SEROLOGIC STUDIES IN COCCIDIOIDOMYCOSIS

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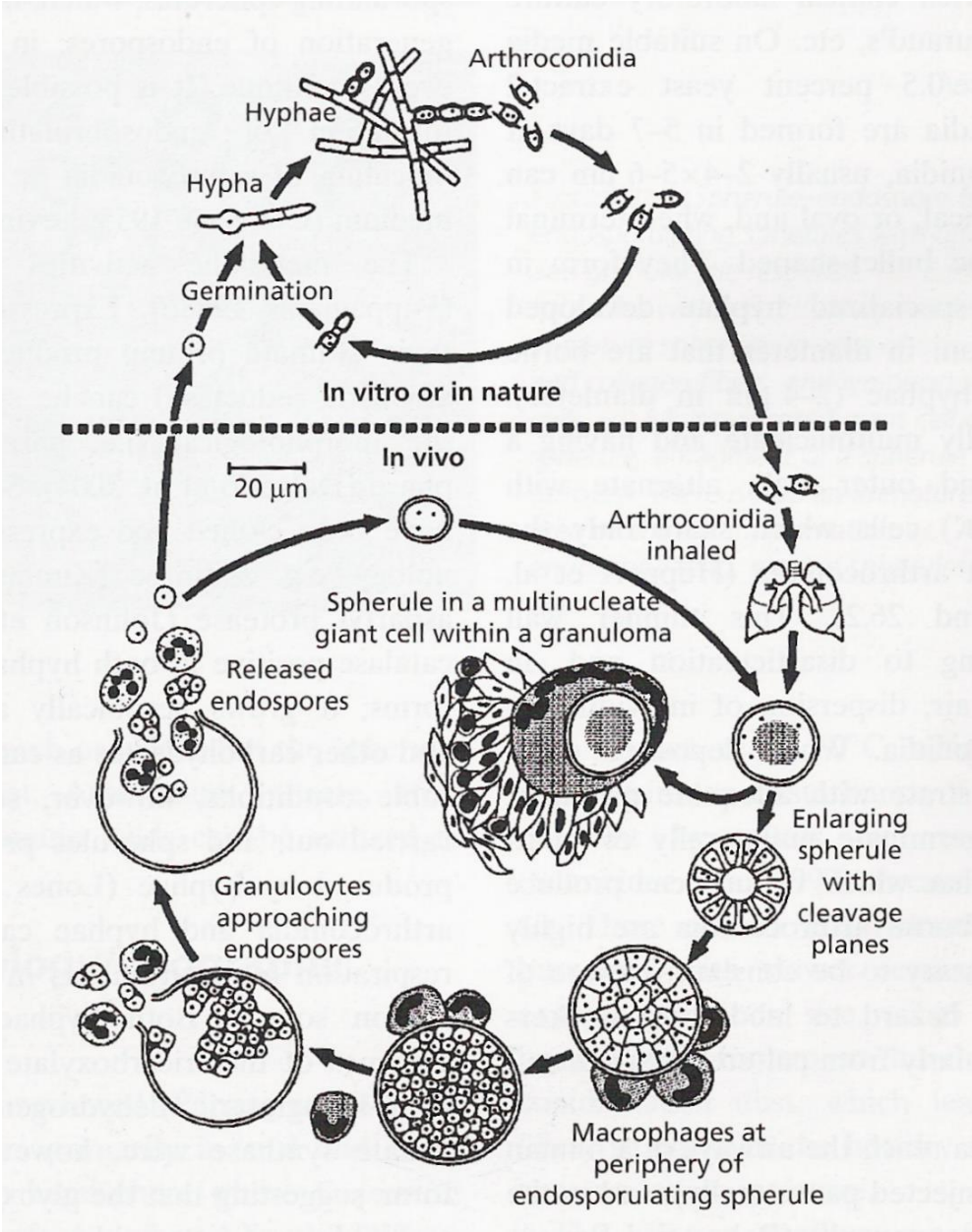
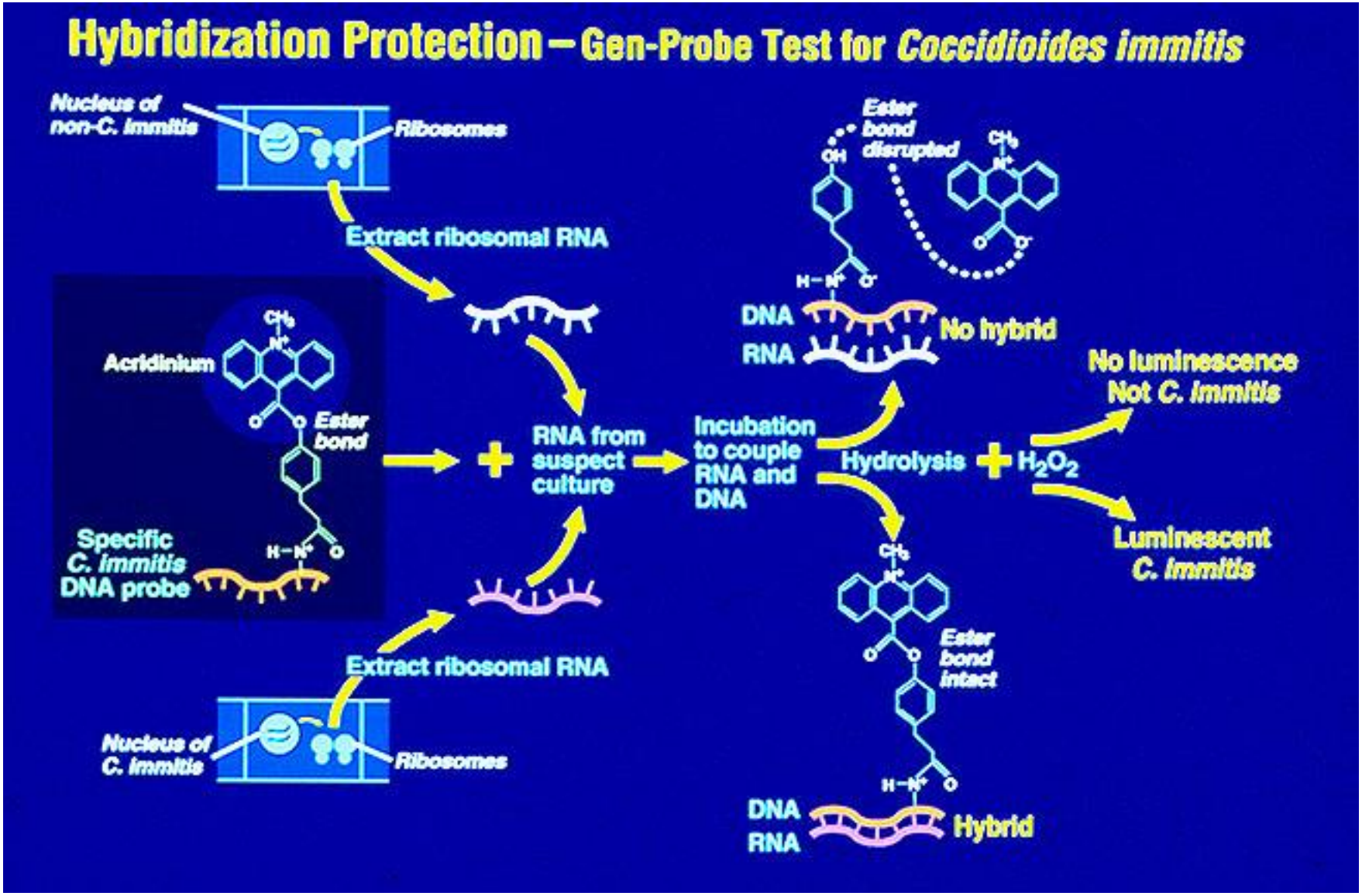
Life Cycle of *Coccidioides*



Diagnostic Tests for Fungal Infection

- Direct Demonstration
  - Microscopic – body fluid, exudates (pus), sputum, biopsy-tissue
  - Molecular – PCR to detect DNA
  - Culture
    - Morphologic identification
    - Molecular identification
      - DNA "probe"; RNA hybridized to compatible DNA
      - Polymerase chain reaction to detect DNA
- Indirect
  - Serologic
    - Detection of antigen of fungus
    - Detection of antibody response to fungus
  - Biochemical
    - Detection of fungal wall beta glucan or galactomannan in body fluids
    - Detection of metabolite, e.g. D-arabinitol by *Candida albicans*

DNA Probe


Hybridization Protection – Gen-Probe Test for Coccidioides immitis
Nucleus of non-C. immitis
Ribosomes
Extract ribosomal RNA
Acridinium
Ester bond
Specific C. immitis DNA probe
RNA from suspect culture
Incubation to couple RNA and DNA
Hydrolysis
H_2O_2
Ester bond disrupted
DNA
RNA
No hybrid
No luminescence Not C. immitis
Luminescent C. immitis
Ester bond intact
Hybrid
Extract ribosomal RNA
Nucleus of C. immitis
Ribosomes

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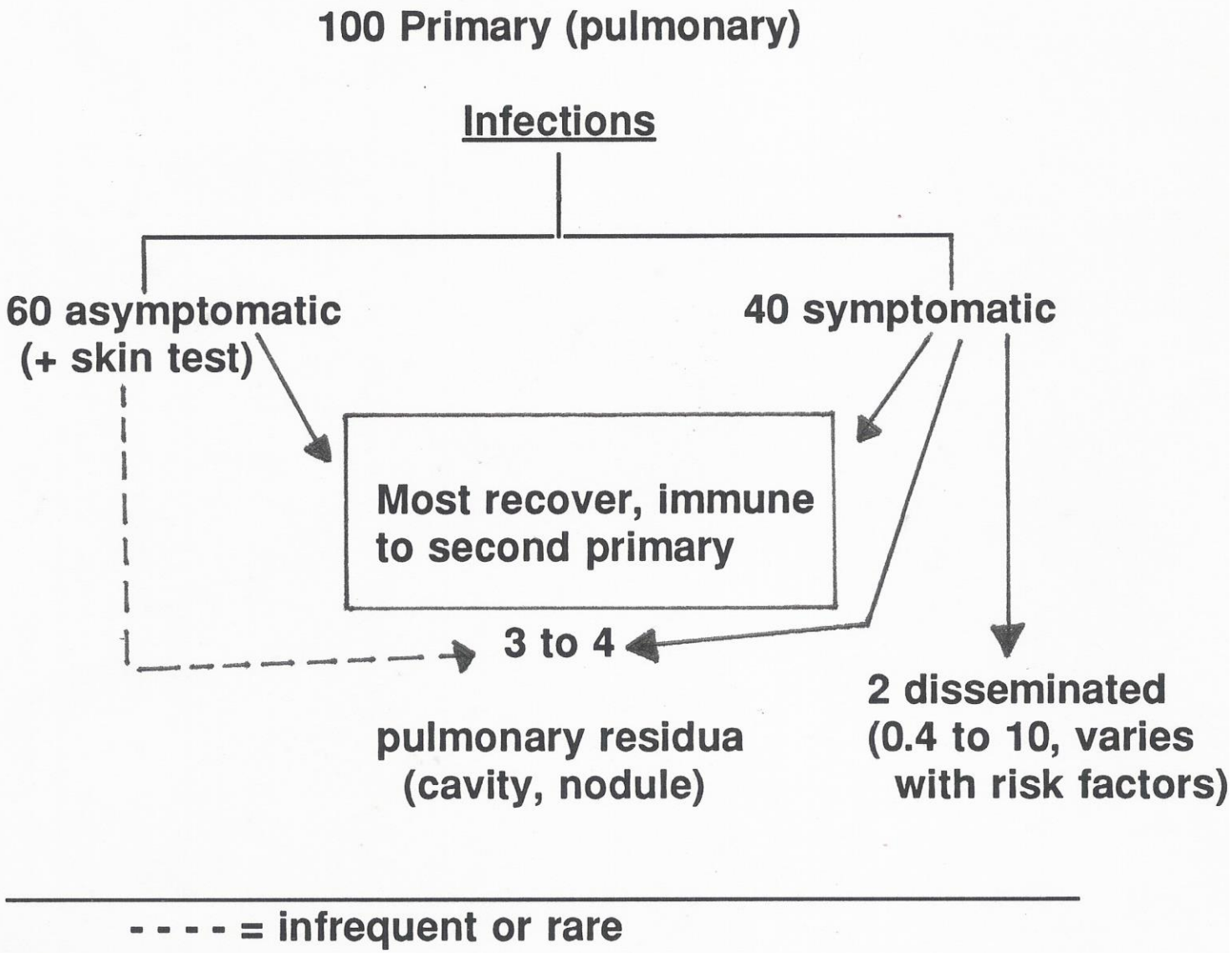
PATTERN OF 39,500 SEROLOGIC TESTS IN COCCIDIOIDOMYCOSIS

Charles E. Smith, M.D., Margaret T. Saito, B.S.
and
Susan A. Simons, M.P.H., Berkeley, Calif.

