

# Neural Tube Defects: Screening and Prevention

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PGY-2, Pathology

# Objectives

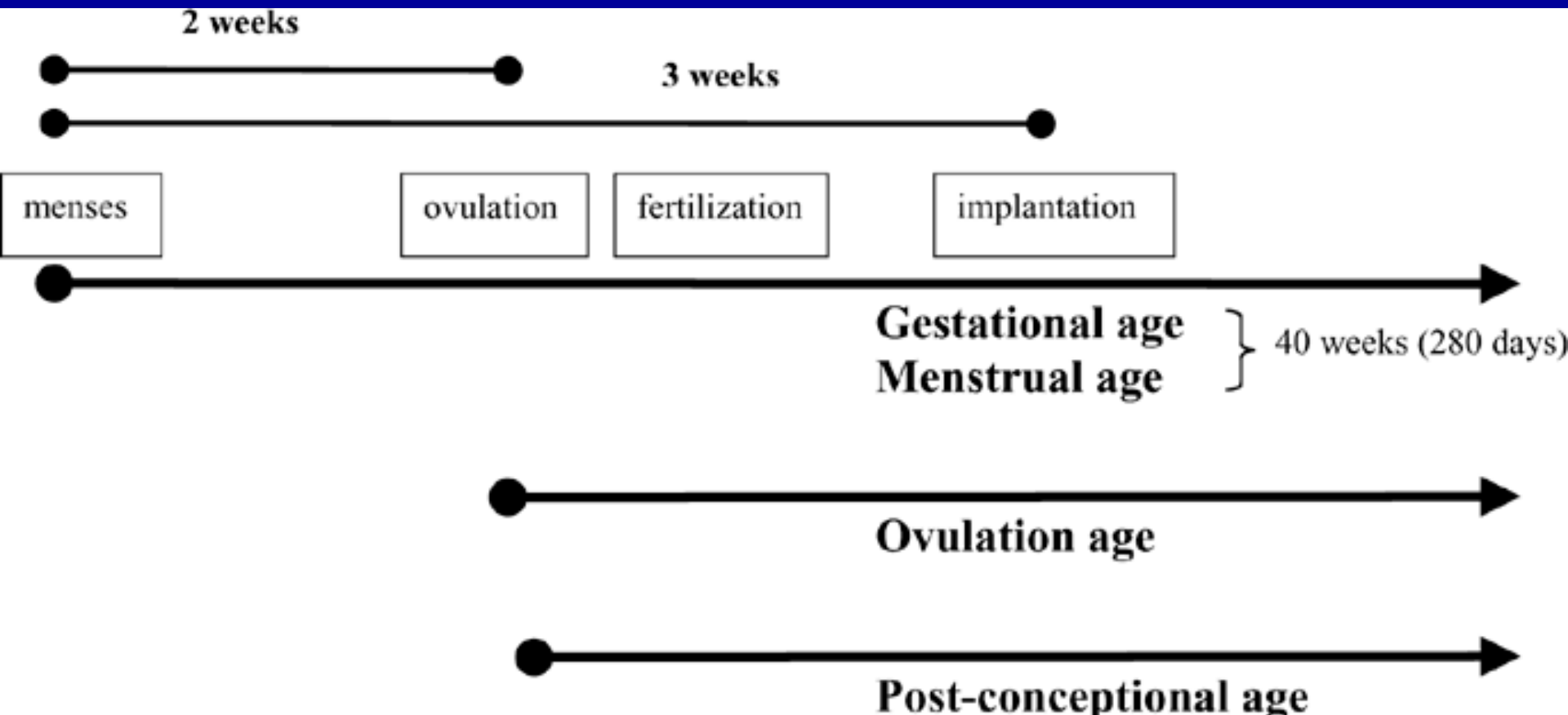
- After attending this presentation, the learner should be able to:
  - Define the most common types of neural tube defects.
  - Describe current prenatal screening for neural tube defects.
  - Explain the impact of folic acid supplementation on neural tube defect incidence.

# Outline- Questions to Answer

- How does the neural tube normally develop?
- What are neural tube defects (NTDs)?
- How can we screen for NTDs?
- How can we prevent NTDs?
- What are some areas for future research?

How does the neural tube  
normally develop?

# Pregnancy Timeline

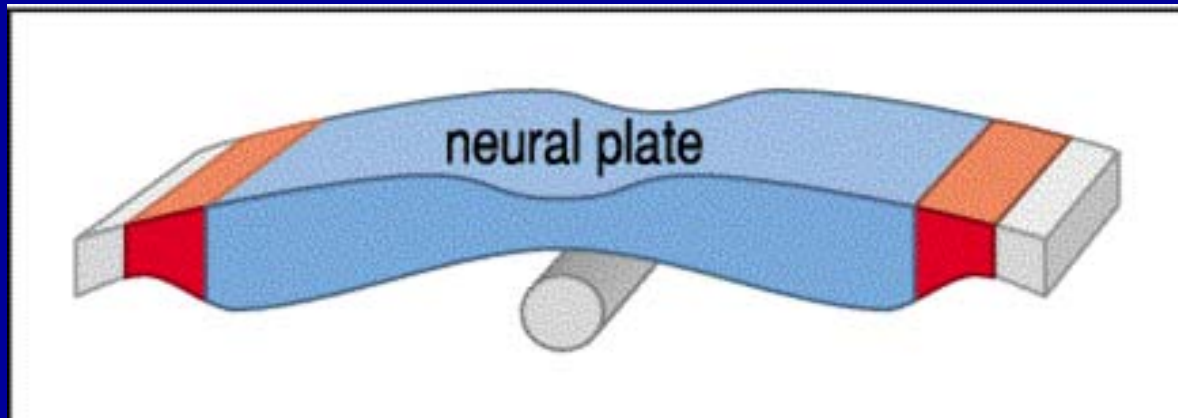


Source: Cunningham FG, Leveno KL, Bloom SL, Hauth JC, Gilstrap LC, Wenstrom KD:  
*Williams Obstetrics*, 22nd Edition: <http://www.accessmedicine.com>

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# Normal Development

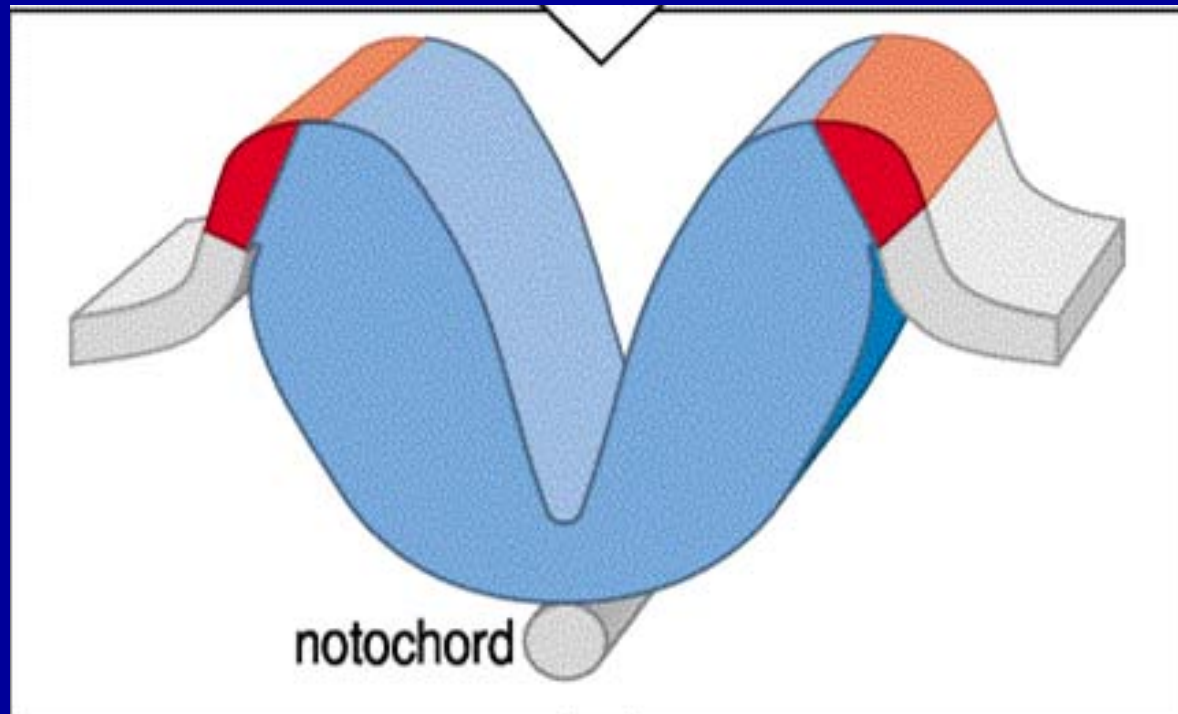
- Nervous system development begins in third week of pregnancy
- Neural plate appears



Figures for next three slides accessed at:  
<https://people.creighton.edu/~idc24708/Genes/Behavioral%20Genetics/3-step%20neurulation.png>

