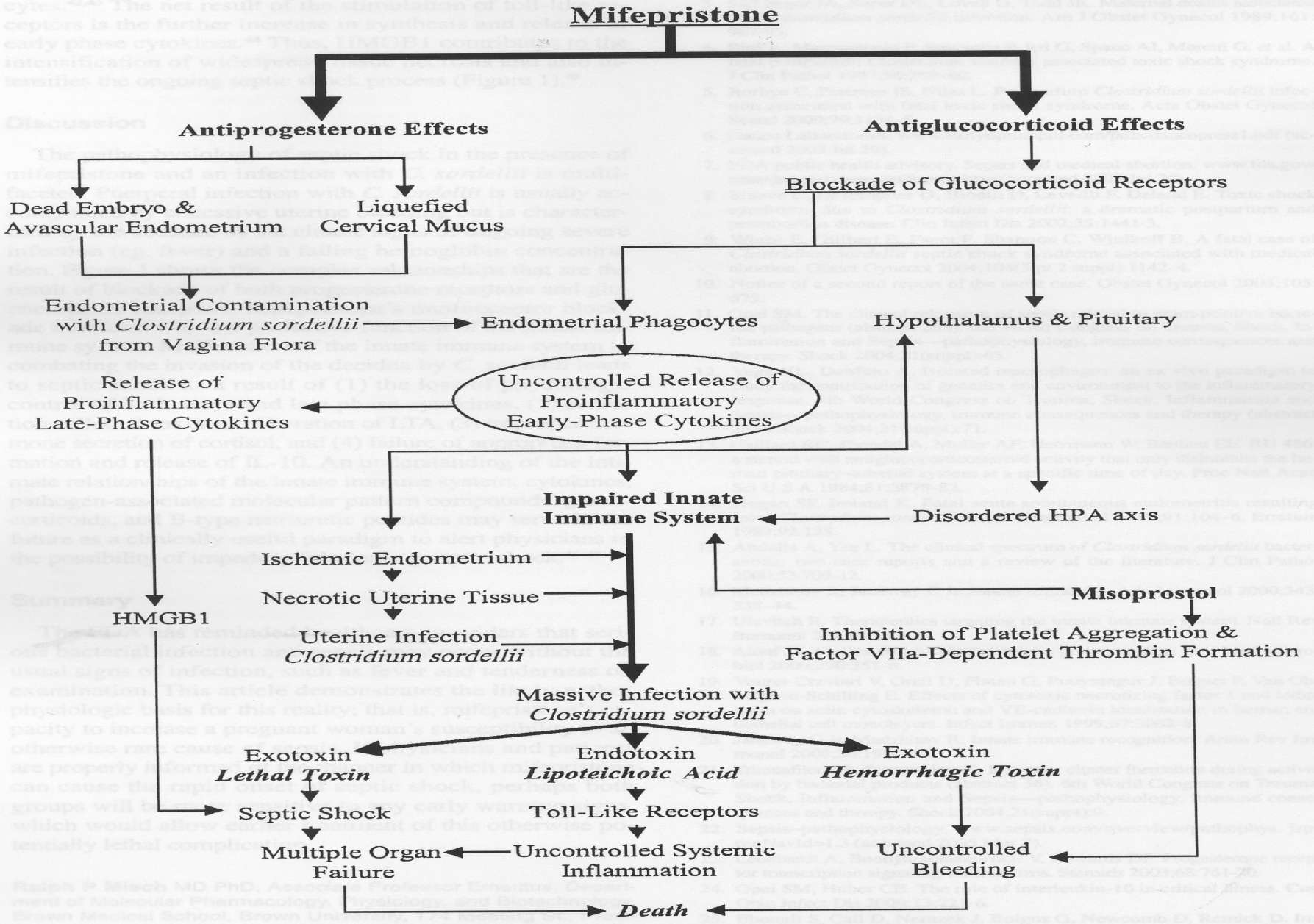


Figure 1. The interactions and complex relations of biologically active ligands in mifepristone-induced abortions. HPA = hypothalamus–pituitary–adrenal; HMGB1 = highly mobile group box 1.

- **“Vaginal application is prohibited in France, where misoprostol can be prescribed only in oral form … no reported cases … in France.”**
- **“… give antibiotic … dexamethasone.”**

Didier Sicard
Annals Pharmacother
2005; 2143

Possible Primary-Prevention Risk Reduction Strategies

- **More likely to be effective:***
  - Surgical termination
  - Eliminate (reduce) mifepristone (RU486)*
    - Safe, specific alternative?
  - Change to oral only (vs. vaginal) administration misoprostol
- **Less likely to be effective:**
  - Antimicrobial prophylaxis; short vs. long course
  - Vaginal/perineal hygiene, cleansing/antisepsis
  - Probiotic(s) vaginal/GI

*No strategies amenable to controlled trial (f) low risk, prevalence

Informed Consent for Mifeprex and Cytotec (Misoprostol) for Medical Abortion

- "I will received Mifeprex 200 mg by mouth (600 mg in FDA protocol) … known side effects include heavy vaginal bleeding, vomiting, diarrhea, abdominal cramping, headache, dizziness, back pain, and/or fatigue.
- "I will be responsible for inserting four tables of misoprostol (800 mcg) into my vagina up to three days from today (2 tables only in FDA protocol) … side effects include nausea, vomiting, diarrhea, abdominal pain, dizziness, hot flashes and/or cramping, bleeding …
- 1) Adverse effects similar, similar to infection
- 2) AEs ≈ Mifeprex (RU486) and Cytotex (misoprostol)
- 3) Complex informed consent/explanation