Table 1. 15-Year Study by C.E. Smith et al. of 39,500 Serologic Tests for Coccidioidomycosis

No. of patients	*(%)*	*Category of Disease*
5,579	(78)	Primary non-disseminating
722	(10)	Disseminated
271	(4)	Non-cavitary pulm. residual
577	(8)	Pulmonary cavities
7,149 cases	(100)	[mean 477 per year]

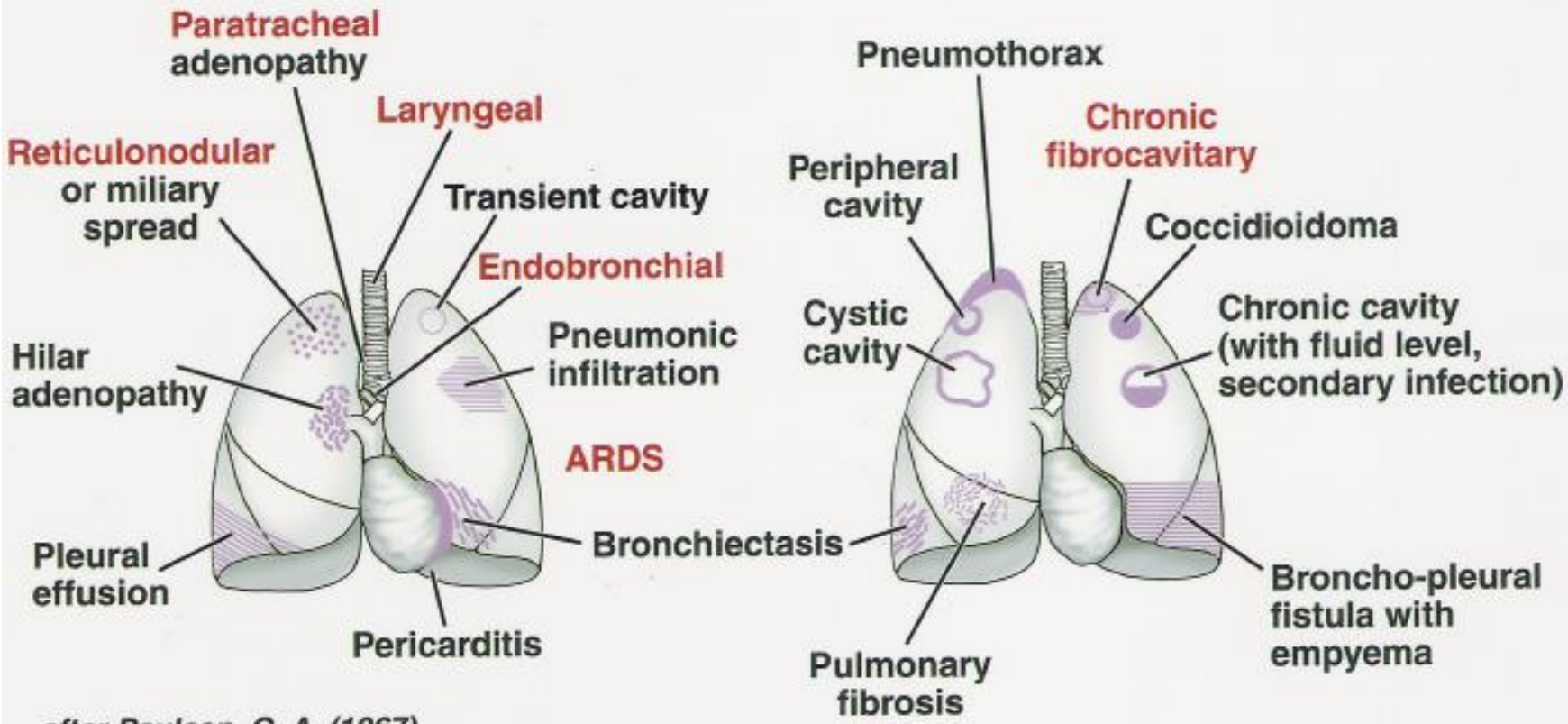
Coccidioidomycosis



Pulmonary Manifestations in ACUTE Coccidioidomycosis

Pulmonary Manifestations in CHRONIC Coccidioidomycosis

Entities added after Paulsen's depiction



after Paulsen, G. A. (1967)

COCCIDIOIDOMYCOSIS---RISK FACTORS FOR METAPULMONARY DISSEMINATION

INNATE

ETHNIC (Genetic)

Filipino, other Asian

African-American

Blood Group B

HLA Class II allele-DRBI*1301

IL-10 (Experimental, Fierer et al)

ACQUIRED

lymphoma

AIDS

immunosuppression

diabetes mellitus

pregnancy

age (with comorbid condition)

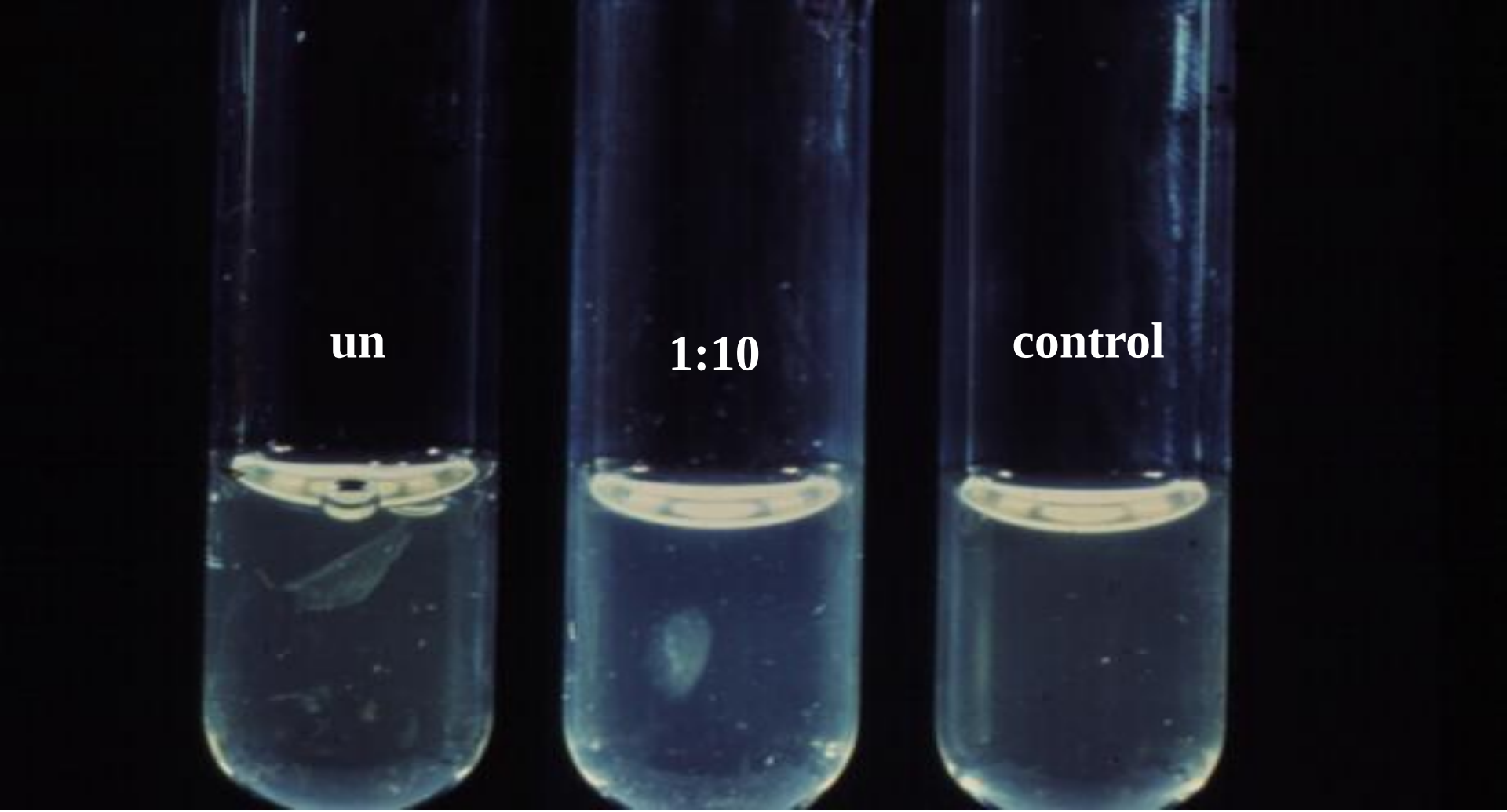
locus minoris resistentiae

Sites involved in disseminated coccidioidomycosis

	Fatal, autopsied series				Survivors
	Forbus and Bestebreurtje (1946)	Huntington, et al. (1967)	Sievers (1974)	Total	Sievers (1974)
Number of Patients:	50	131	20	201	16
	%	%	%	%	%
Lung	86	86	60	84	25
Spleen	70	51	25	53	0
Liver	60	45	35	48	0
Kidney	60	35	48	41	0
Skin &Subcut.	64	21	0	29	31
Bone	48	16	22	22	13
Leptomeninges	36	*63	**55	55	13
Adrenal	32	17	20	21	0

Heart (pericardium), pancreas, psoas and retroperitoneal, thyroid, prostate, larynx, scrotum, optic nerve, bladder, penis, placenta, parotid gland, tongue, mastoid bone, periosteum of orbit.