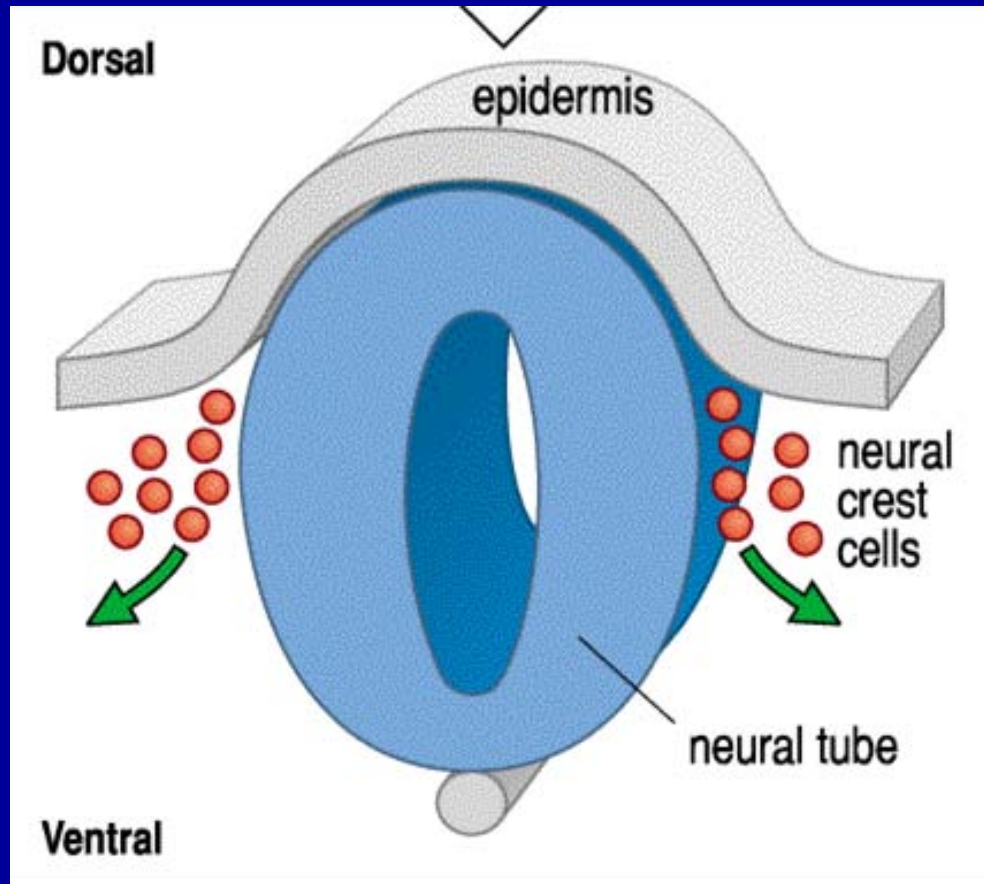
# Normal Development

- Edges of neural plate elevate to form neural folds



# Normal Development

- Neural folds continue to raise and eventually join to create the neural tube





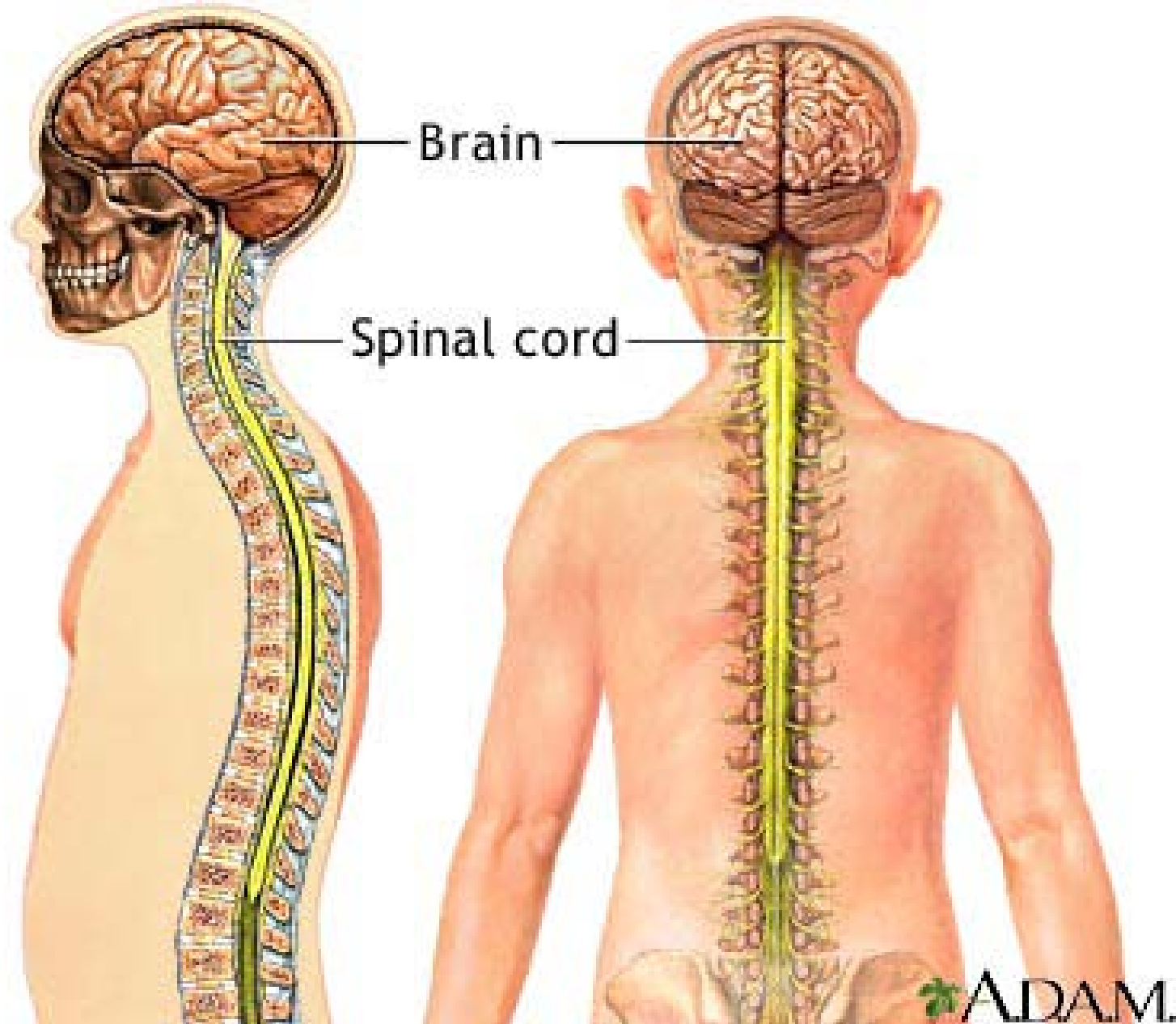
# Neural Tube Animation

From Wesleyan University, Middletown CT

<http://learningobjects.wesleyan.edu/neurulation/animation.php>

# Normal Development

- Neural tube closure is complete by 27 days
- This is so early, many pregnancies are not recognized at this point!

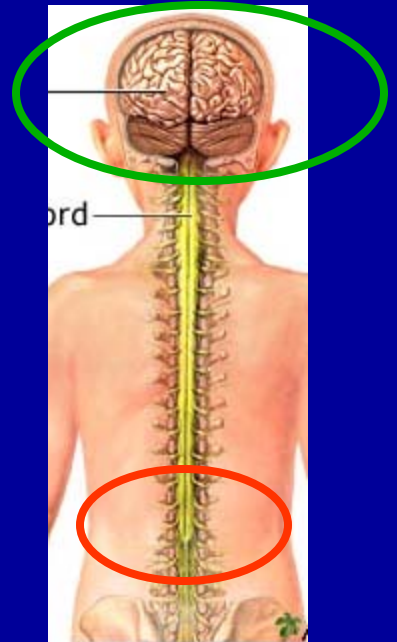


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<http://www.nlm.nih.gov/MEDLINEPLUS/ency/images/ency/fullsize/19588.jpg>

What are neural tube defects  
(NTDs)?

# Neural Tube Defects

- Caused by problems in closing of the neural tube during development
- May affect any part of the brain or spinal cord
- Many different types
  - Spina bifida = most common
  - Anencephaly
  - Many other variants