C. Sordellii assoc Toxic Shock (CATS)

CLINICAL CARE

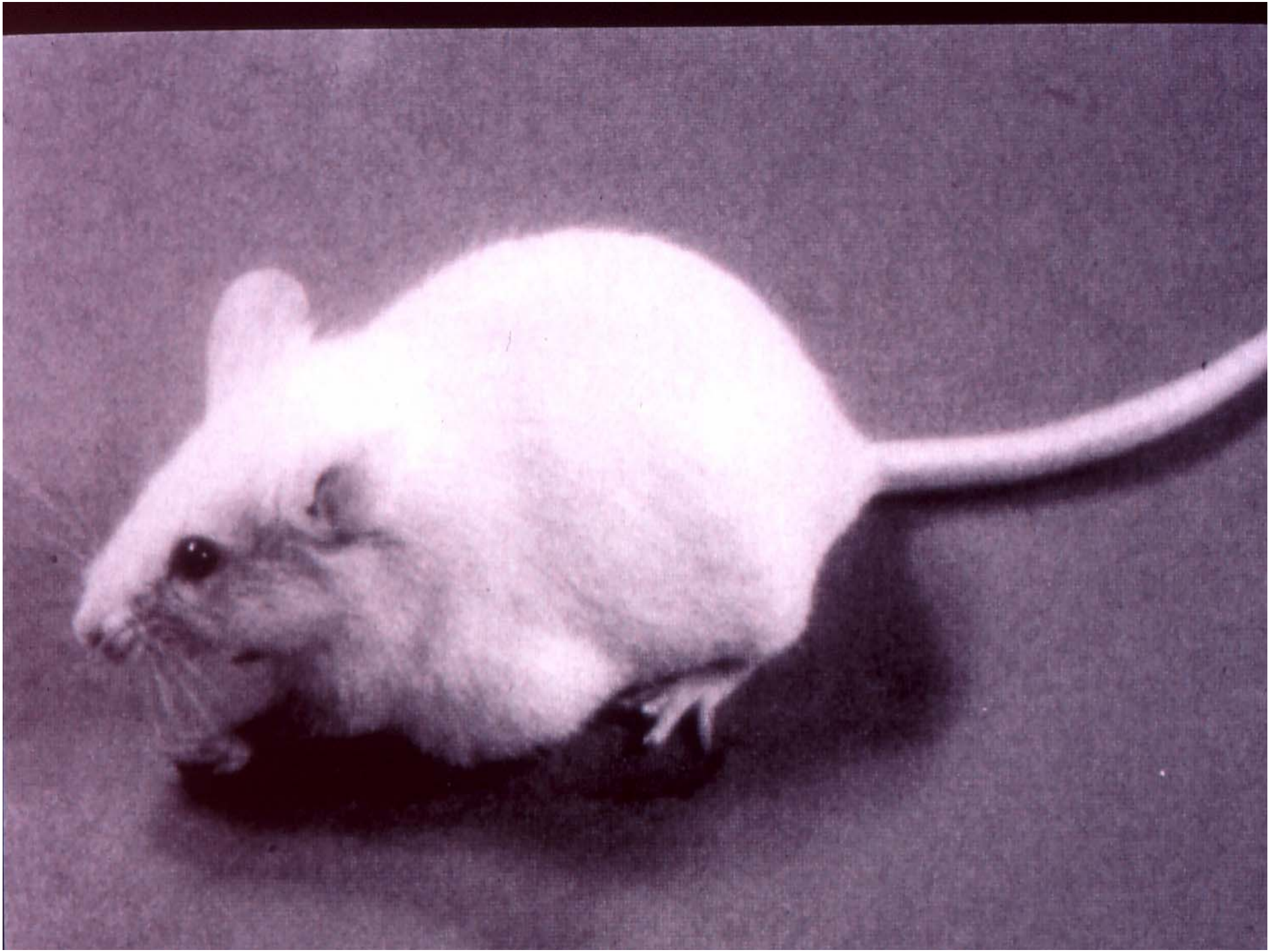
- Symptoms: <7d post event, "weak", "dizzy", "abdominal pain", vaginal bleeding
- Signs: ↑ HR, ↓ R, BP ↓↓, T ↓ or →

LABORATORY

- Hct/Hbg ↑↑
- Wbc ↑↑↑ … "leukemoid reaction"
- LFTs ↑, Creatinine/BUN ↑, others?
- Gram stain D/C, POC