*36 or **27% with solitary meningitis



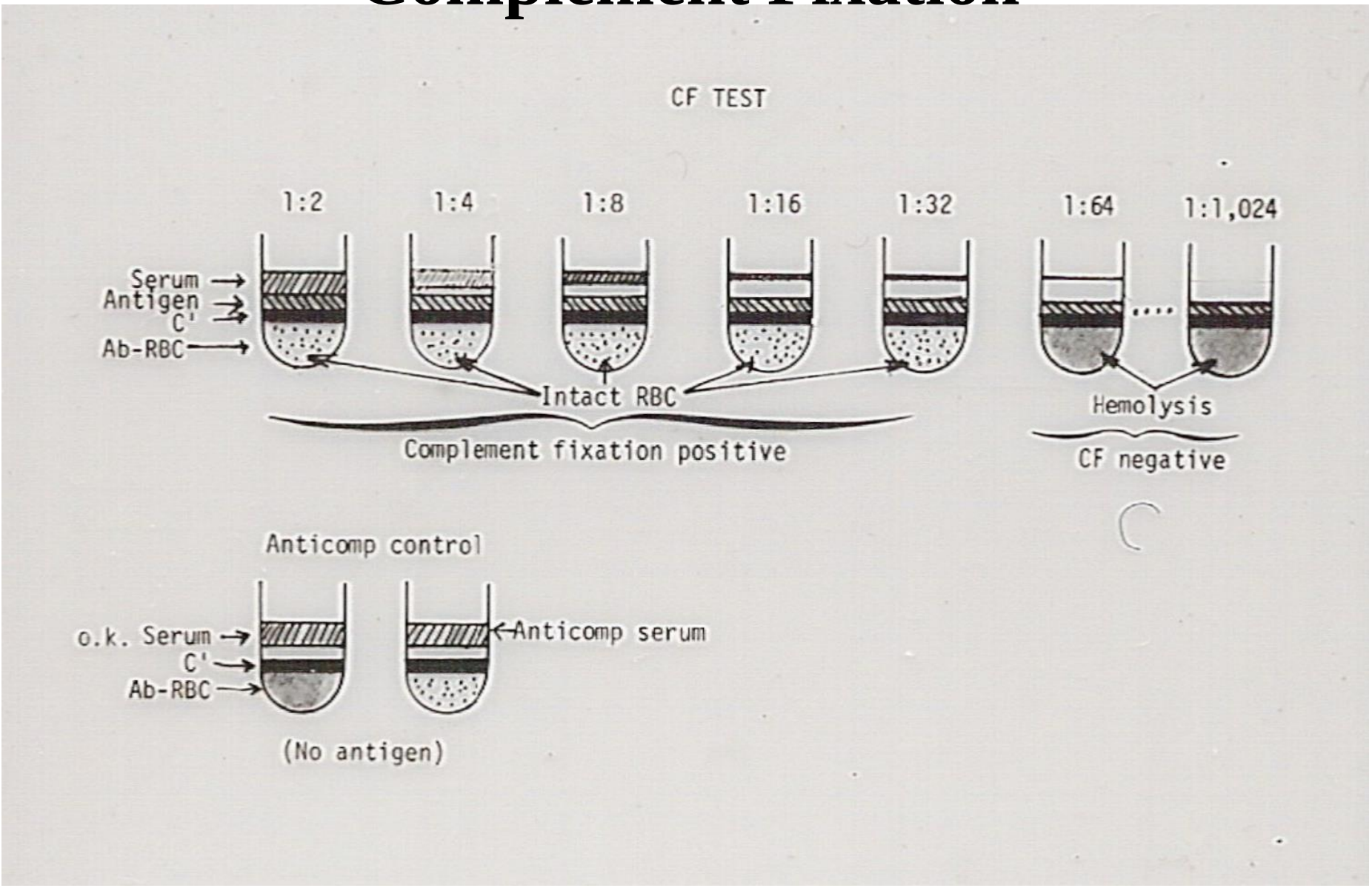
Tube precipitin test

Serum: Constant volume

Antigen: Undiluted, 1:10

Control: No antigen

Components and Reactions in Complement Fixation



Examples of Complement Fixation Results

Negative:

SPEC #	SOURCE	DRAWN	TESTED	1:2	1:4	1:8	1:16	1:32	1:64	AC Control 1:2
G4293	BLD	7-13	7-20	0	0	0				0

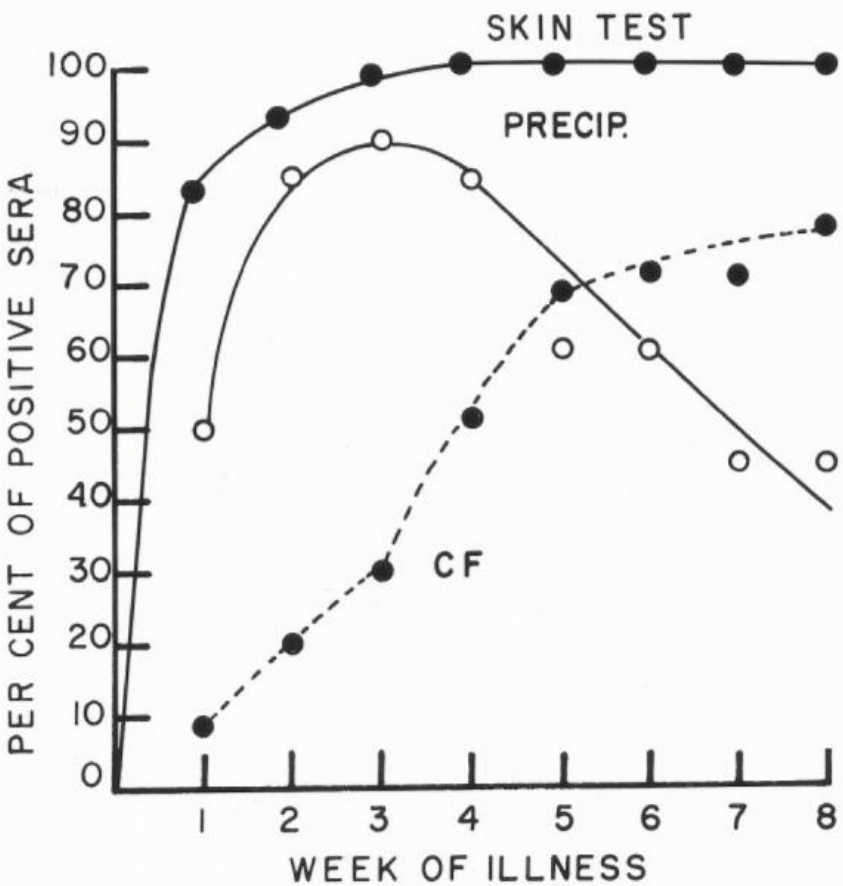
Positive:

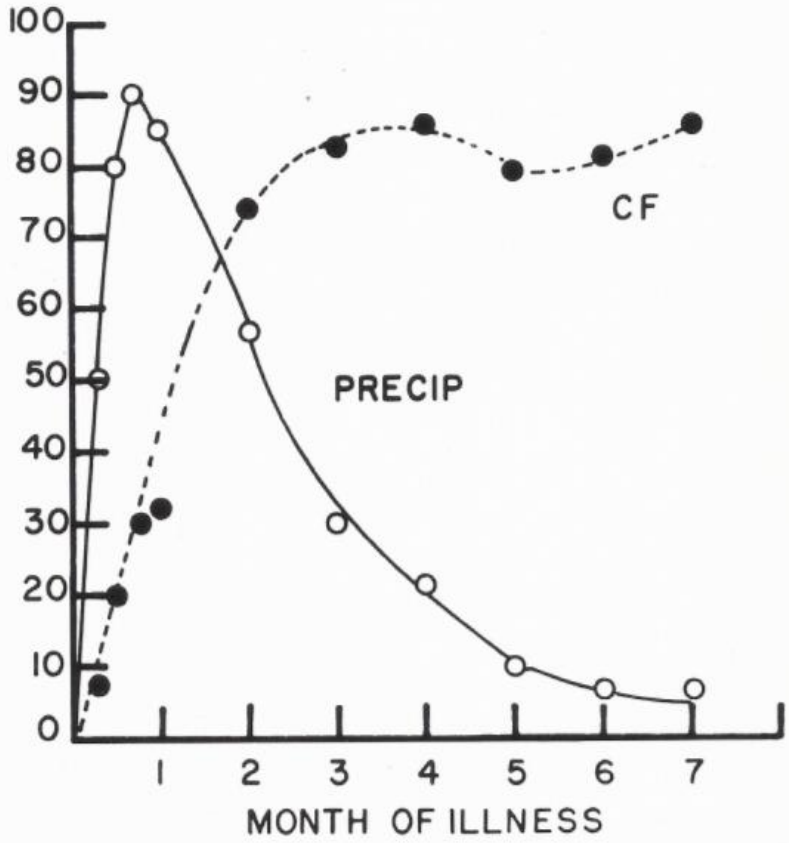
SPEC #	SOURCE	DRAWN	TESTED	1:2	1:4	1:8	1:16	1:32	1:64	1:2
G2763	BLD	4-1	8-5	4	4	3	2	0		0
G5673	BLD	8-1	8-5	4	4	4	3	0		0

"ZONING"

SPEC #	SOURCE	DRAWN	TESTED	1:2	1:4	1:8	1:16	1:32	1:64	1:2
G3294	BLD	3-17	8-5	1	1	2	2	1	0	0
G7294	BLD	8-1	8-5	1	2	2	1	0	0	0