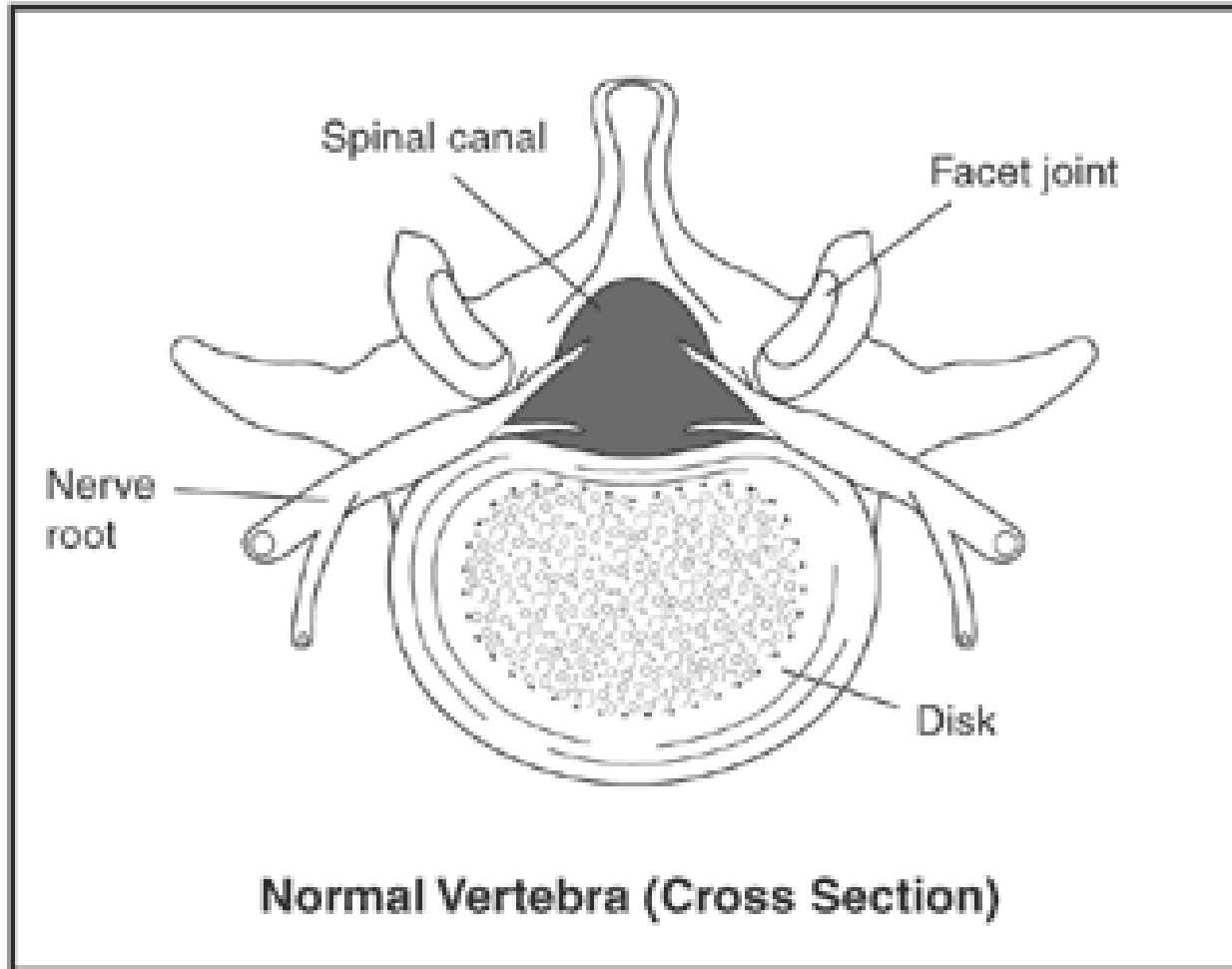


# Spina Bifida

- Also called “open spine”
- Affects vertebrae and spinal cord
- CDC estimate- About 1 in 2,500 US babies affected yearly
- Main types
  - Spina bifida occulta
  - Spina bifida cystica / aperta
    - Meningocele
    - Myelomeningocele

# Normal

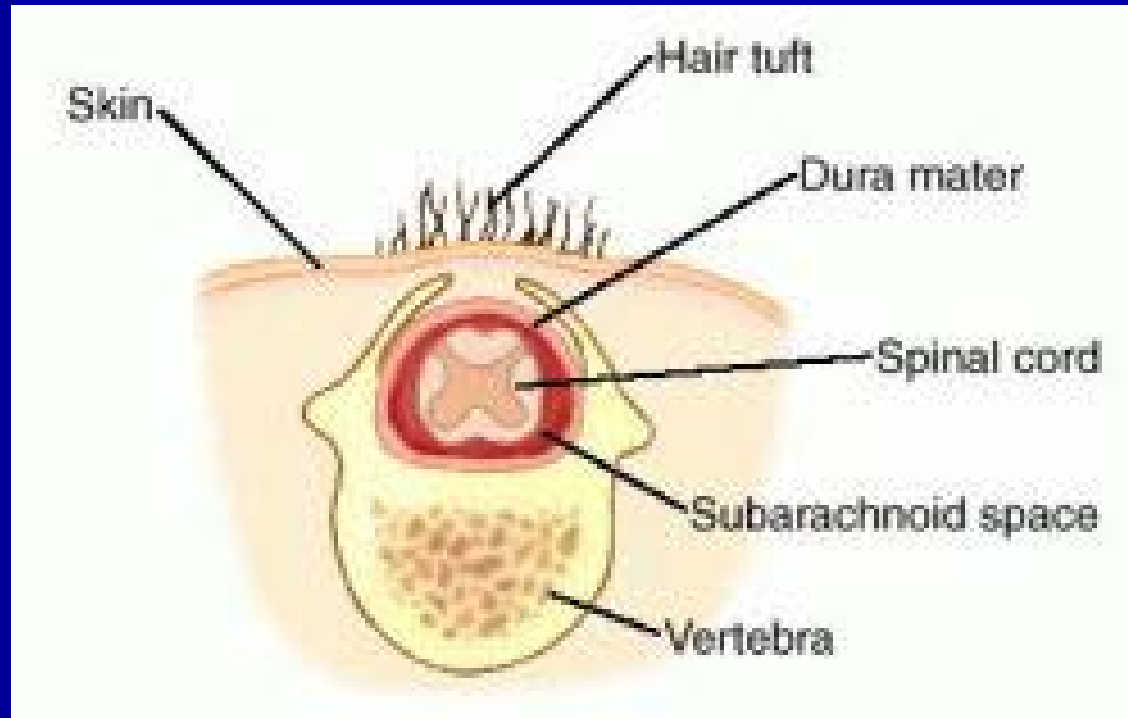
Figure 4



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[http://www.niams.nih.gov/Health\\_Info/Spinal\\_Stenosis/images/Fig4\\_Spinal\\_Sten.gif](http://www.niams.nih.gov/Health_Info/Spinal_Stenosis/images/Fig4_Spinal_Sten.gif)

# Spina bifida occulta



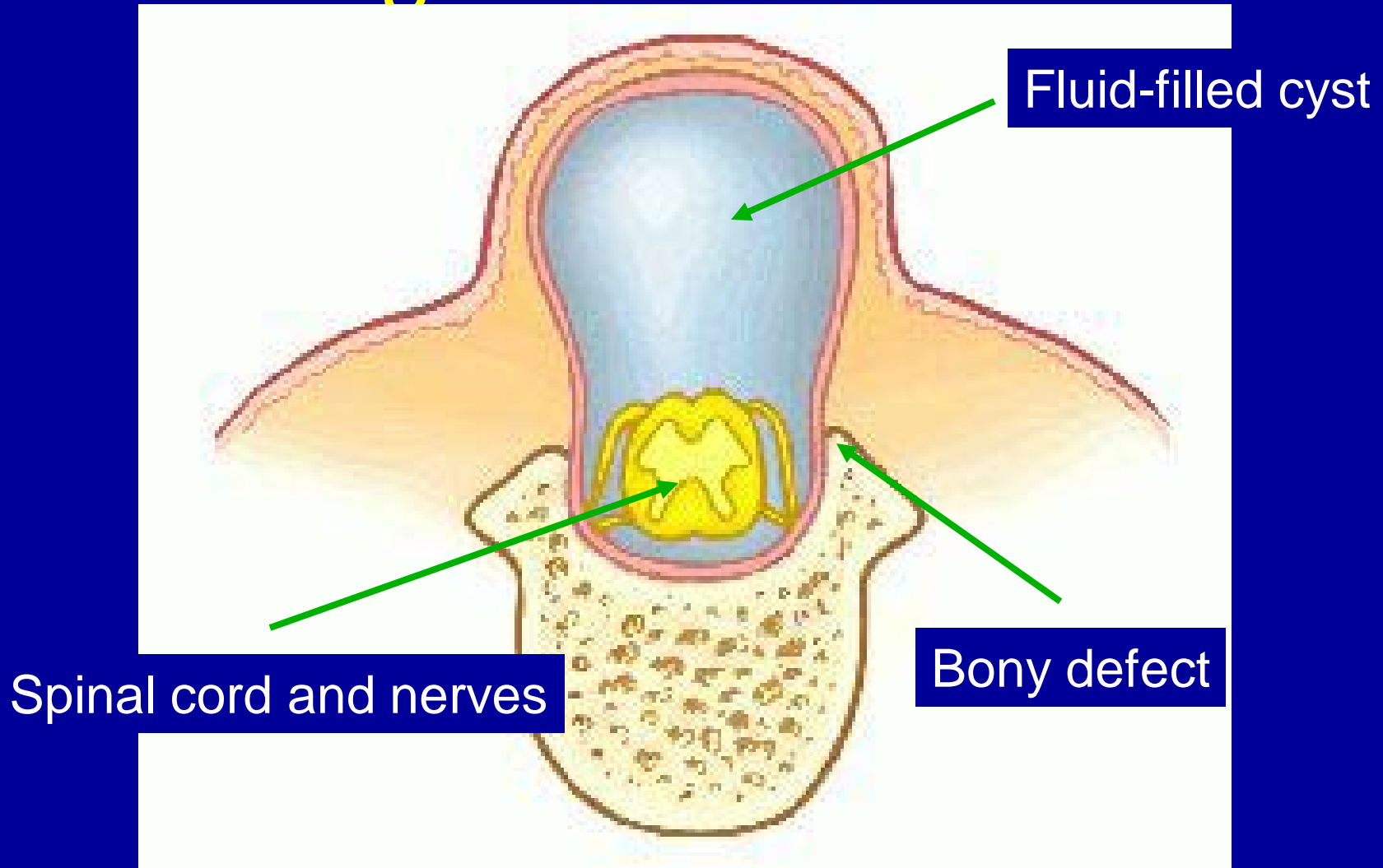
- Cannot be detected by screening
- Patients do not have symptoms
- Does not require treatment