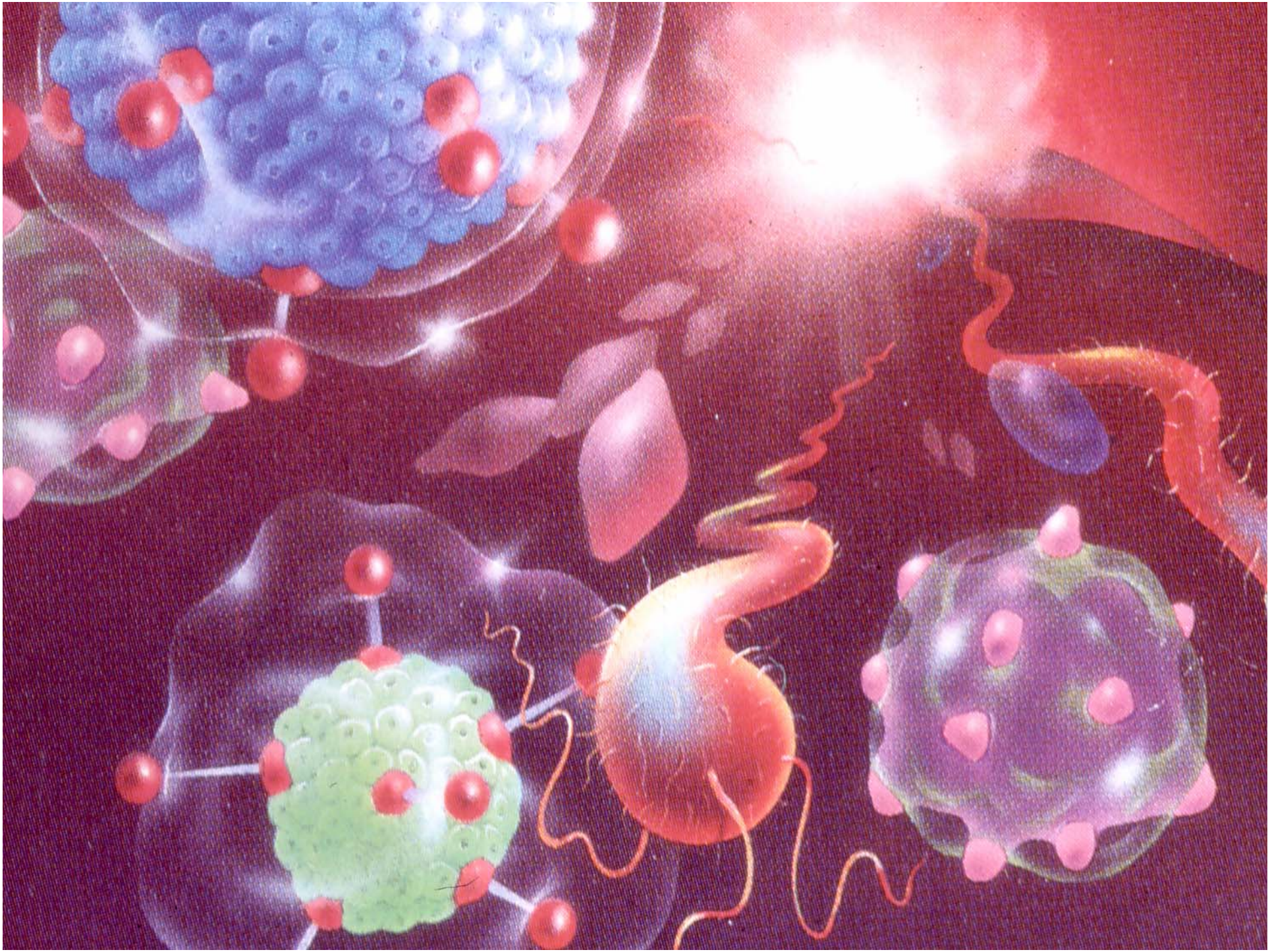
TREAT

- Admit; consultation(s)
- Multi-organ support
- Surgical extripation: TAH ± BSO, D+C "early", TAH ± BSO "late"
- Antibiotics: broad spectrum*
- Steroids (f) (cortisol) ↑ or ↓
- Anti I sera? IgG? Anti-toxin inhibitors, toxin inhibitors
- (hPC)
- Component support
- Anti-TNF, IL-1?, IL-10?
- *clindamycin, imipenen



Research Imperatives

- **Evaluate:**
  - Sub-cellular, cellular, organ, organism effects mifepristone/misoprostol
  - Epidemiology C. sordellii (culture, nucleic acid technologies)
  - Models of RU486
    - Pathophysiology infection/inflammation
    - Intervention – early/late
  - Mifepristone/misoprostol metabolism
    - Metabolites, pharmacokinetics, pharmacogenomics (P-450, other metabolic systems)
  - Active surveillance for adverse effects/changes in protocols
  - Optimized early medical termination protocols (f) safety, efficacy, AEs, designs



Plasma Concentrations and Receptor Binding of RU486 and Its Metabolites in Humans

O. Heikinheimo, et al. J Steroid Biochem 1987; 26: 279-84

- The affinity of RU486 for human uterine P receptor was highter than P, but lower than OR 6-2058 – as were primary metabolites
- RU486 had approx 4x higher binding affinity to GC receptor than dexamethasone (or cortisol)
- √ metabolites contribute to anti-P and even greater to anti-GC effects
- Mifepristone metabolization by CYP3A4

Glucocorticoids Play a Fundamental Role in Protecting the Brain During Innate Immune Response

"Mifepristone (RU486) increases inflammatory reaction in the brain following injection LPS."

Nadeau S. J Neurosci 2003; 23; 5536-44

Hemodynamic Hallmarks of Sepsis

Robert W. Schrier. NEJM 2004; 351: 159-169

- ↑ generalized arterial vasodilatation
- ↓ systemic vascular resistance
- LPS activates (TOLL-4)
  - → cytokines (TNFα, IL-1, IL-6), ↑ NO
  - → neurohumeral axis responses
    - HPA responses (cortisol)
    - Sympathetic
    - Renin-angiotensin-aldosterone
  - ↑ CO_2 ~ ↓ after load

C. Difficile Toxin-Induced Colitis After Use of Clinda Vaginal Cream

AM Meadowcroft, G Latham. Ann Pharmacother 1998; 32: 309

- **Case report:**
  - 25 yo postpartum woman with BV, Rx'd clindamycin cream 7 day
  - ⊕ C. difficile watery stool
  - Disappeared d2 without treatment

Theobold Smith's Equation:

$$\text{Infection (Outcome)} = \frac{\text{Inoculum x Virulence}}{\text{Host Defenses}}$$

1. Innate immunity
2. Acquired immunity
3. Nutrition
4. Medication(s)
5. Prior conditions

RU486 (Mifepristone) Mechanisms

- **Multiple and Interacting:**
  - hP receptor, hGC receptor blockade
  - Multiple steps (f) innate immune responses (Miech)
  - Interactions with C. sordellii/anthrax toxins on hPr, hGCr and nuclear hormone receptor transactivation (Sternberg)

"The effects of neural (endocrine) factors on inflammatory responses, which rapid in onset, vary over time, enhancing or dampening responses (and outcomes)."

EM Sternberg
Nature Reviews Immunology
2006; 5: 318-328

Feed Back Loop: Neuronal (HPA / PNS / SNS)*

- **Optimize inflammatory responses**
  - ↑ initial local efficacy
  - ↓ "dampen" proinflammatory cells/molecules
- **Natural Experiments**
  - **Too much: overwhelming** virulence/proinflammatory responses
    - ↑ shock, damage, lethality
  - **Too little:** ↓ dampening effects

Mifepristone (RU486) Adverse Effects in Animal Models

Effects

J Fan. Circ Shock 1994; 42: 76-82	GR blockade worse, rats, exacerbates effects endotoxins
P Brouckaert. Eur J Imm 1992; 27: 981-986	Mimics IL-1 in rats sensitization to lethal effects TNFα
G Lazar. Circ Shock 1992; 36: 180-184	RU486 lowered survival 71% $\rightarrow$ 15%
*A Hawes. Inf Imm 1992; 2641-2647	Lewis rates $\uparrow$ lethality to 91%
G Fantuzzi. J Imm 1995; 105; 3552	Mice, GC $\downarrow$ TNF