Sequence of Immune Responses in Coccidioidomycosis



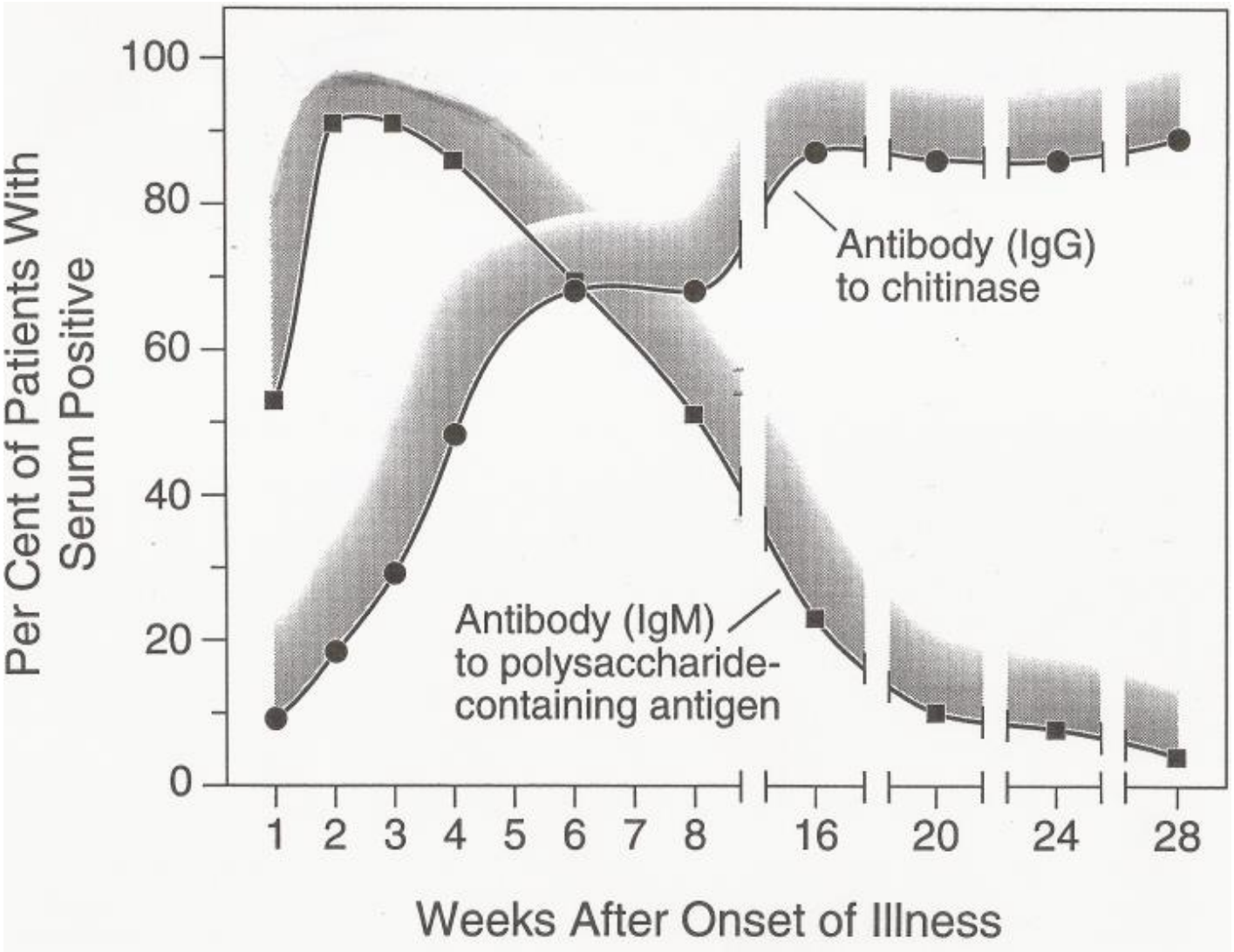


Precip = tube precipitin test

CF = complement fixation test

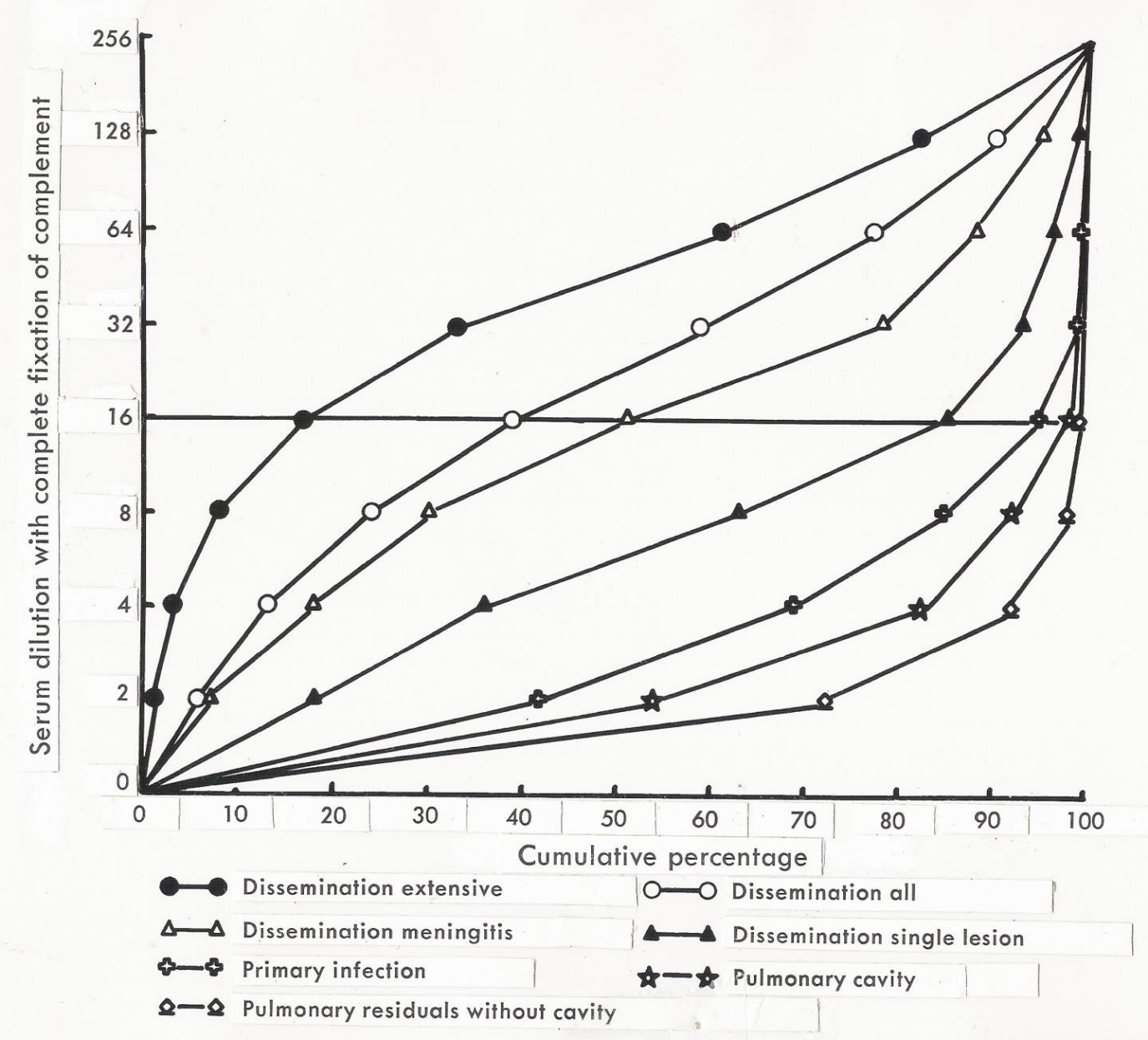
Smith, et al (1950)

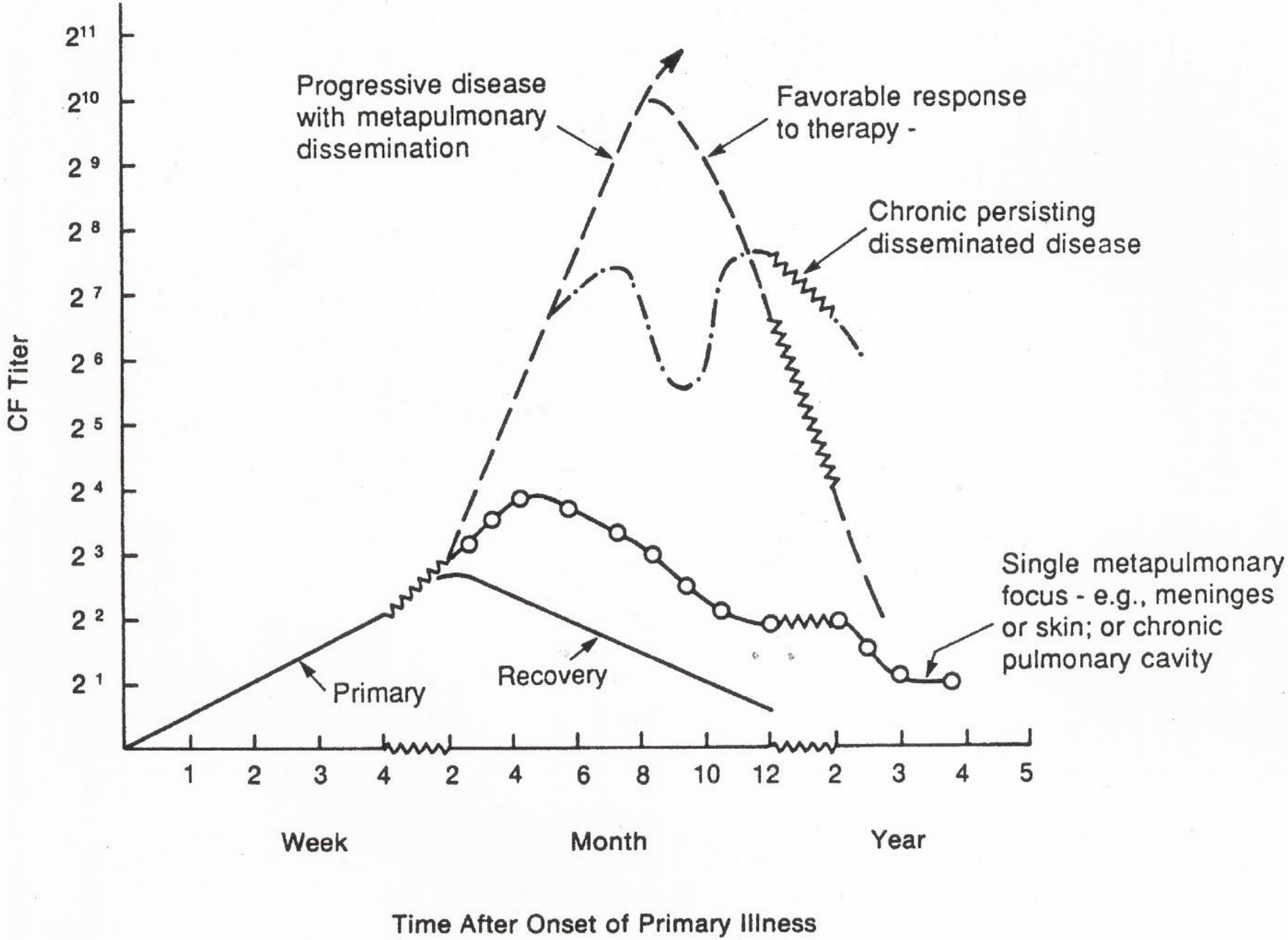
Sequence of Antibody Responses



Shaded areas for immunodiffusion with concentrated serum, or EIA

CF titer in Various Clinical Forms




CF Titer
2¹¹
2¹⁰
2⁹
2⁸
2⁷
2⁶
2⁵
2⁴
2³
2²
2¹
Progressive disease with metapulmonary dissemination
Favorable response to therapy -
Chronic persisting disseminated disease
Single metapulmonary focus - e.g., meninges or skin; or chronic pulmonary cavity
Primary
Recovery
1 2 3 4 2 4 6 8 10 12 2 3 4 5
Week
Month
Year
Time After Onset of Primary Illness

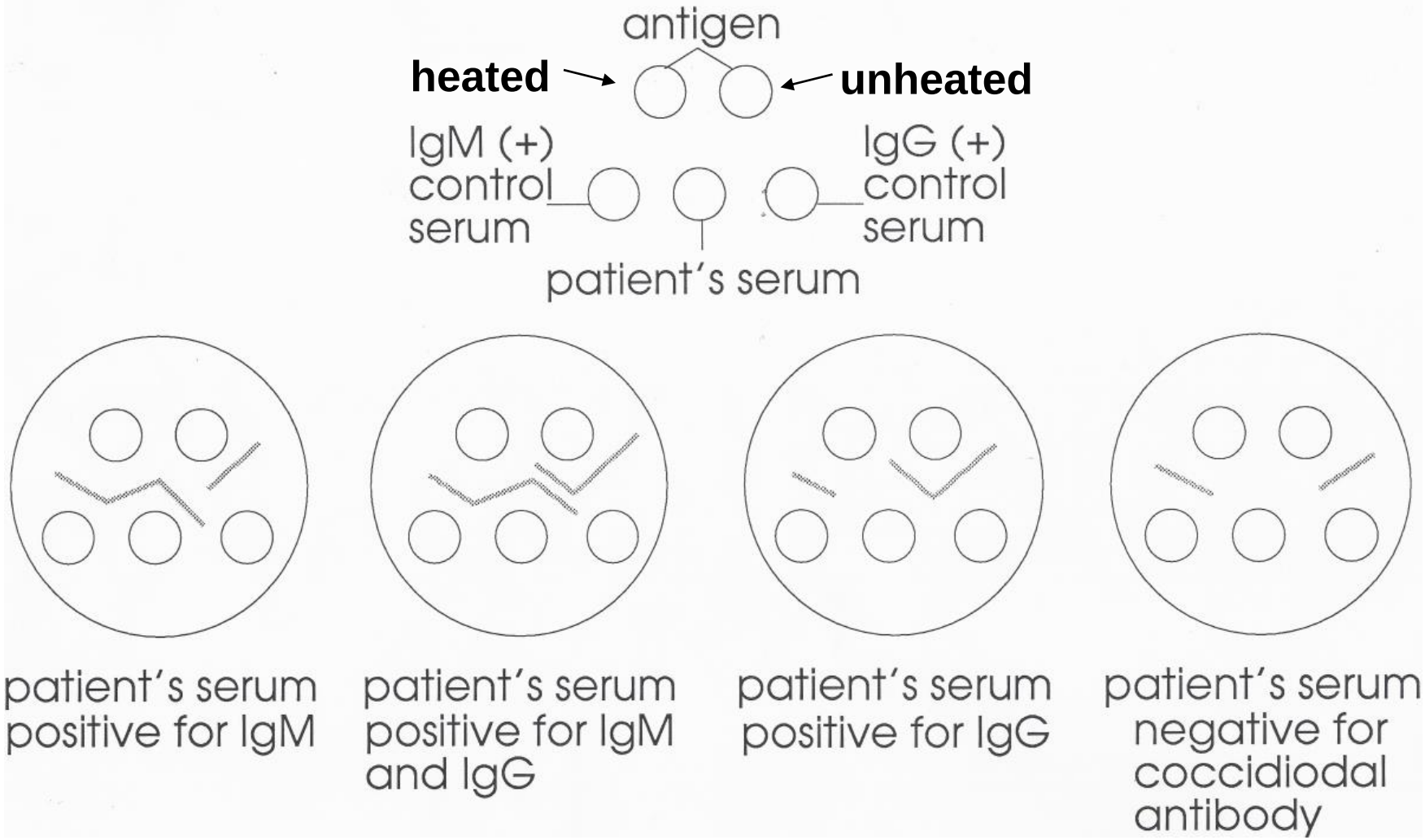
The declining complement fixation titer is favorable, but in an immunocompromised patient a decrease in titer may be the result of a diminished humoral responsiveness.