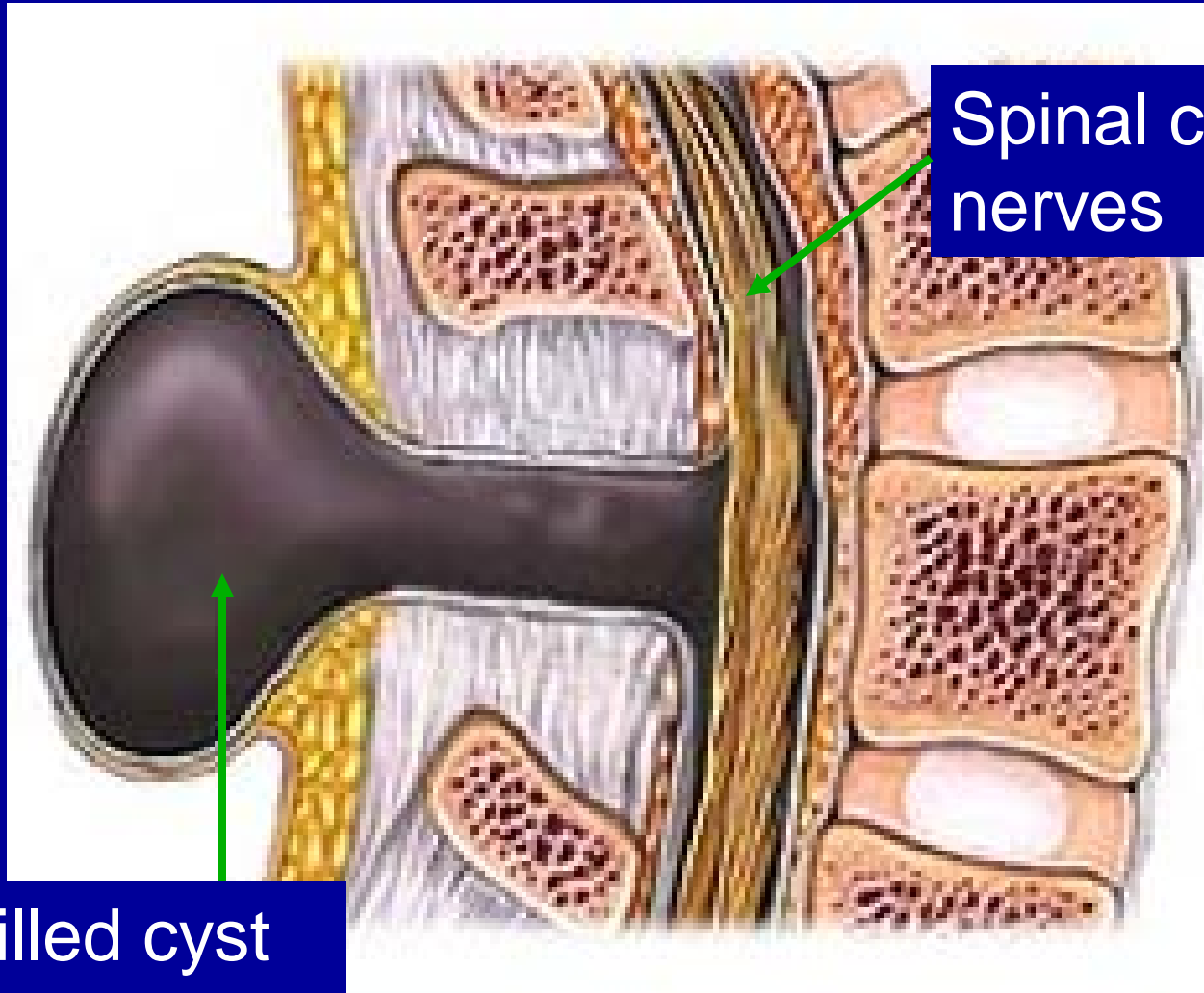
# Spina bifida occulta



# Meningocele – Cross Section



# Meningocele- Side View



Spinal cord and  
nerves

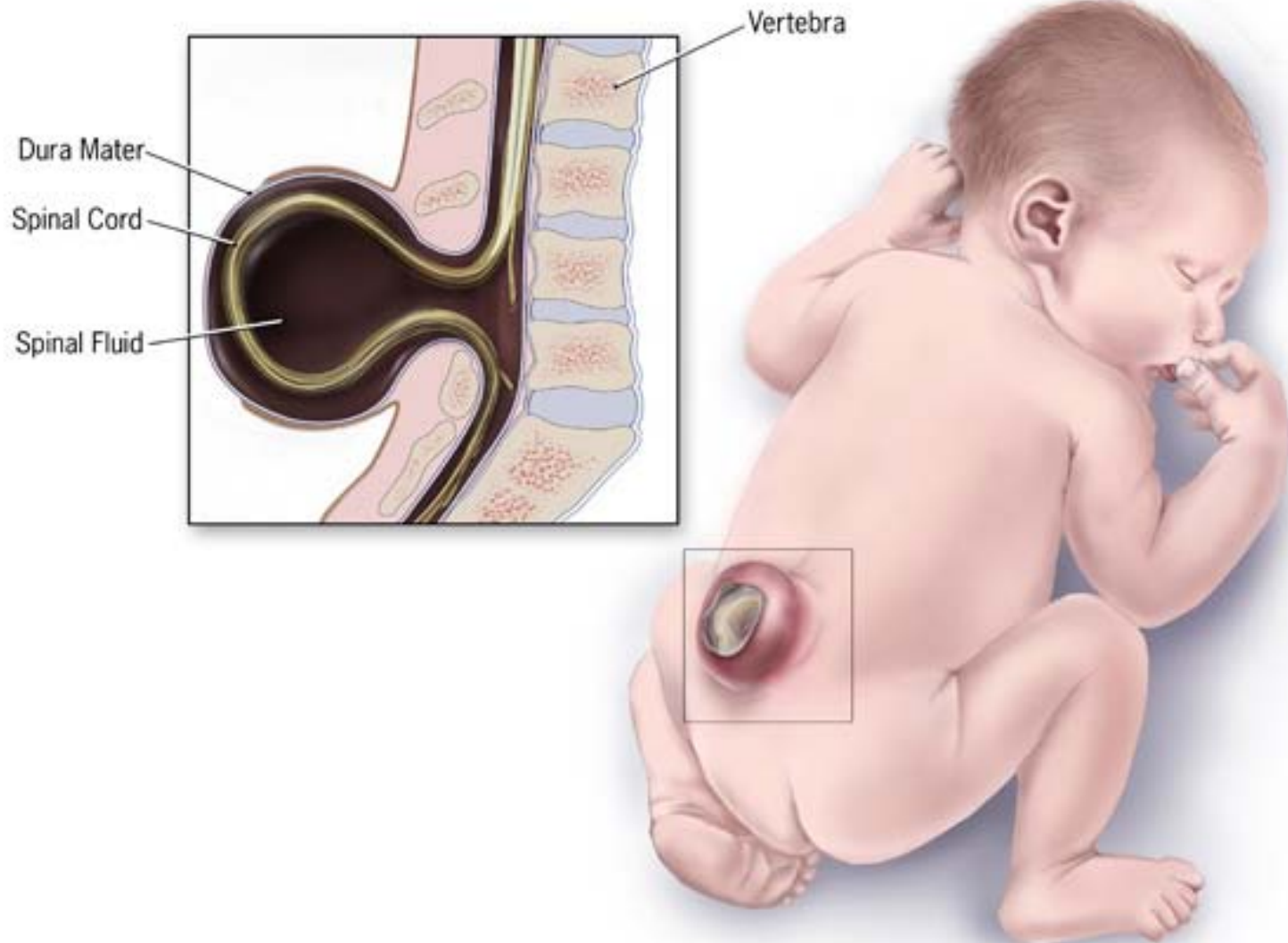
Fluid-filled cyst

# Meningocele

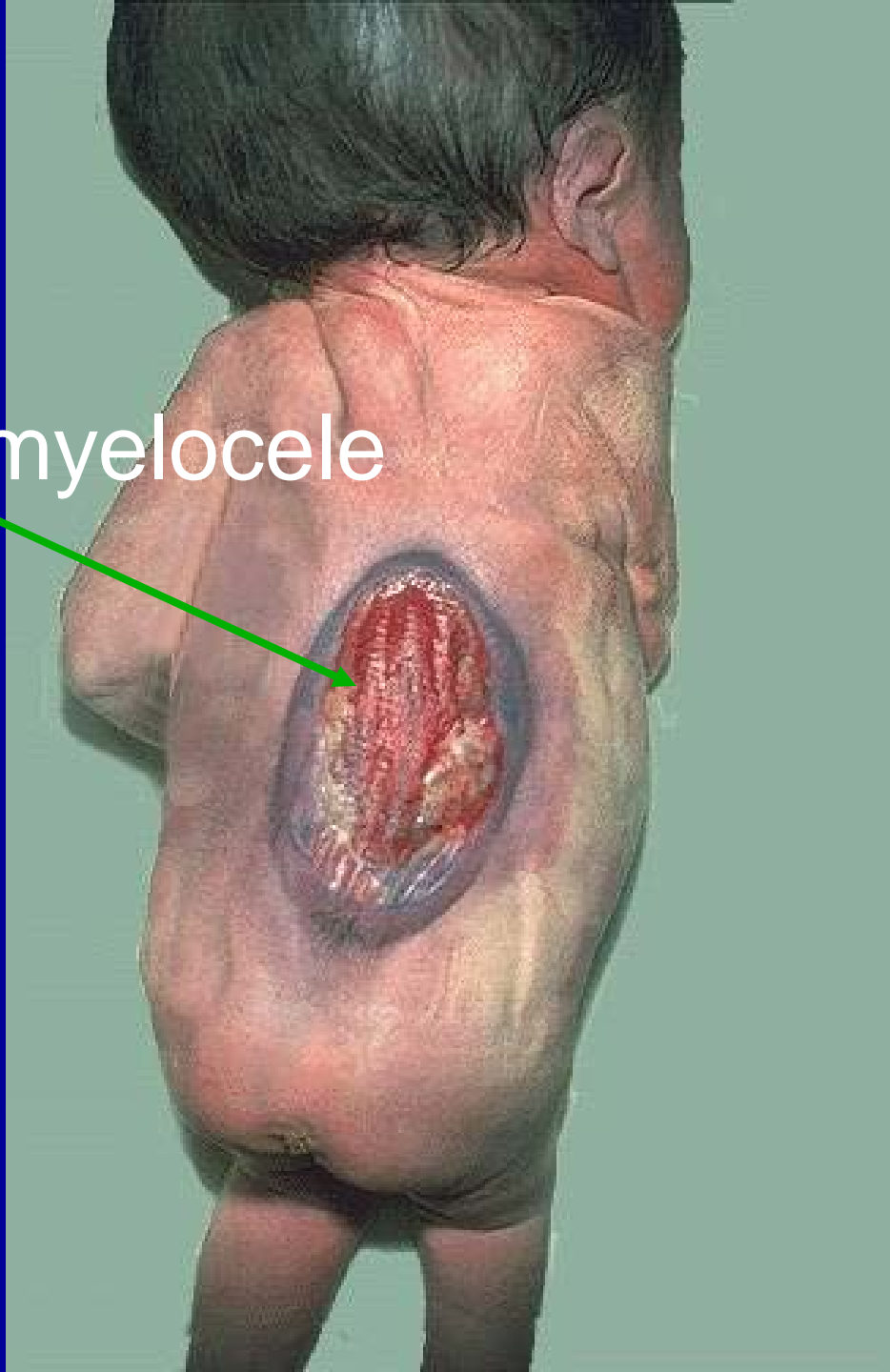
- Cyst must be surgically removed
- Patients usually have normal development