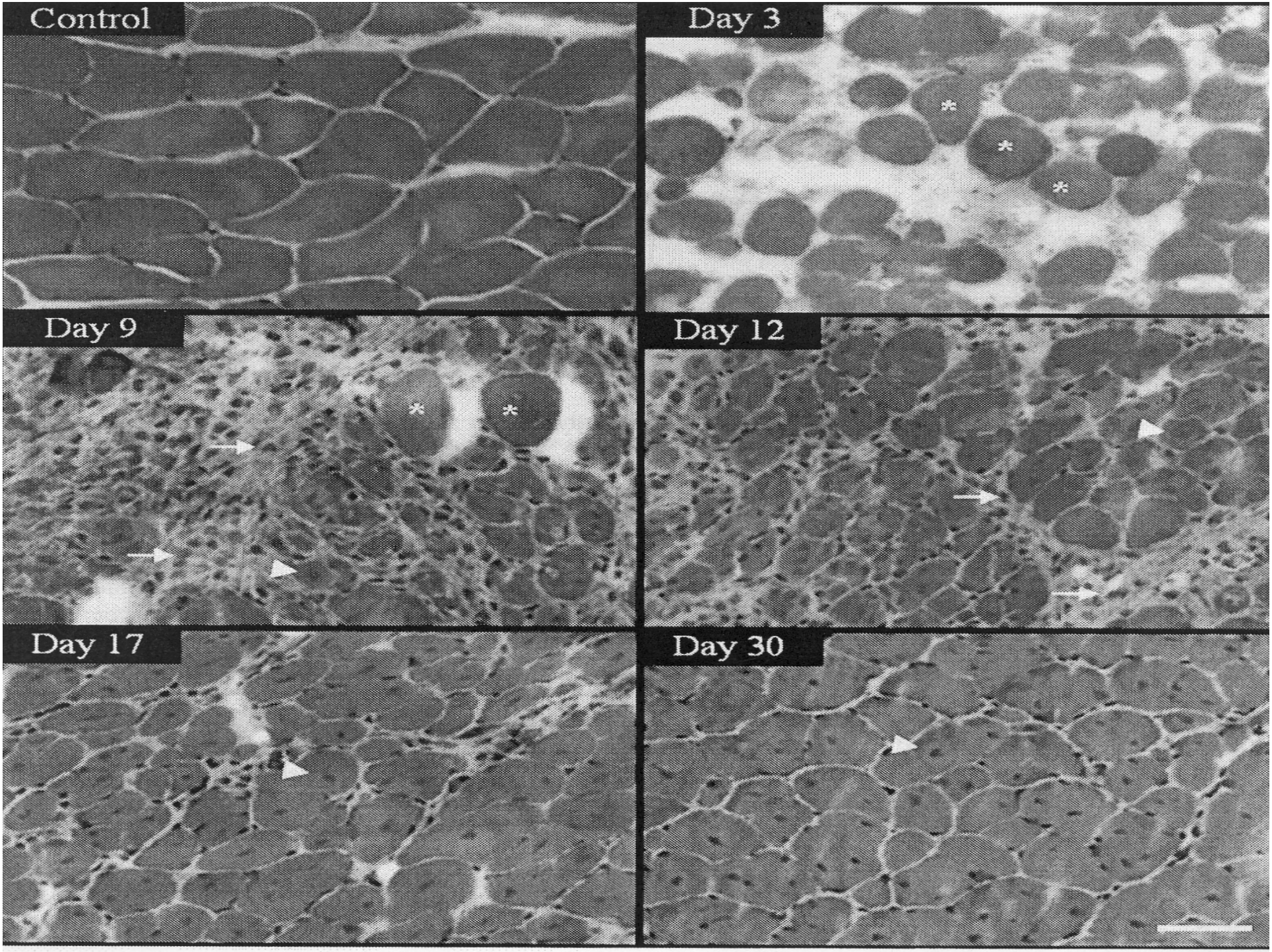
Vaginal Carriage and Neonatal Acquisition of Clostridium difficile

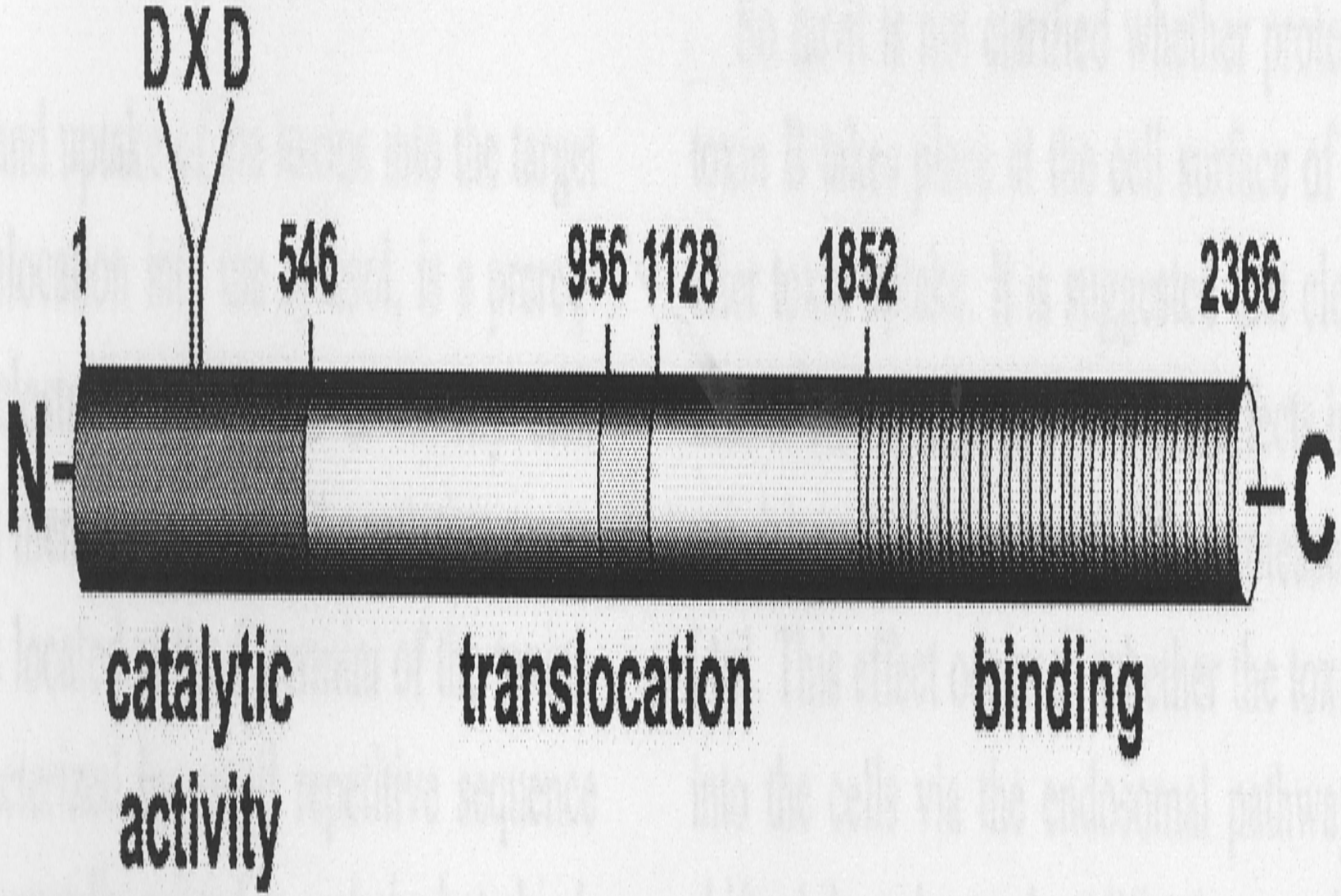
S Tabagchali, M Wolks. J Med Microbiol 1984; 18: 47-53