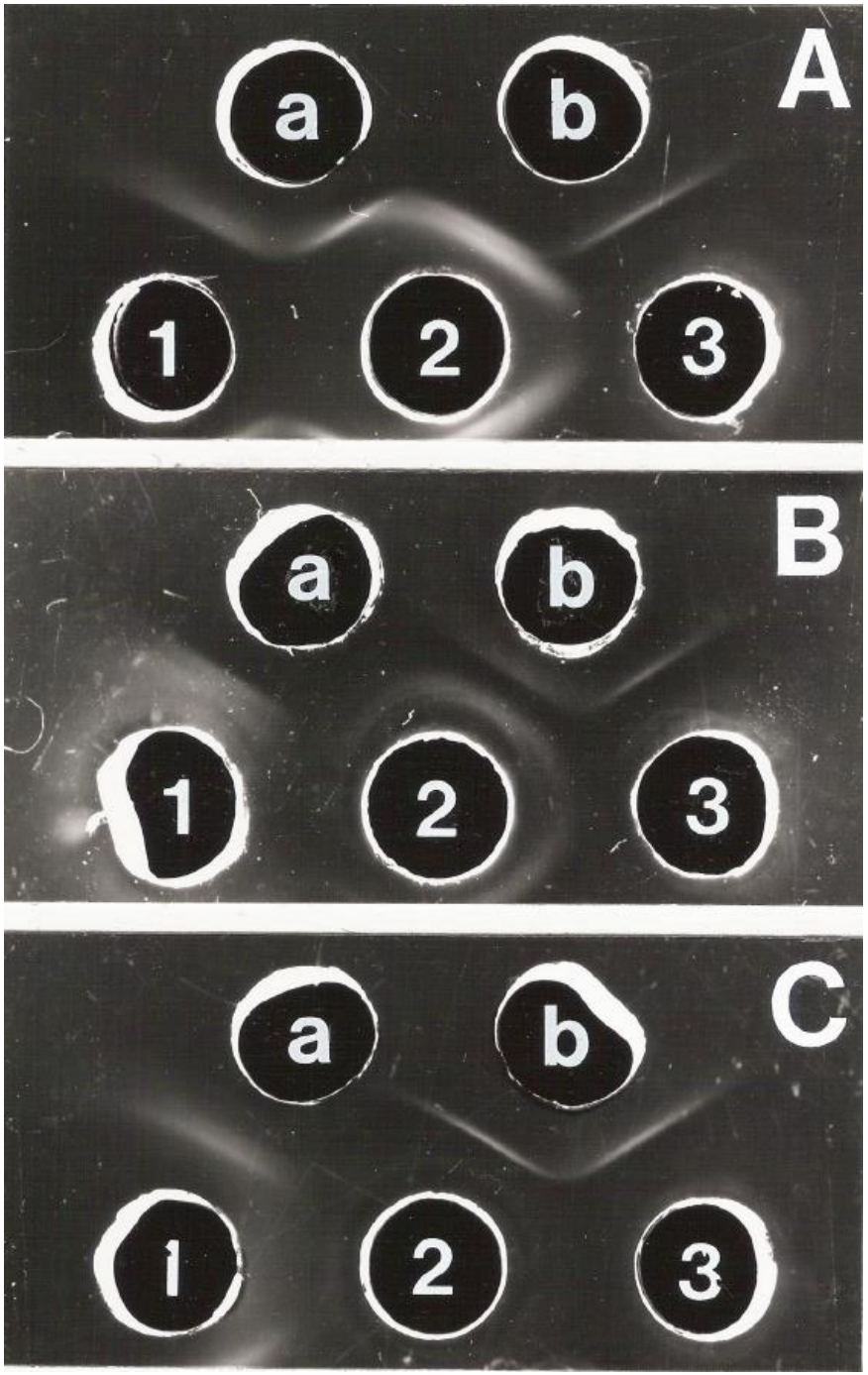
The decreasing complement fixation titer may be helpful in following the effects of therapy. However, attempting to treat until the patient is seronegative may be too rigorous a goal. The titer may persist at a low level – 1:2 to 1:4 – long after the clinical resolution of disease.

Gel Immunodiffusion

("Double Diffusion")

for Antibody in Coccidioidomycosis





Qualitative immunodiffusion

CF titer

1:128

1:64

1:32

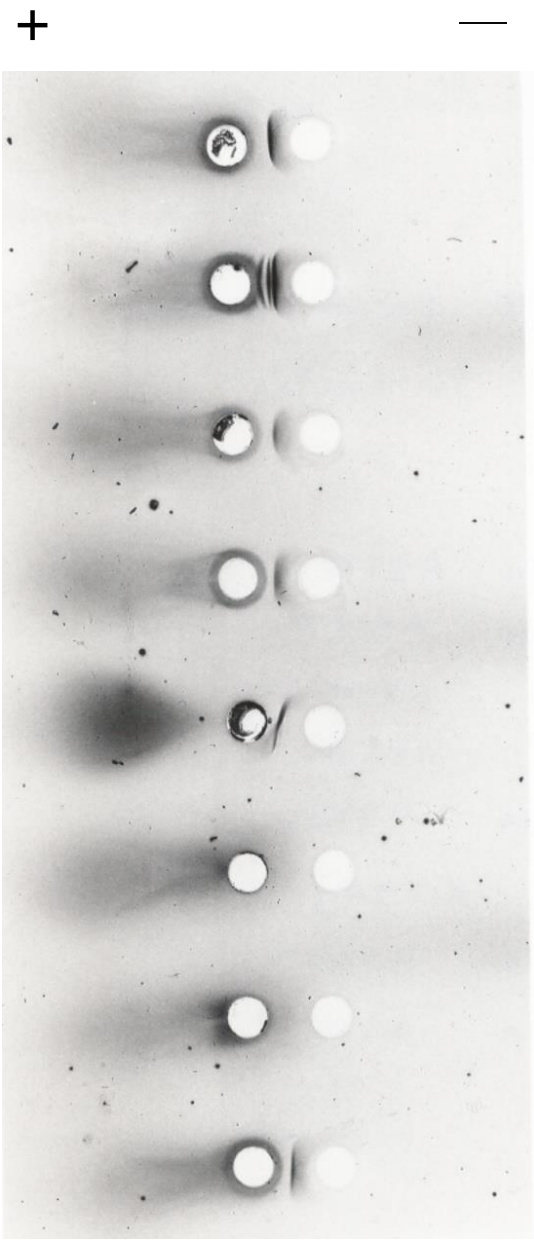
1:16

1:8

1:4

1:2

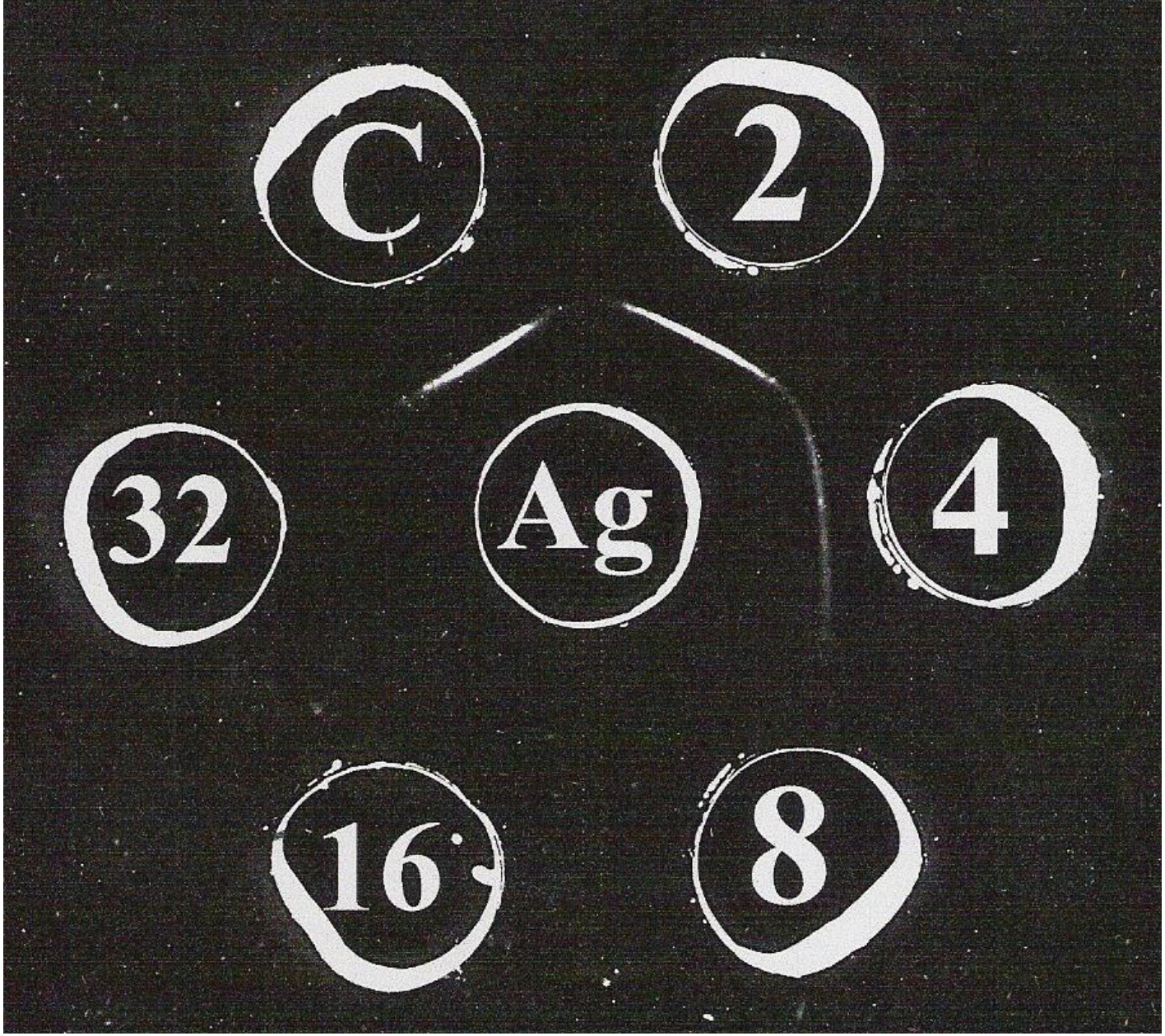
>1:2



TS58 Antigen

1:10

Counterimmunoelectrophoresis (CIE)