# Meningomyelocele

## Spina Bifida (Open Defect)



# Meningomyelocele



Accessed at:  
<http://library.med.utah.edu/WebPath/jpeg3/PERI094.jpg>

# Meningomyelocele

- Must be closed surgically
- Thin cover = high infection risk if not treated
- Patients often have leg paralysis or bowel/bladder control problems
- Seizure disorders, kidney problems also seen

# Anencephaly

- CDC Estimate- about 1 in 4,000 US babies affected yearly
- Severe problem with brain and skull development
- Top part of head and brain does not form
- Patients are blind, deaf, unconscious
- Patients do not usually survive longer than days



# Anencephaly



# Risk Factors

- NTDs more common in North America and UK
- NTDs less common in Asian countries
- Independent risk factors
  - Prior affected pregnancy
  - Drugs that affect folic acid metabolism
  - Maternal diabetes
  - Maternal obesity

# Risk Factors

- However...
  - About 90% of cases of NTDs occur in children of women with no risk factors!

How can we screen for NTDs?

# Alpha Fetoprotein (AFP)

- Glycoprotein (70,000 Da)
- Made by fetal liver
  - Small contributions from yolk sac and other fetal organs
- Concentrations
  - Highest in fetal serum
  - Moderate in amniotic fluid
  - Lowest (but still detectable) in maternal serum

# Implications for screening

- There are three samples we could use to screen
  - Fetal serum- cordocentesis
  - Amniotic fluid- amniocentesis
  - Maternal serum- maternal blood draw
- The last option is by far the least invasive and least risky, so this is where we start

# AFP Patterns

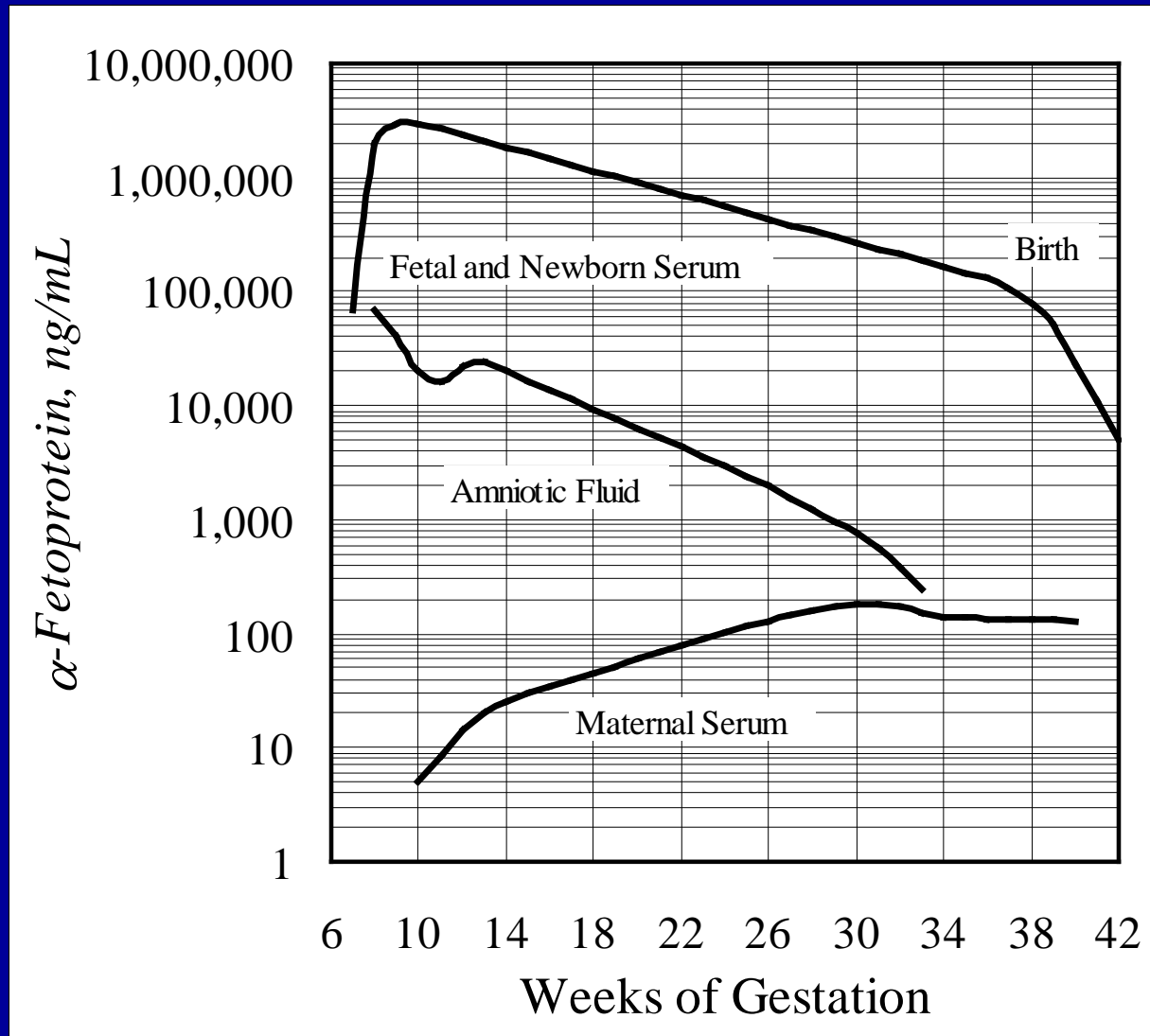


Figure 54-12, Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, 4<sup>th</sup> ed., p. 2182.

# A Brief History of Screening

- 1972- Brock et al.
  - Amniotic fluid AFP ↑
- 1973- Three groups (Brock, Hino, Leek)
  - Maternal serum AFP ↑
- 1977- Wald et al.
  - Population screening with maternal serum AFP



# A Brief History of Screening

- 1980s- maternal serum AFP screening standard of care in US
- Maternal serum screening now also uses additional markers to describe risk of Down syndrome and Trisomy 18

# How we measure risk

- Best time for screening - 16 -18 weeks
- Why?
  - At this time in pregnancy, we can reliably see a difference between NTD and normal groups

# How we measure risk

- Many factors affect maternal serum AFP
  - Number of fetuses ↑
  - Weeks gestation ↑
  - Maternal size ↓
  - Ethnic background ↑ **in African-Americans**
  - Maternal diabetes ↓
  - Open neural tube defects ↑
- We cannot just measure AFP without correcting for variation

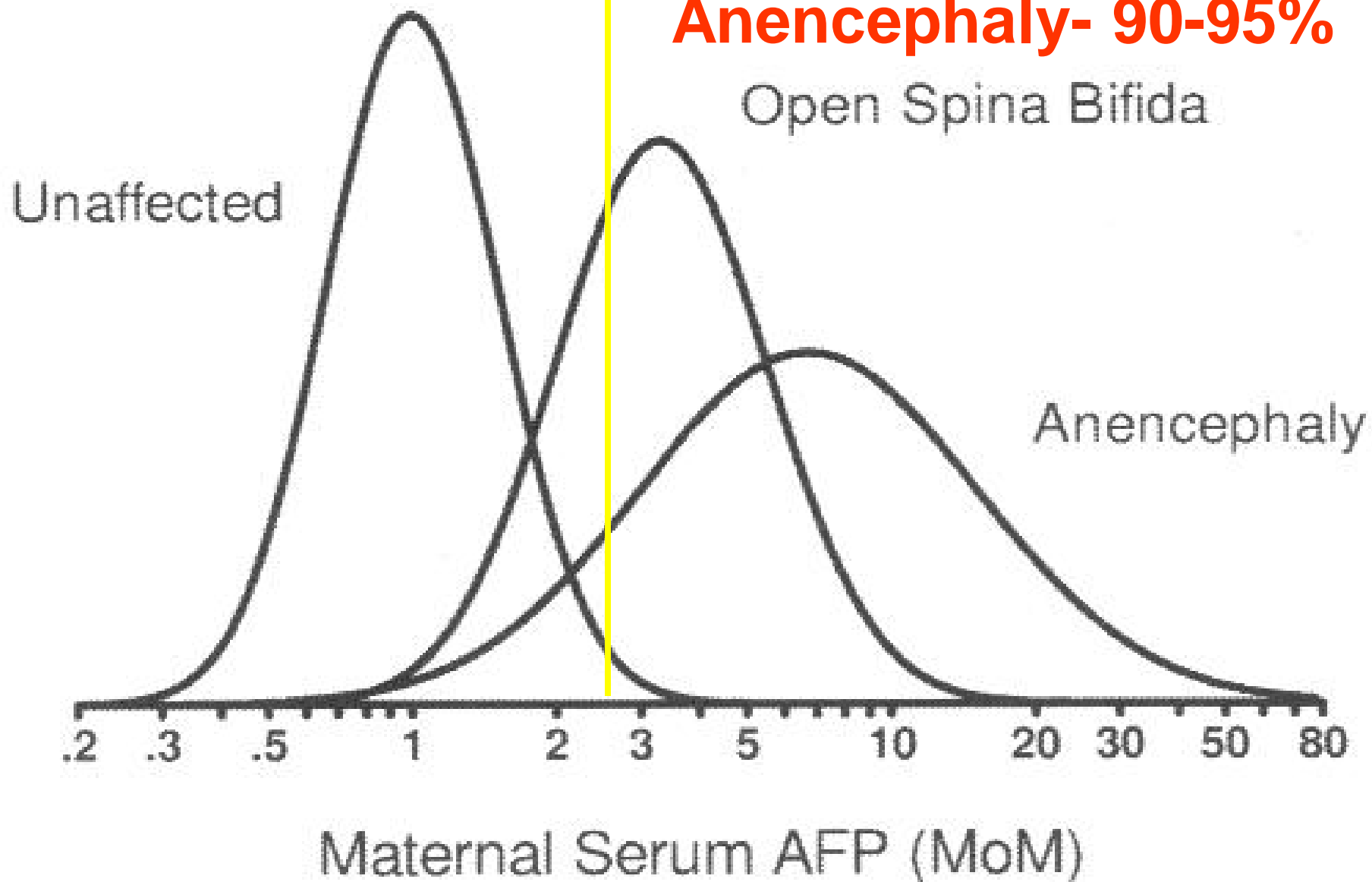
# How we measure risk

- AFP measured with sandwich immunoassay
- To correct, we use Multiples of the Median (MoM)
  - Median values for week of gestation calculated in performing lab
  - MoM calculation
    - Maternal test result / median for gestational age
  - Additional corrective factors are used to account for other sources of AFP variation