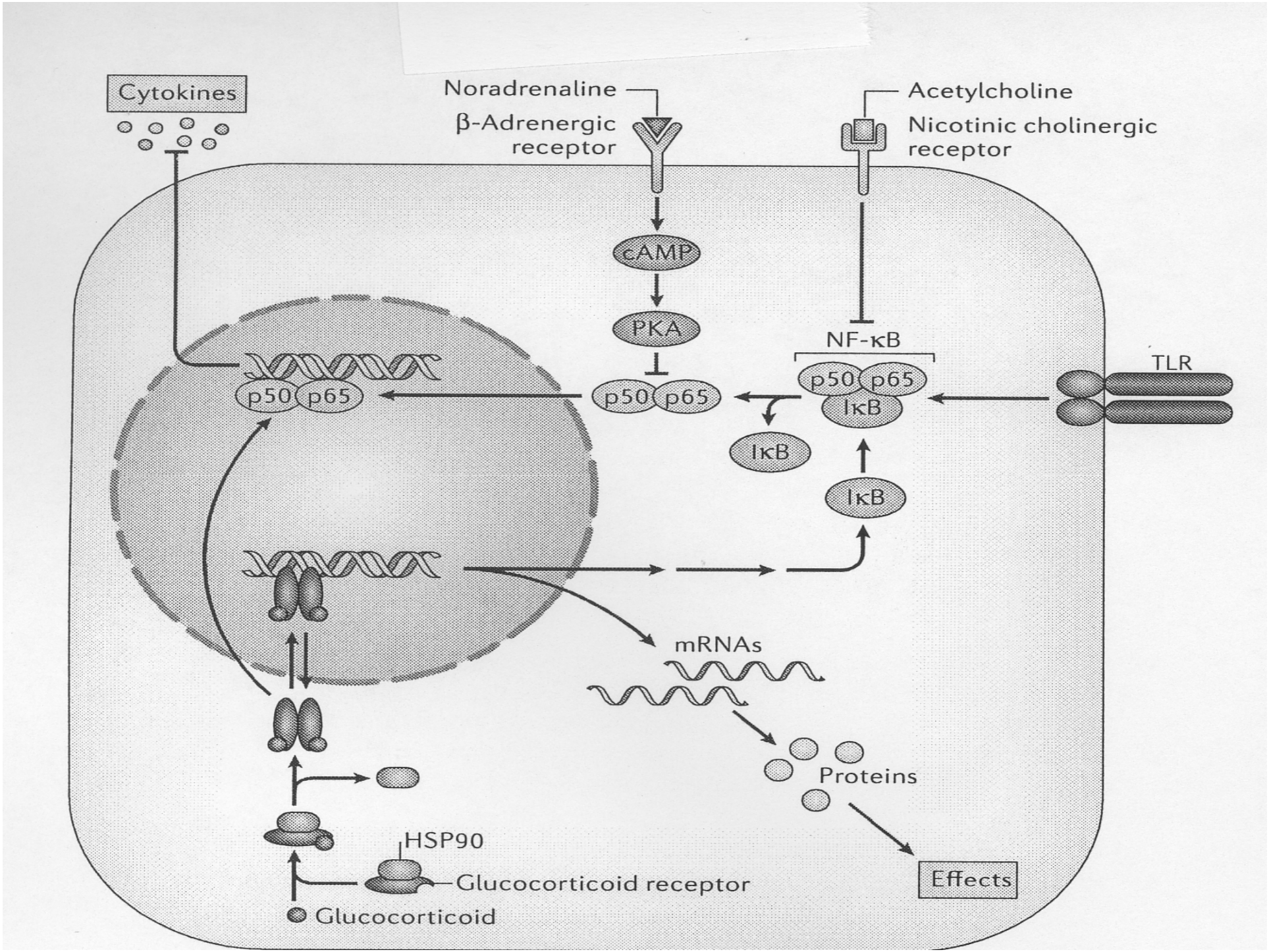
Broth/plate investigation maternal/neonatal colonization, #50 pairs	
Mother, prior birth	18%
Mother, after birth	8%
Neonatal stool	62%
Mothers + prior to birth; baby colonized	8/9
Mothers, prior	33/41
"Both vagina and environment sources of *C. difficile* in baby"*	
*I Al-Jumaili. J Clin Micro 1989; 19: 77 - *no maternal/nurse carriage but similar newborn colonization*	
*D Thirkell, Ant van Lee 1984; 50: 355-360 – *91% C. difficile vaginal carriage with CCFA media, 2d incubation*	