Quantitative immunodiffusion

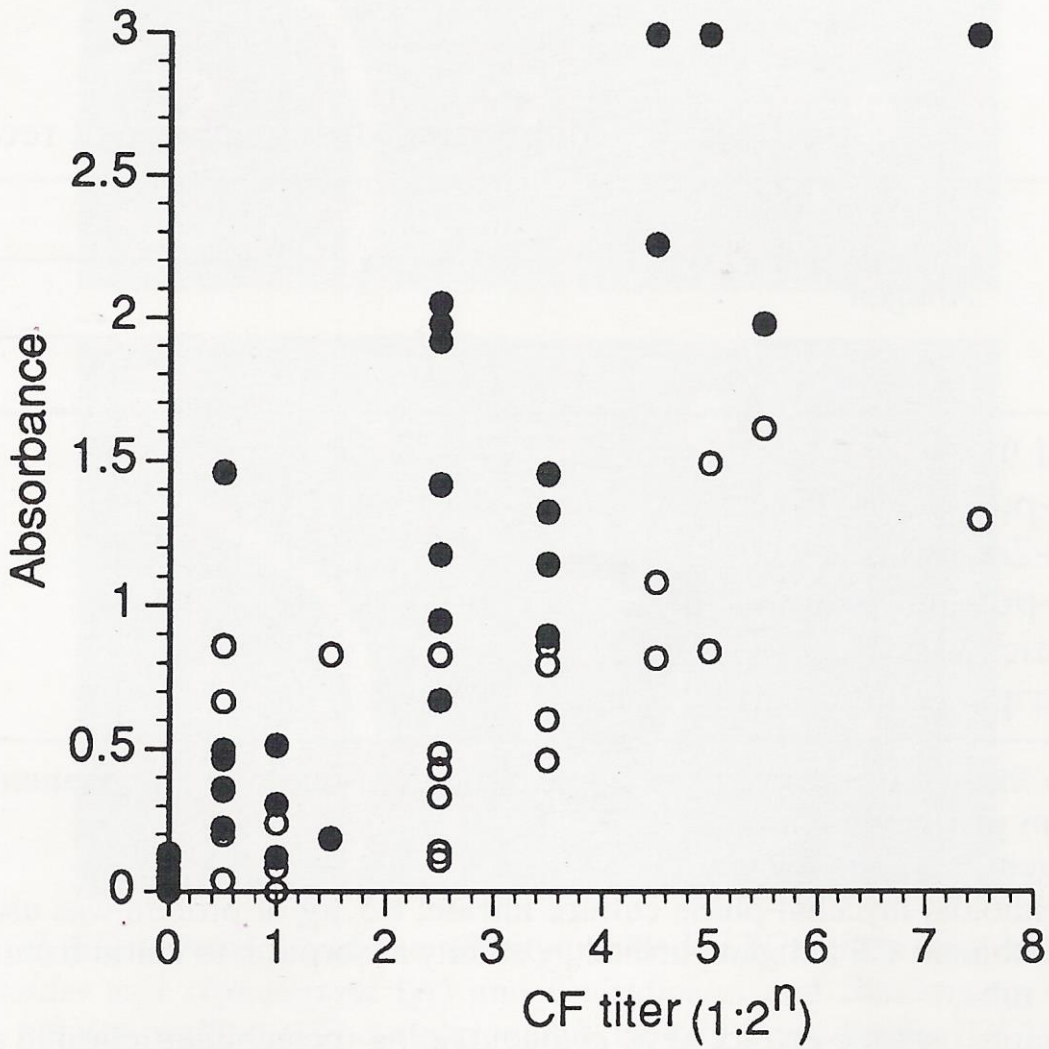


FIG. 4. Reactivity of the affinity-purified rCF EIA and the Meridian Premier *Coccidioides* EIA with respect to previously determined CF titer. Symbols: ○, Meridian Premier EIA; ●, affinity-purified rCF EIA.

Johnson, S. et al 1996

TABLE 2. Comparison of complement-fixing activity obtained with the AP rCF and coccidioidal antigens

Serum specimen[a]	Antigen	CF score									
		Serum dilution									Serum control[b]
		1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	
Z4766	AP rCF[c]	4	3	0							0
	CF pool[d]	4	3	0							0
Z4853	AP rCF	4	4	4	4	2	0				0
	CF pool	4	4	4	4	3	0				0
Z4765	AP rCF	2	3	3	4	4	3	3	1	0	0
	CF pool	4	4	4	4	4	4	3	3	0	0

[a] Sera from three different patients.
[b] Anticomplement serum control; serum plus complement.
[c] Affinity-purified (AP) rCF antigen/chitinase.
[d] Coccidioidal mycelial CF pool 93 antigen.

Johnson, S. et al 1996

Comparative Reactivity of Sera of a Patient with Coccidioidomycosis by Enzyme Immunoassay (EIA), Complement Fixation (CF) and Immunodiffusion (ID)

Date of Serum	ID IgM	ID IgG	CF titer	EIA Optical density
2-20-06	(+)	Negative	Negative	IgG 0.594
4-7-06		(+)	1:8	IgG 0.446
4-19-06	ND		1:32	IgG 0.281
5-24-06	ND		1:128	IgG 0.654
6-7-06	ND		1:128	IgG 0.818
6-20-06		(+)	1:128	ND
7-11-06		(+)	1:64	ND
7-24-06	ND		1:32	IgG 0.703

APPLICATION OF SEROLOGIC TESTS IN COCCIDIOIDOMYCOSIS

	DETECTION OF	
	IgM	IgG
Tube precipitin (TP)	yes	no
Latex particle agglutination (LPA)	yes	no (±)**
Immunodiffusion = TP (IDTP)	yes	no
Complement fixation (CF)	no (rare*)	yes
Immunodiffusion = CF (IDCF)	no	yes
Counterimmunoelectrophoresis(CIE)	yes	yes
Enzyme-linked immunoassay (EIA)	yes	yes

* rarely positive complement fixation in presence of positive IDTP (IgM, and absence of detectable IgG by IDCF).

** occasional positive LPA in presence of detectable IgG but not IgM by immunodiffusion.

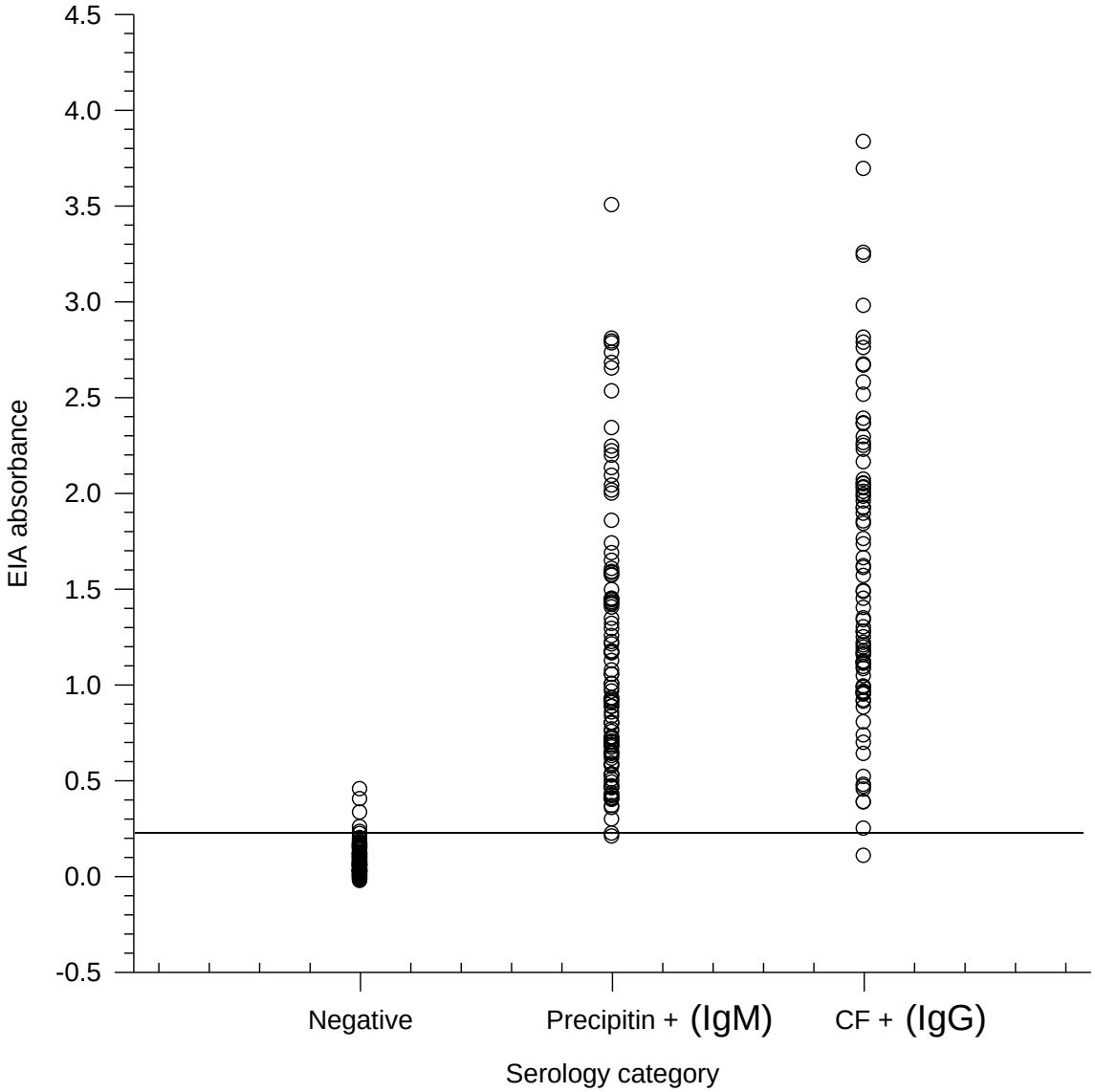
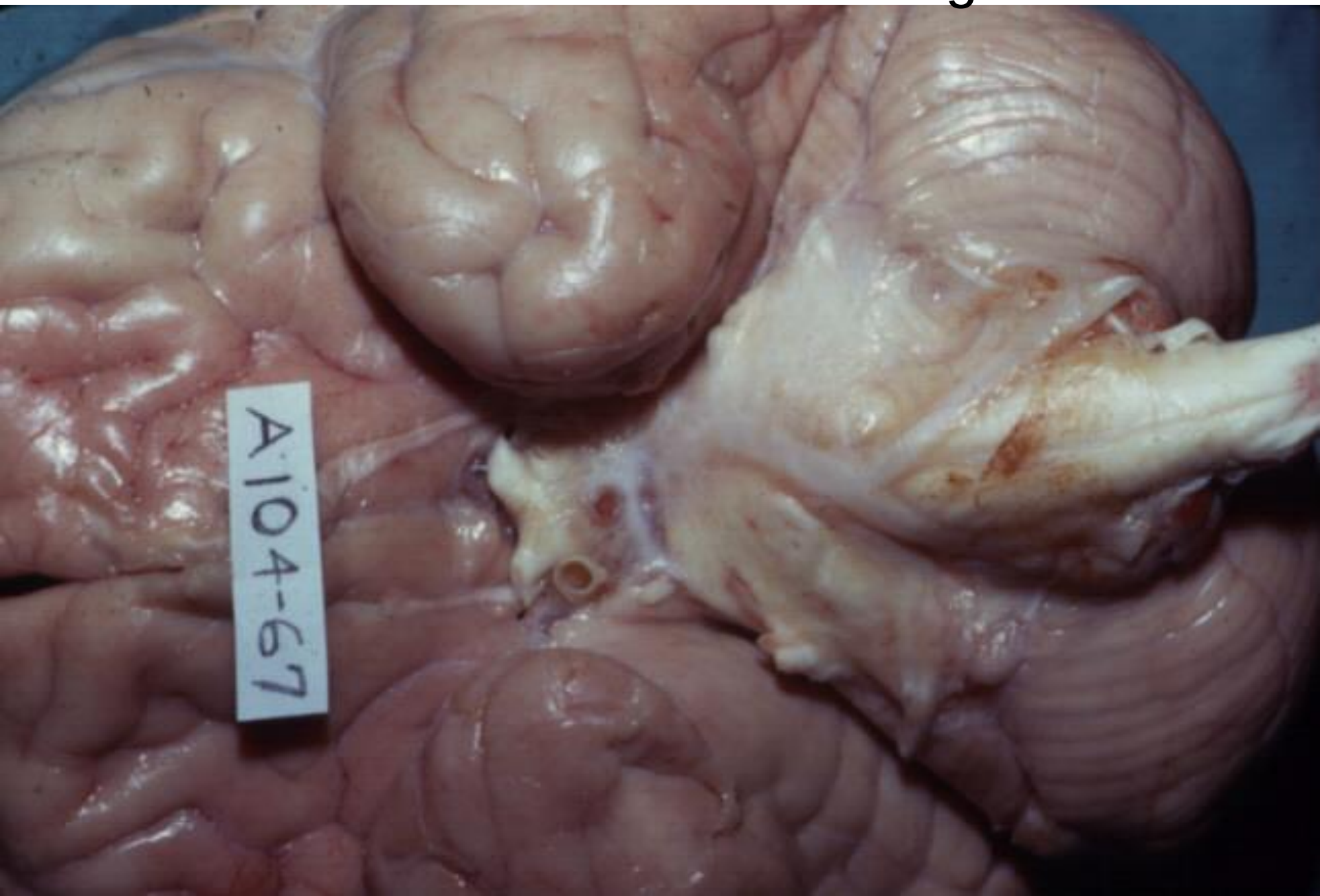


Figure 1. Reactivity of coccidioidal IgA in sera of patients with coccidioidomycosis positive for IgM, IgG or both.