**FP- 1.5-2.5%**

**Open SB- 80-85%**

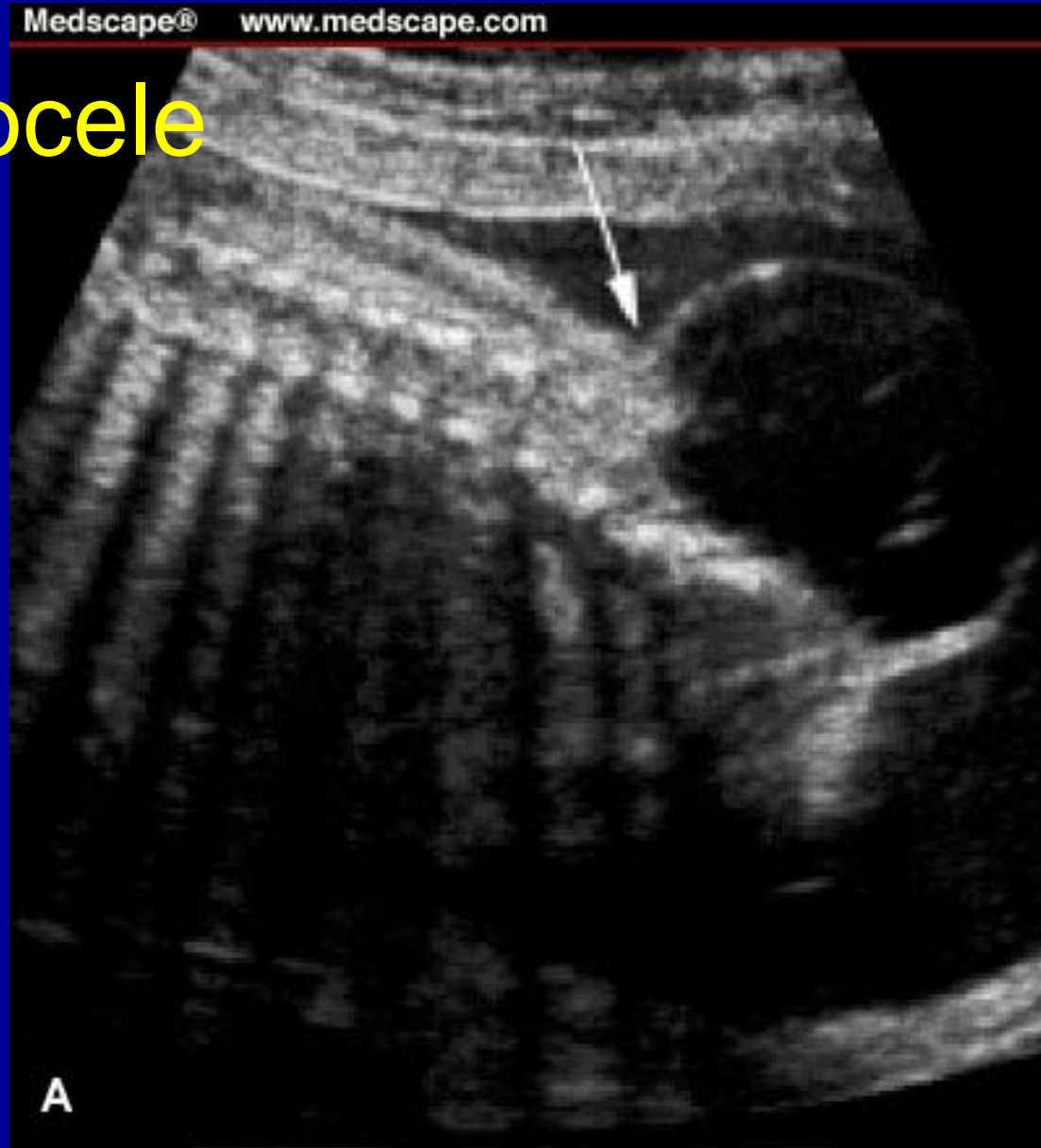
**Anencephaly- 90-95%**



# What happens if the screen is positive?

- Some recommend to re-test AFP (if  $<3.0$  MoM)
- Level II (targeted) ultrasound
  - Confirms gestational age and number of fetuses
  - Specifically looks at fetal head and spinal cord

# Meningocele



Accessed at:  
[medgenmed.medscape.com/viewarticle/406646\\_print](http://medgenmed.medscape.com/viewarticle/406646_print)



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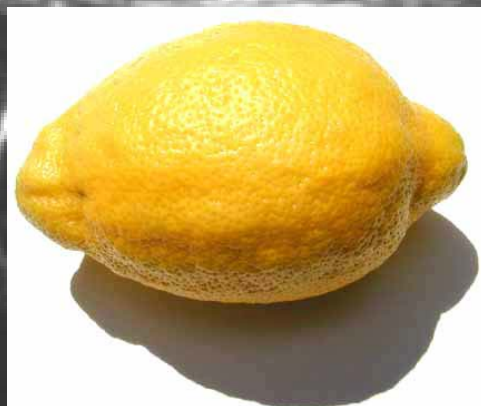
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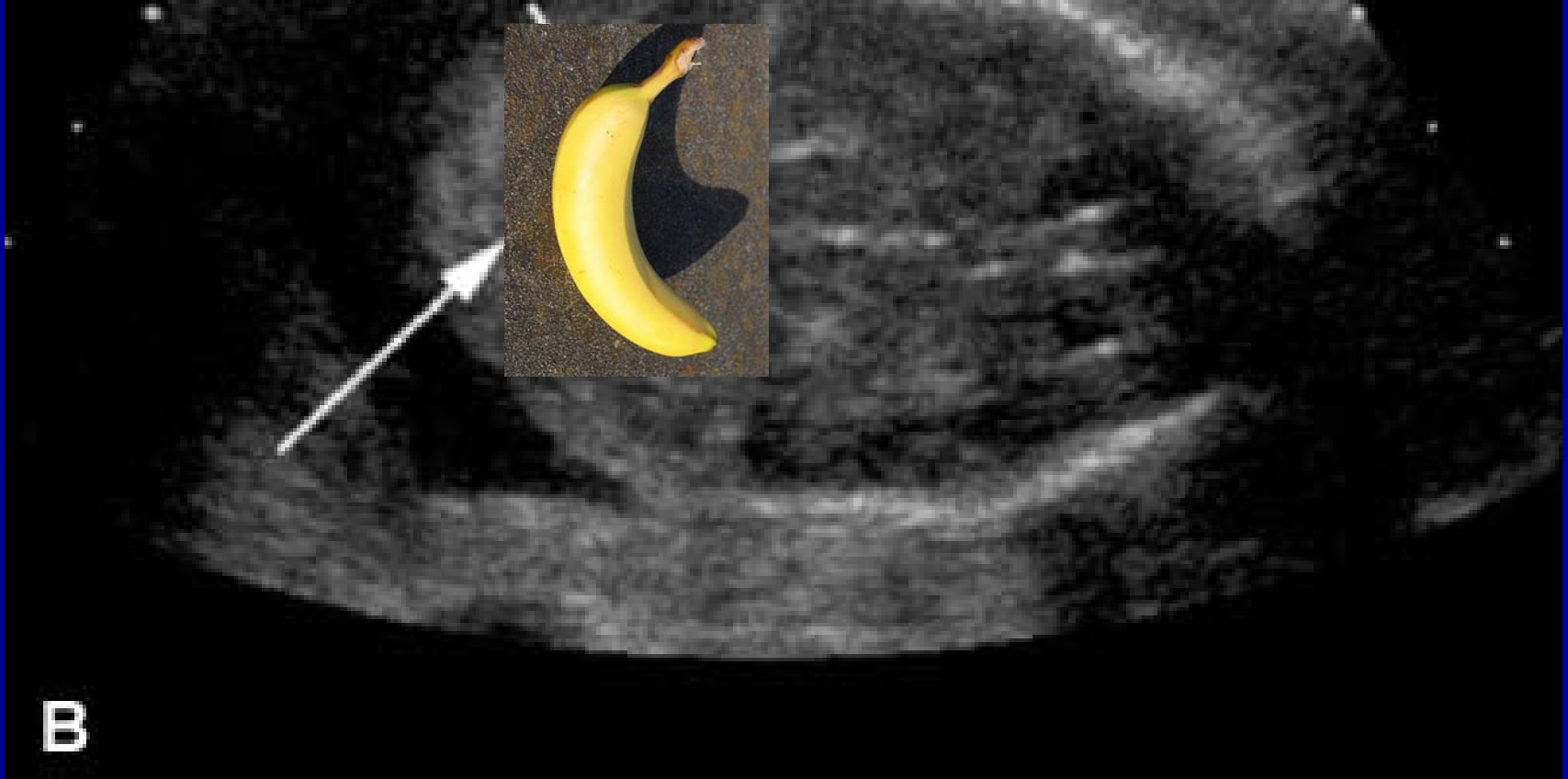
**“Lemon Sign”**