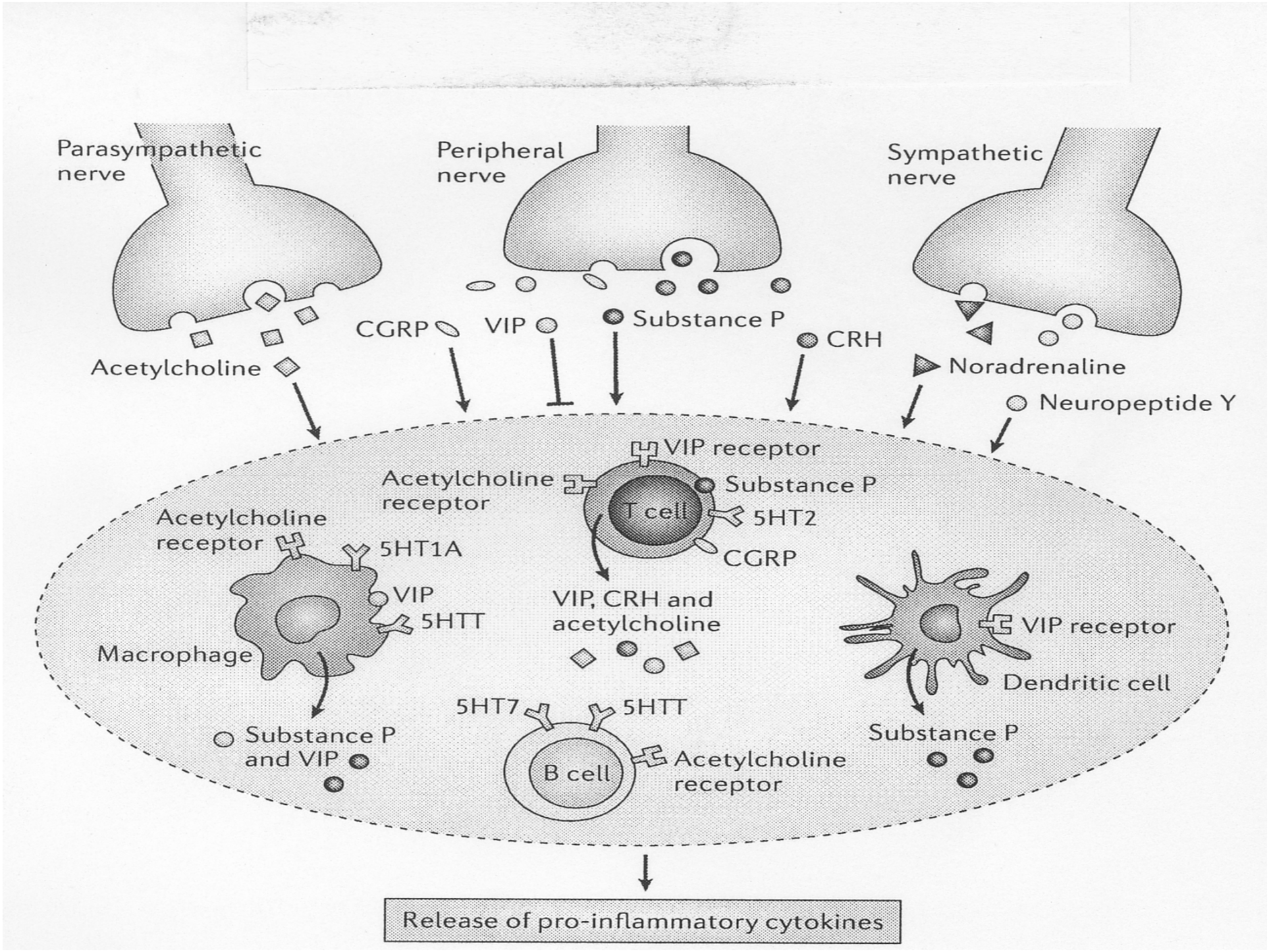
TSST-1 Biologic Effeccts

- Direct, pyrogenic activities
- T-cell mitogenicity
- Inhibits IgG secretion
- Inhibits PNM migration
- Enhances endotoxin susceptibility


Control
Day 3
Day 9
Day 12
Day 17
Day 30

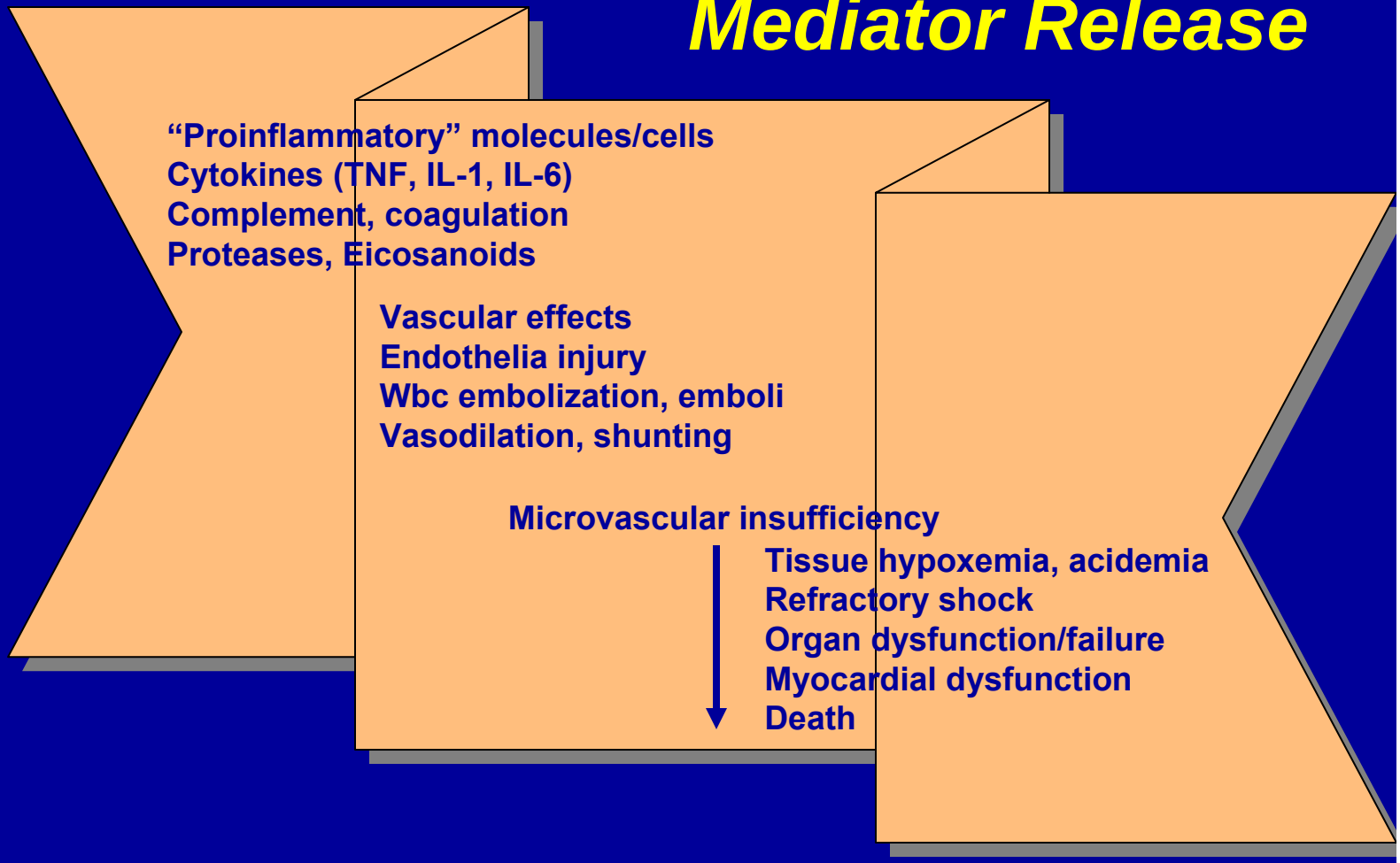

DXD
1
546
956
1128
1852
2366
N-
-C
catalytic activity
translocation
binding


Cytokines
Noradrenaline
β-Adrenergic receptor
Acetylcholine
Nicotinic cholinergic receptor
cAMP
PKA
NF-κB
p50
p65
IκB
TLR
mRNAs
Proteins
Effects
HSP90
Glucocorticoid receptor
Glucocorticoid


Parasympathetic nerve
Peripheral nerve
Sympathetic nerve
Acetylcholine
CGRP
VIP
Substance P
CRH
Noradrenaline
Neuropeptide Y
VIP receptor
Acetylcholine receptor
Substance P
T cell
5HT2
CGRP
Acetylcholine receptor
5HT1A
VIP
5HTT
Macrophage
VIP, CRH and acetylcholine
VIP receptor
Dendritic cell
5HT7
5HTT
Substance P and VIP
B cell
Acetylcholine receptor
Substance P
Release of pro-inflammatory cytokines