Coccidioidal Lesions of the Central Nervous System

- leptomeningitis
- ventriculitis/ependymitis
- meningeal/cerebral vasculitis
- cerebral abscess
- syringomyelia

Basilar coccidioidal meningitis



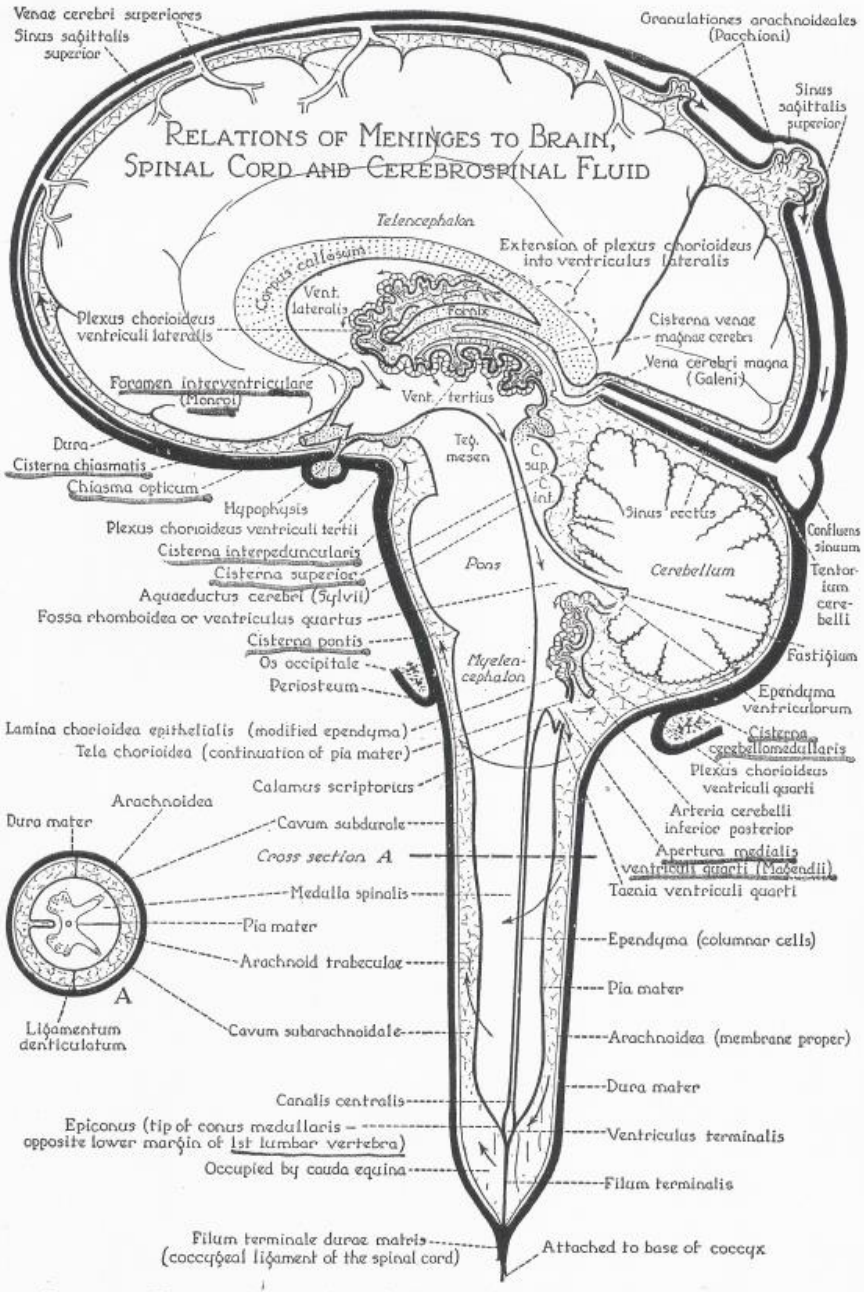
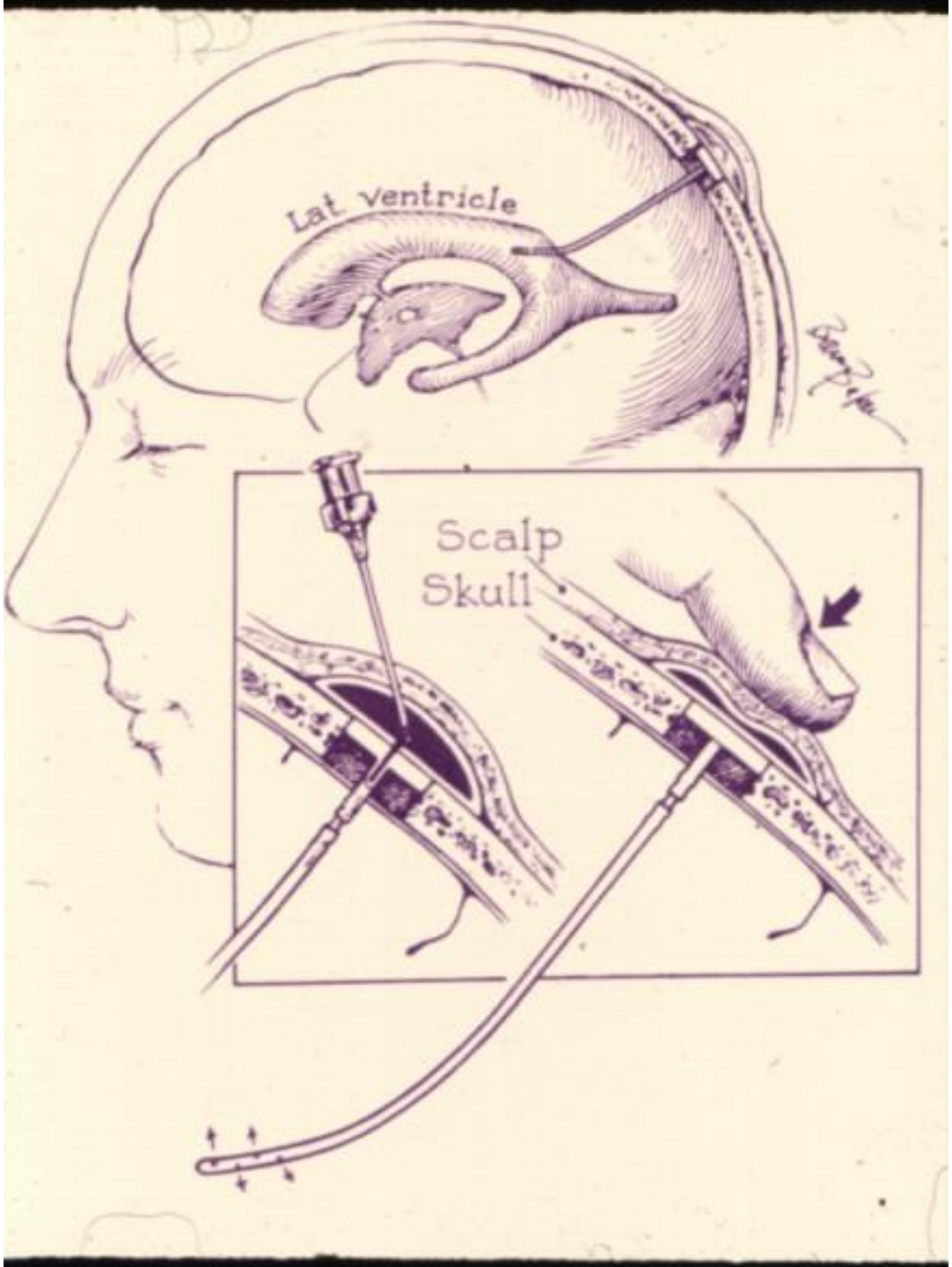
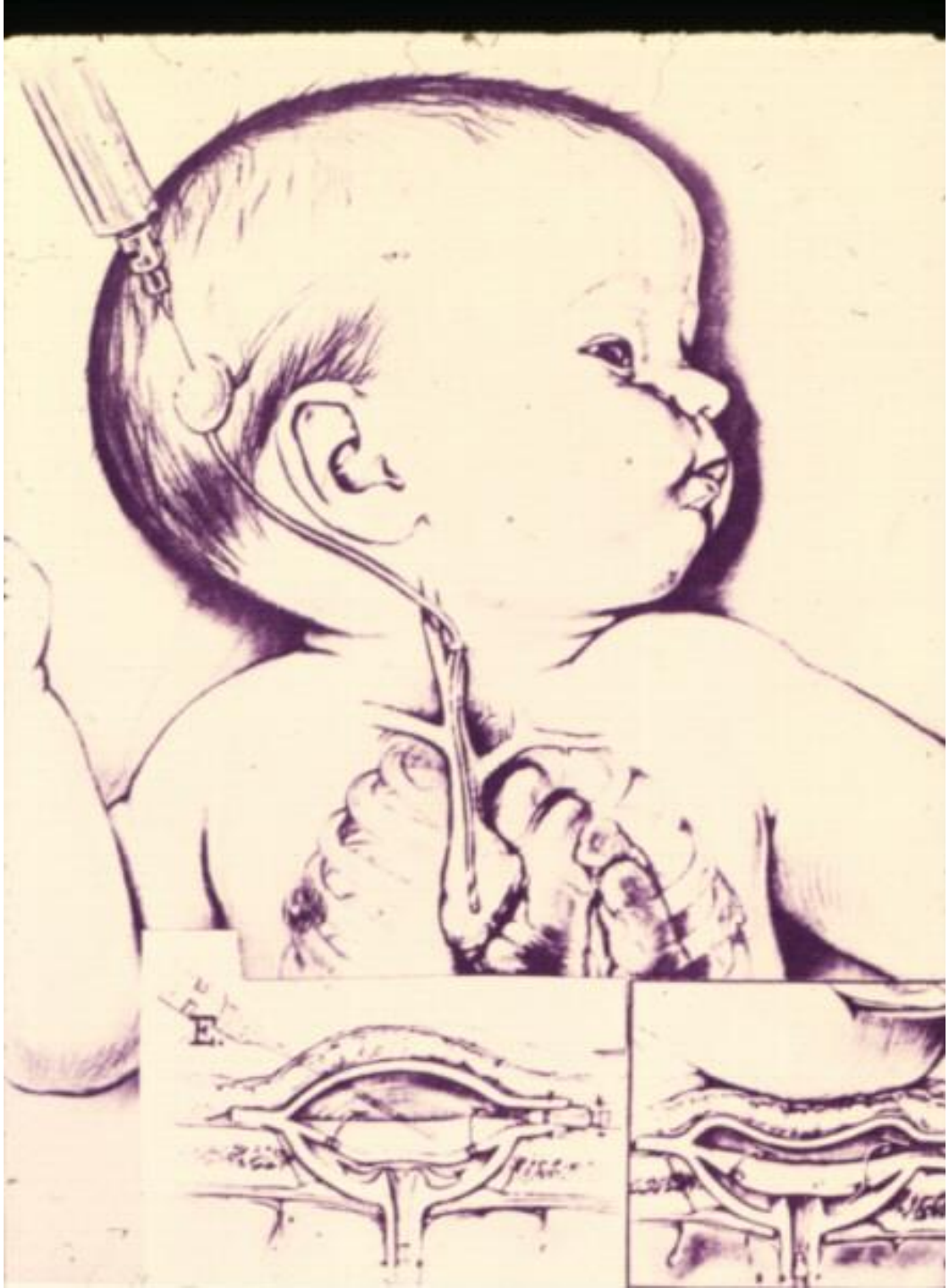


FIGURE 63. Diagram of the meninges, brain ventricles, and subarachnoid spaces. The arrows indicate the direction of the flow of the cerebrospinal fluid. (From Rasmussen, The Principal Nervous Pathways. By permission of The Macmillan Co., Publishers.)