**Lemon Shaped Skull**

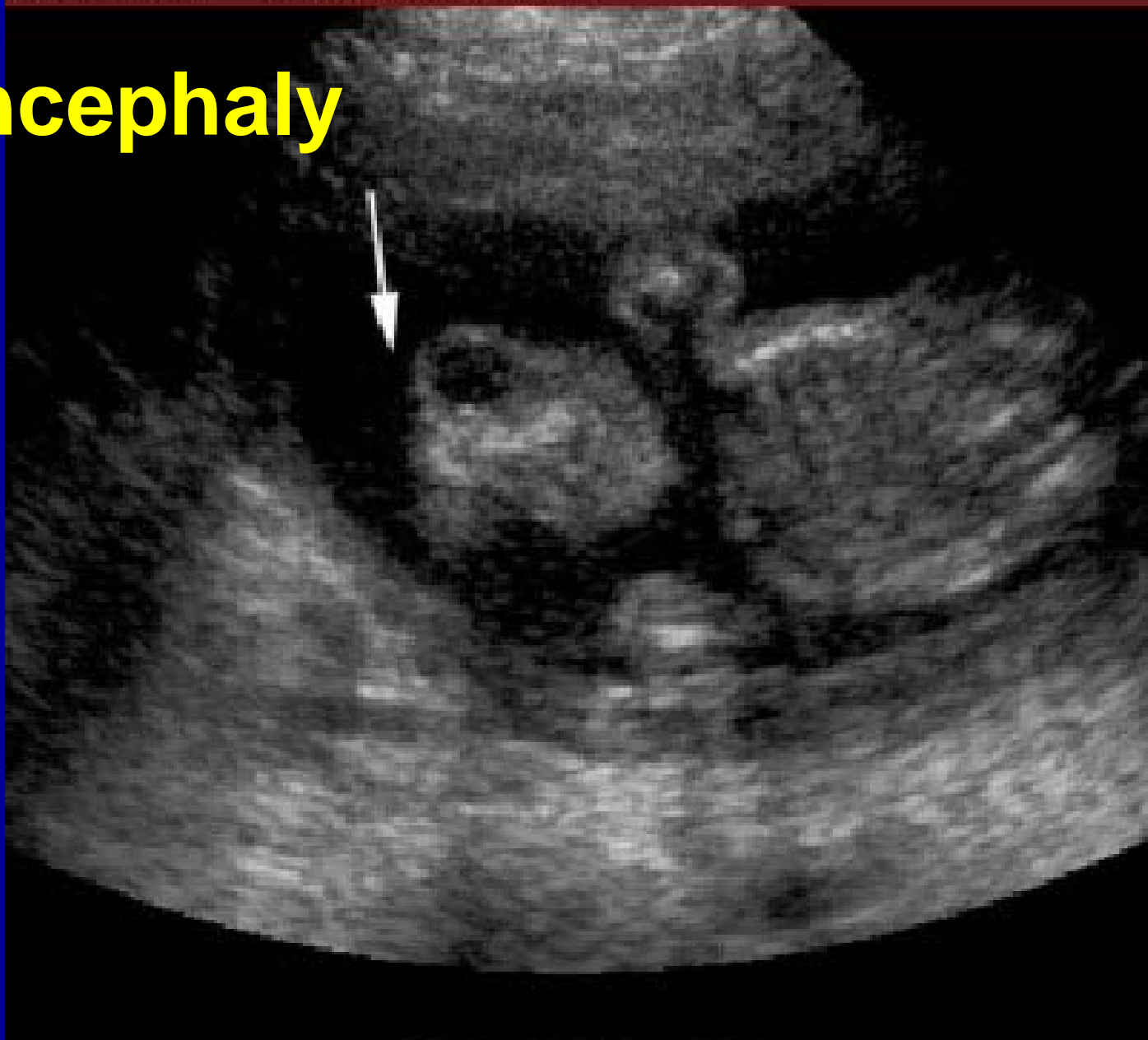




# “Banana Sign”



# Anencephaly



Accessed at:  
[medgenmed.medscape.com/viewarticle/406646](http://medgenmed.medscape.com/viewarticle/406646) print

# What happens if the screen is positive?

- Amniotic fluid AFP
  - May be false positive (up to 2-3%) if there is fetal blood contamination
- Amniotic fluid acetylcholinesterase (AChE)
- Genetic counseling
- Chromosomes (karyotype) may also be useful

How can we prevent NTDs?

# What are folates?

- Definitions
  - Folate and folic acid- general terms for a family of compounds
  - Foods naturally high in folates include
    - Leafy greens
    - Legumes
    - Liver

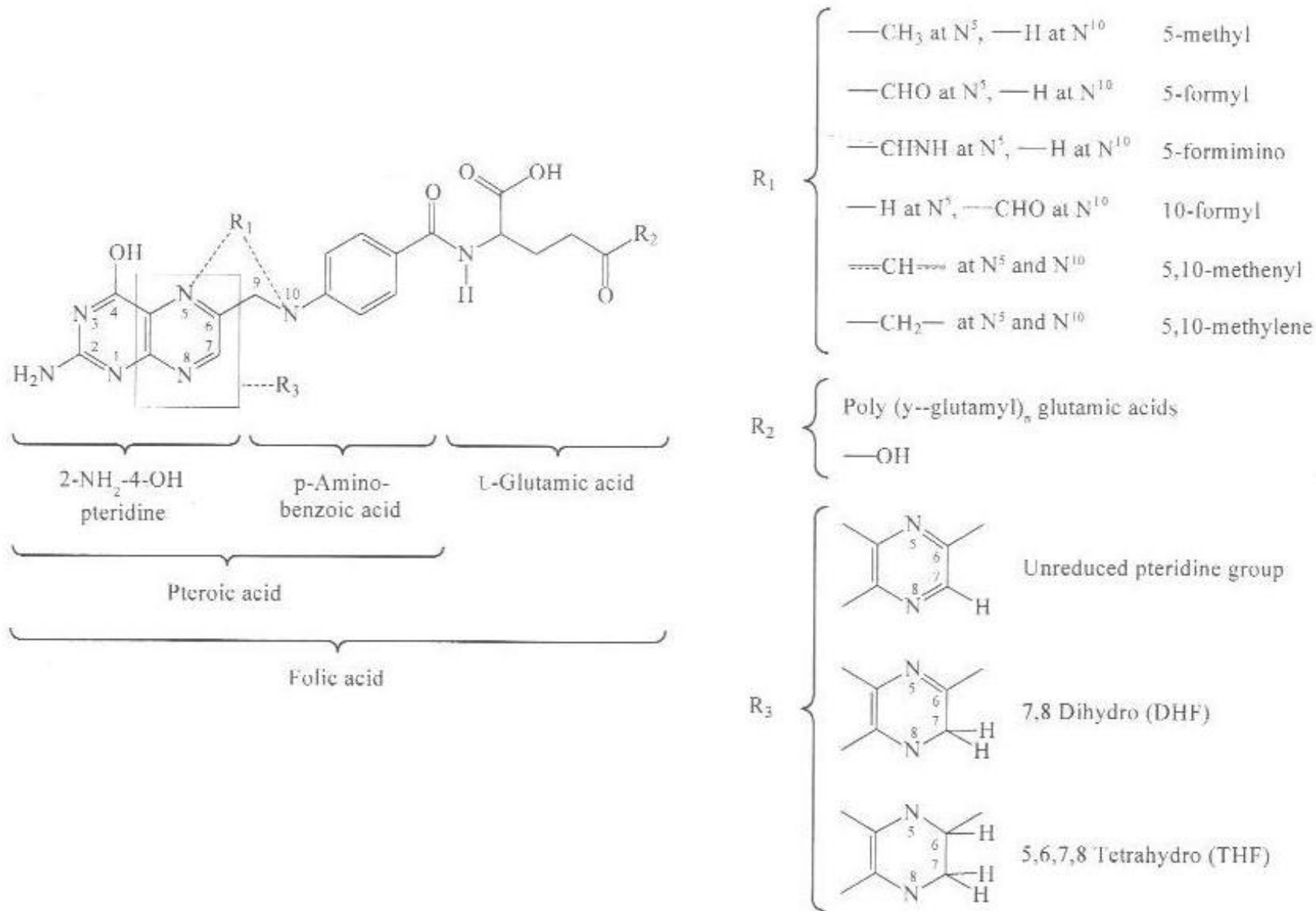
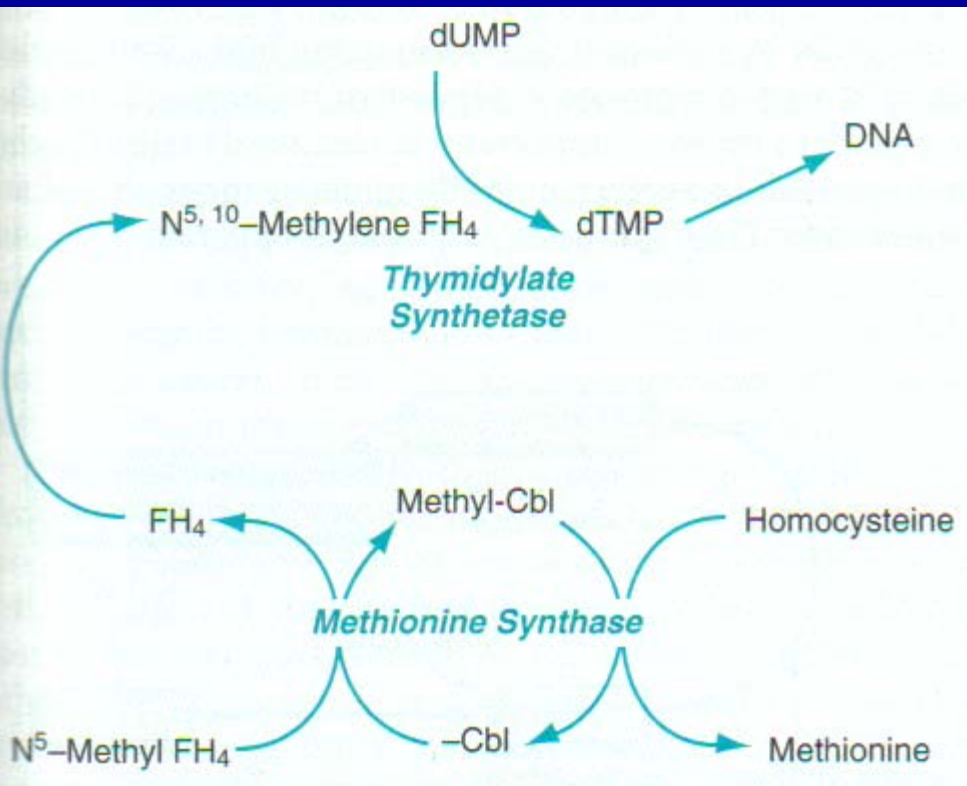