Septic cascade

- Invasive infection (bacteria, viremia, Rickettsiaemia, fungemia, parasitemia) or other cell damaging events/processes
- (f) innate (initial/non-specific) immunity



Molecular Identification of Bacteria and Bacterial Vaginosis (BV)

David Fredricks, TL Fredler, JM Marrazzano. NEJM 2005; 353: 1899-11

- Analyzed BV vs. central vaginal microflora ribosoma DNA sequences 16S rDNA, PCR assays, FISH ($\geq 10^6$), 27 subjects BV, 46 w/o BV
- Results: 73 women evaluated
  - w/o BV 1-6 bacterial spps ($\bar{x}$=3.3)
  - Lactobacilli predominate *83-100%)
  - BV a) ↑ diversity 9-17 phylotypes ($\bar{x}$=12.6)
  - Newly recognized spps (A vaginae, etc.)
  - 35 unique bacterial spps + no close relatives!
  - 3 “new” unrecognized Clostridials highly specific for BV, BV assoc Anaerobes 1-2-3
- (No C. sordellii, 3 C. perfringens)

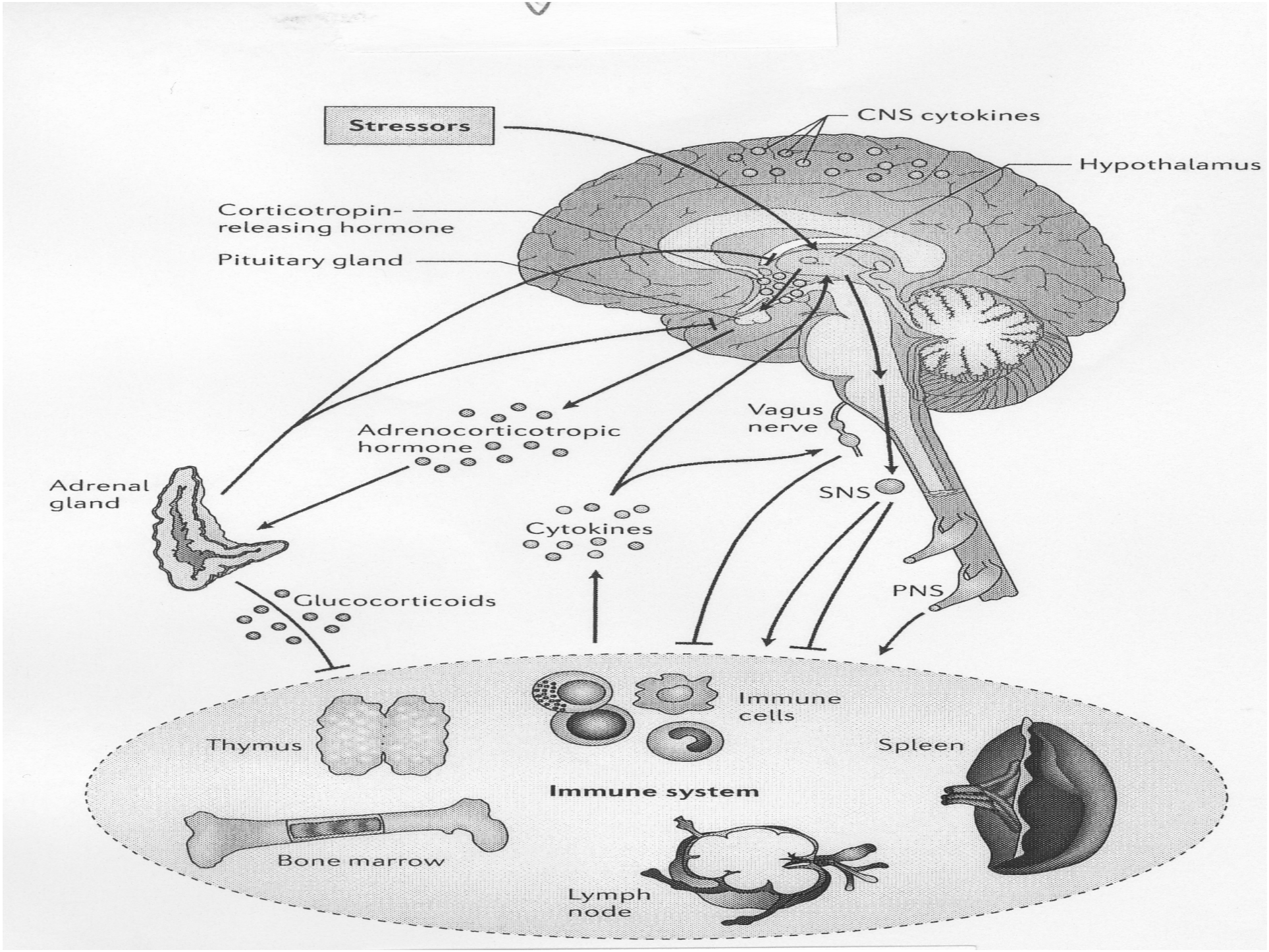
"It must be emphasized that septic shock … in **NOT caused directly** by the **invading pathogen**, … rather (septic shock) results from an overwhelming (pro) inflammatory mediator response .. that induces pathologic change in the host."

Roger C. Bone
Sepsis and Shock Complications
Crit Care Med 1994; 22

RU486 (Mifepristone) Increases Lethality/Severity in Infection/Inflammation Model

- Lewis and other rat model of strep cell wall and HPA axis conditions. Intact HPA-axis feed back loops protective
- "RU486 (which had no effect alone) has highly toxic when administered in rat with SCW (streptococcal cell wall) … resulting in 100% mortality."