CSF Compartments

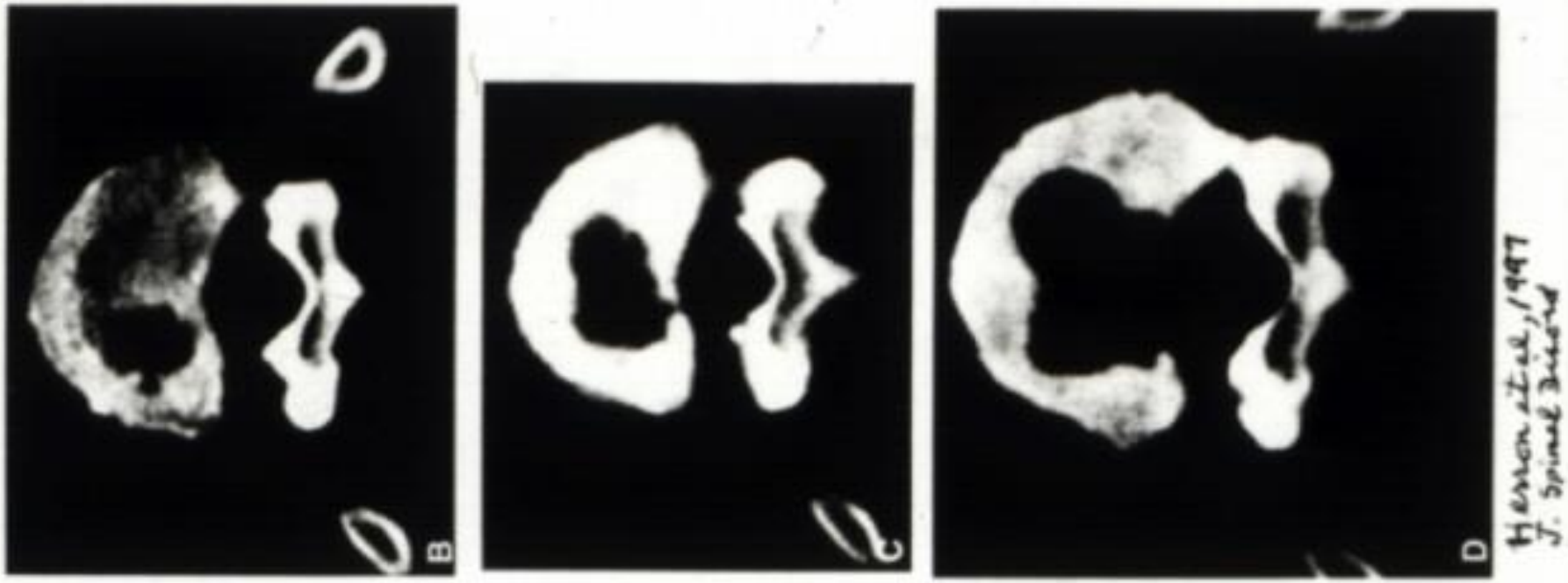


Ommaya reservoir and catheter in lateral venticle



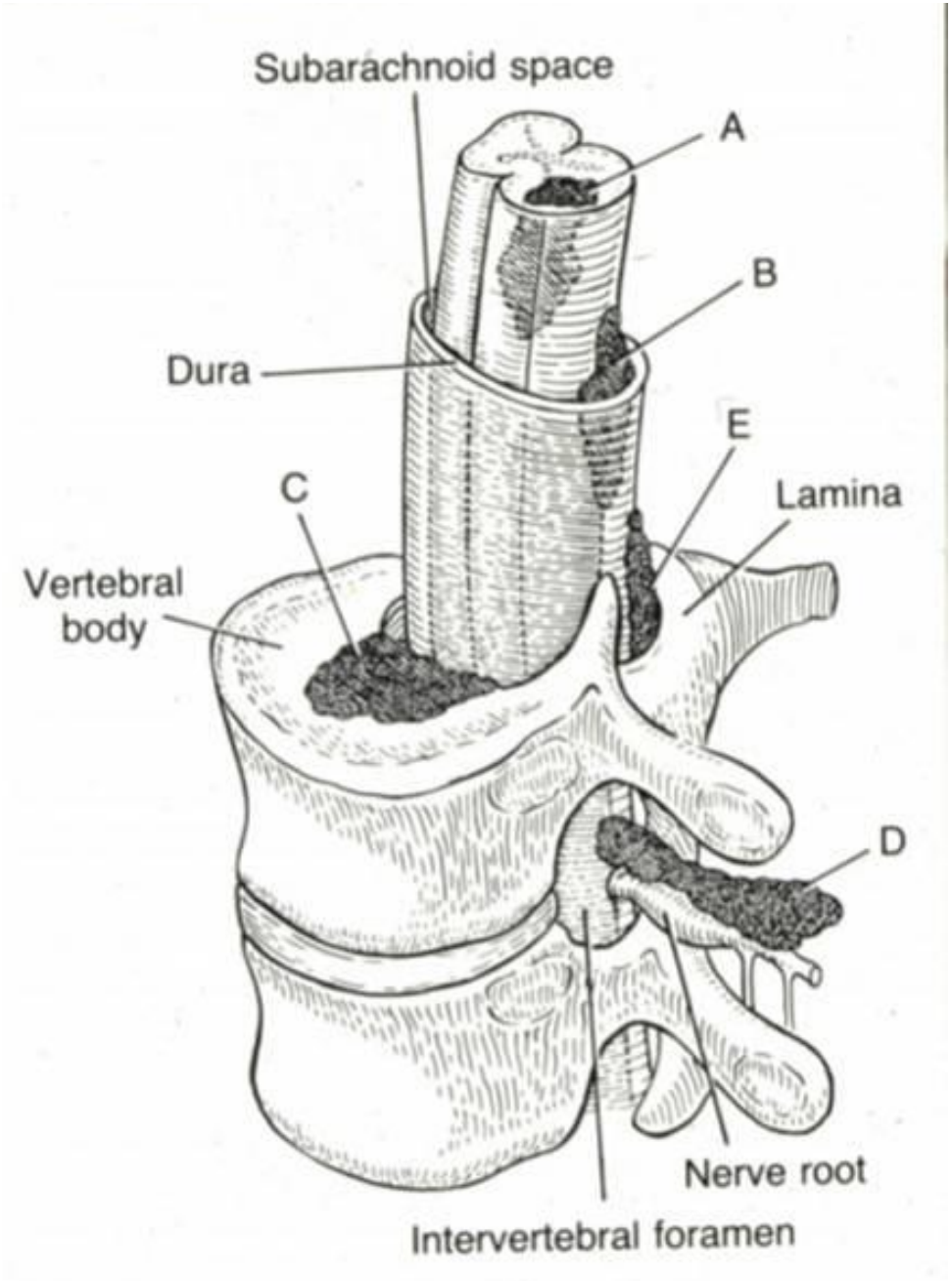
Ventriculo-atrial shunt

Lytic coccidioidal lesion of a vertebra



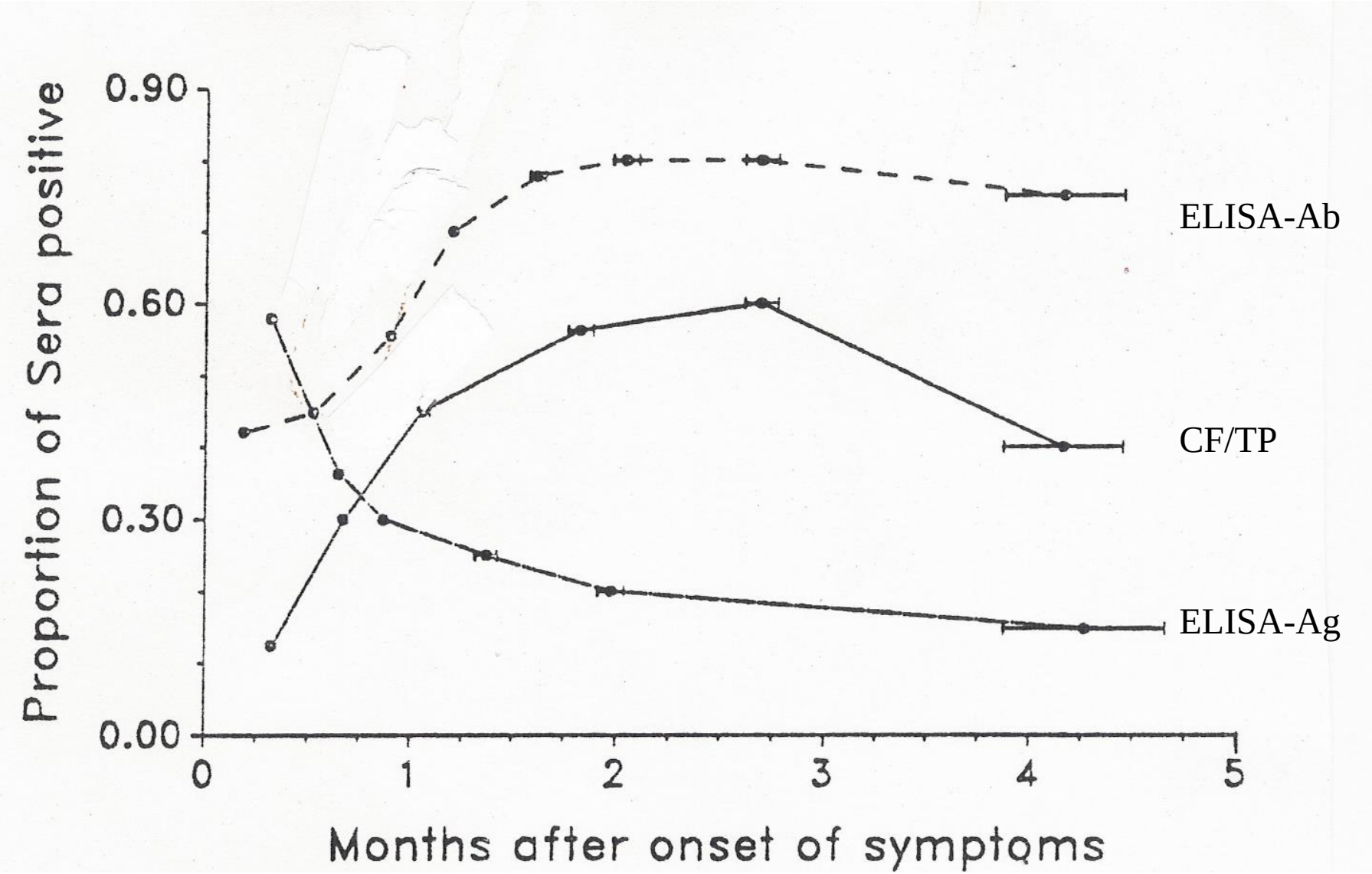
Herron et al., 1997 Spinal Disorders

Juxtadural vertebral lesion



Cerebrospinal Fluid Findings

Coccidioidal	**CSF**		
	glucose	**protein**	**cells**
meningitis	↓	↑	↑
juxtadural lesion	**normal**	↑	**normal**



Coccidioidal antigenemia

Galgiani et al. 1991

Additional Tests for Coccidioidal Antigen in Humans

- Antigenuria and antigenemia using cross-reactions between coccidioidal antigen and anti-histoplasma antibody.~Kuberski, Wheat et al., 2007
- Antigenemia using equine coccidioidal antibody. ~ Miller, C.M., Johnson, S.M., Pappagianis, D.,2007