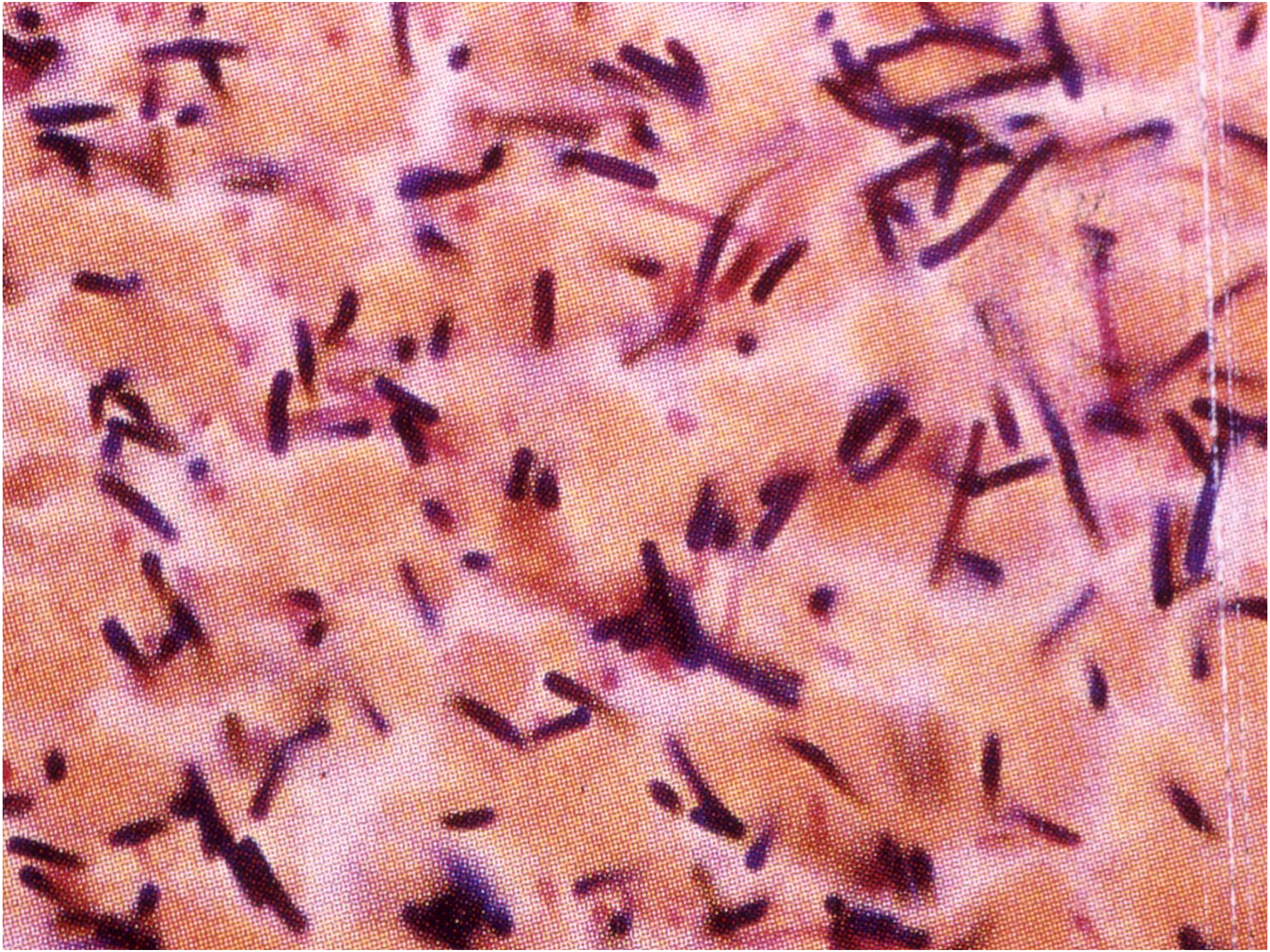
Sternberg E, Hill J, Chrousos GP, Gold PW. Inflammatory mediator-induced HPA Axis activation in Lewis rats. Proc Nat Acad Sci 1989; 86: 2374-78


Stressors
CNS cytokines
Hypothalamus
Corticotropin-releasing hormone
Pituitary gland
Adrenocorticotropic hormone
Vagus nerve
Adrenal gland
SNS
Cytokines
Glucocorticoids
PNS
Immune cells
Thymus
Spleen
Immune system
Bone marrow
Lymph node

Lower Reproductive Tract Microecology*

- What's in a name? Statistical (f) population, personal
  - √ endogenous microflora
  - √ resident microflora
  - "normal flora"
- Multiple variables
  - Life cycle/hormonal status
  - "Day-to-day"
- Type studies
  - Prevalence, any
  - Quantitative, > 10^5 CFUs
- Technologies
  - Selective/non-selective media
  - Nucleic acid techniques
  - FISH, visual, other

*Fredricks DW. NEJM 206; 353: 1899
Hammill H. Ob Gyn Clin NA 1989; 16: 355
Bartlet J. JID 1977; 127: 8015



A RCT of mifepristone in combination with misoprostol administered sublingually or vaginally for medical abortion up to 13 wks of gestation

- 340 women, 200 mg mifepristone plus misoprostol (600 mg) sublingual vs. 800 mg vaginally x2 or 3 doses*

Misoprostol #	"Sublingual"* 171	"Vaginal" 169	P
"Dissatisfied"	12%	4%	<0.01
Diarrhea	71%	52%	<0.01
Shivering	84%	64%	<0.01
Unpleasant taste	71%	32%	<0.01
Surgical evacuation	1.3%	2.6%	ND
Doses	1.28	1.35	ND

•Most decline F/U

•** T ½ sublingual ↓, bioavailability ↑ to 6h, AUC ↑ sublingual

Hamoda H (Aberdeen) BJOBG 2005; 112: 1102-8

Proposed Scenario @ Mifepristone (RU486) and C. sordellii toxic shock

JA McGregor, E Equils. Contraception 2005; 71: 161

- P withdrawal initiates intrauterine apoptosis/necrosis, local bleeding
- Blood, necrotic tissue, pH, ↓ redox enhance clostridia growth, exotoxin effects
- Uterine activity transports C. sordellii into uterus → infection, toxin production/cell death, systemic dissemination
- L/H toxins cause multi-organ cell (heart, vessels); "cytokine storm" undamped by blocked GCRs
- Death

Innate Immunity: Neural (CNS, PNS, Hormonal) Regulation @ Non-specific Host Responses to Pathogens*

- "First line" defenses vs. "invading" microbes or "damaging" factors
  - PAMPs; pathogen-associated molecular patterns, i.e., TLRs 1-11
  - Initial non-specific cell/humeral responses
- Immune mediators → rapid CNS/endocrine responses
  - Amplify local clearance
  - "Dampening" responses → "resting state"

Risks of Mifepristone Early Pregnancy Termination in Context

J McGregor, O Equils. Contraception 2005; 71: 161

- Mifepristone impairs innate host responses and may predispose to lethal infections
- Mifepristone competitive inhibitor with PR and GC receptors – interferes GC effects genomic, cellular, organism levels
- Sepsis animal models: blocks endocrine stress responses and ↑ lethality
- Toxigenic Clostridium sordellii: lethal, hemorrhagic toxins

Operating Instructions, IBM

Thomas J. Watson