Figure 30-20, Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, 4<sup>th</sup> ed., p.1110

# What are folates?



- Folates important for
  - Amino acids
    - Methionine synthesis
    - Histidine metabolism
    - Serine-glycine metabolism
  - DNA
    - Thymidylate synthesis
    - Purine synthesis

Figure 13-21, Robbins and Cotran  
Pathologic Basis of Disease, 7<sup>th</sup> ed. p. 641

# What is the role of folates?

- We are not entirely sure
  - There is definitely an association between folic acid supplementation and decreased rates of NTDs
  - The exact biochemical mechanism for this is unclear



# Definitions

- Fortification
  - Addition of folic acid to food products during manufacturing
- Supplementation
  - Addition of folic acid to diet by taking vitamin pills

# History

- 1960s – possibility of folic acid to prevent NTDs suggested
- 1980s – small studies of folic acid supplementation in pregnant women appeared to decrease NTD risk

# History

- MRC Vitamin Study – 1991
  - International, multicenter trial
  - 1817 high risk women (previous child with NTD)
  - 4 treatment groups
    - Folic acid only
    - Other vitamins only (A, D, B1, B2, B6, C, nicotinamide)
    - Folic acid + other vitamins (both)
    - Placebo (neither)

# History

- MRC Vitamin Study Conclusions
  - Decrease in NTDs in two groups treated with folic acid
  - 72% of NTDs were prevented with treatment
  - Recommended supplementation for high risk women
  - Normal risk women thought to benefit from supplements as well

# History

- 1992 - US Public Health Service recommended that all women of childbearing age ingest 400  $\mu\text{g}$  folic acid daily
- January 1, 1998 – US FDA mandated grain fortification
  - 140  $\mu\text{g}$  folic acid / 100 g grain

# Current Recommendations

- Women of childbearing age - 400 µg/day
- High risk women - 4000 µg/day
- Use of supplements does not mean we do not need to screen for NTDs

Complete list at  
<http://www.cdc.gov/ncbddd/folicacid/cereals.html>

# Has fortification made a difference?

- Prefortification (1995-1996)
  - Spina bifida - 2,490
  - Anencephaly – 1,640
  - Total – 4,130
- Postfortification (1999-2000)
  - Spina bifida – 1,640
  - Anencephaly – 1,380
  - Total – 3,020
- Approximately 27% decline in NTDs

CDC. Spina bifida and anencephaly before and after folic acid mandate- United States, 1995-1996 and 1999-2000. MMWR 2004; 53:362-5.



# Has fortification made a difference?

- Boulet et al., 2008
  - Prevalence of neural tube defects per 10,000 births in US
  - Three time periods
    - 1999-2000
    - 2001-2002
    - 2003-2004

# Has fortification made a difference?

	1999-2000	2003-2004
Prevalence	5.98	5.37
Spina bifida	3.51	3.39
Anencephaly	2.47	1.98
Hispanic	7.48	6.91

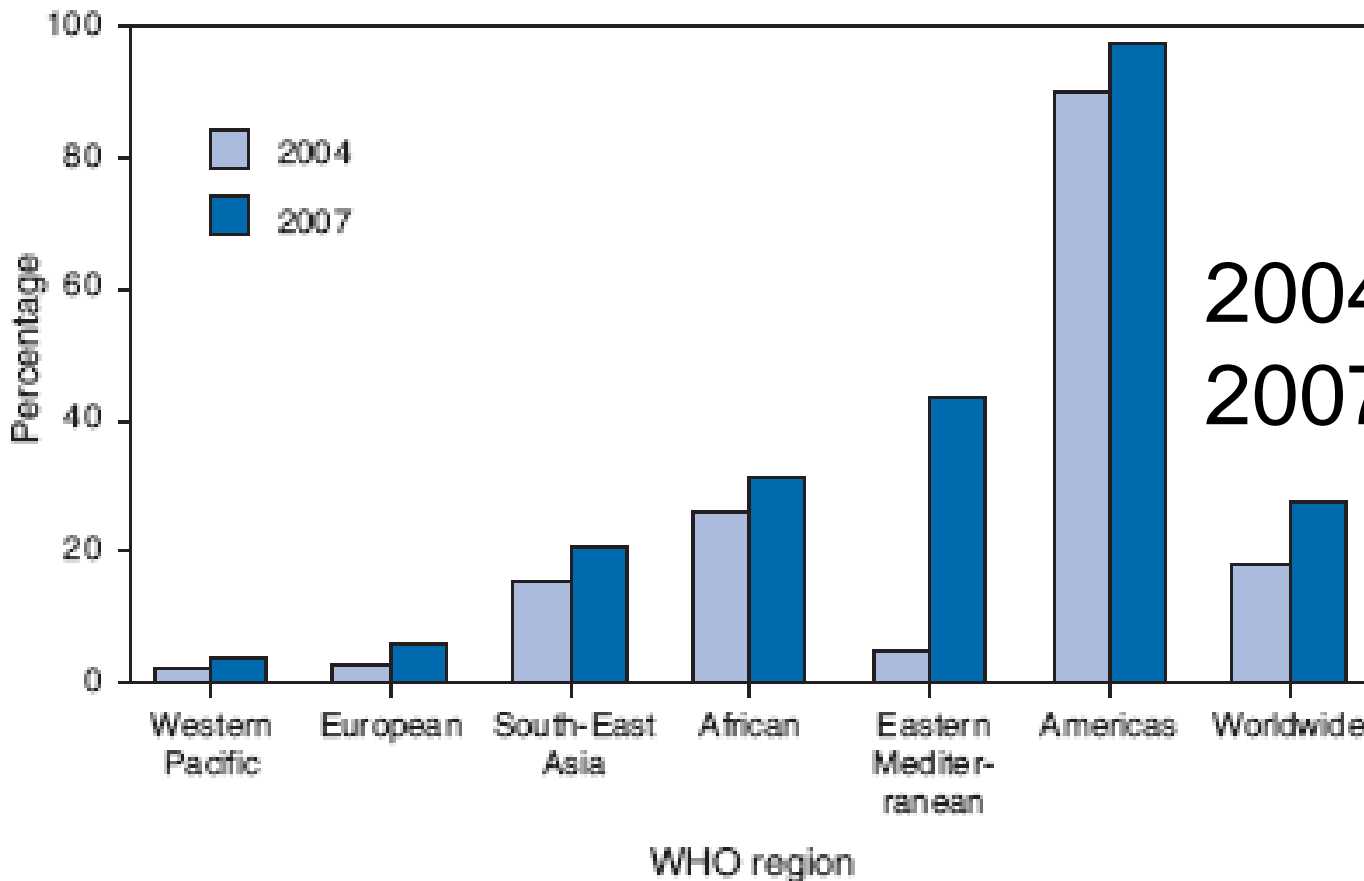
Boulet S et al. Trends in the postfortification prevalence of spina bifida and anencephaly in the United States. Birth Defects Res A Clin Molec Teratol. 2008;82:527-32.

# Has fortification made a difference?

- DeWals et al. *NEJM* 2007.
  - Review of all live births and stillbirths in 7 Canadian provinces
  - Total number of births = 1,909,741
  - 1993-2002 (Canada mandated folic acid fortification on November 11, 1998)
  - Found 46% decrease in NTD prevalence after fortification

# Are we fortifying enough?

**FIGURE. Percentage of wheat flour processed in roller mills that was fortified — worldwide and by World Health Organization (WHO) region, 2004 and 2007**



2004- 18%  
2007- 24%

CDC. Trends in wheat-flour fortification with folic acid and iron – worldwide, 2004 and 2007. MMWR. 57(1); 8-10.

# Are we fortifying enough?

- Changes from 2004-2007 in fortification
  - Number of countries increased from 33 to 54
  - 540 million additional persons had access to fortified flour in 2007

# Are women using supplements?

- CDC national study, 2007
  - Surveyed 2,003 women 18-45 years
  - 40% of all women using folic acid supplement
  - Non-Caucasian women had lower rates of supplement use

# Are women using supplements?

- Women 18-24 years (1/3 of all births in US, most unplanned pregnancies)
  - Aware of need for folic acid use – 61%
  - Know when to take folic acid – 6%
  - Use daily folic acid supplement – 30%
  - Lowest awareness, knowledge and use of all age subgroups in survey

CDC. Use of supplements containing folic acid among women of childbearing age – United States, 2007. MMWR 2008 57(1):5-8.

# Inequalities in folic acid use

- Bentley et al., 2006
  - Consumption of folate by age and race
  - 15-44 year old women consuming  $\geq 400$   $\mu\text{g}/\text{d}$ 
    - Whites - 39%
    - Blacks - 26%
    - Hispanics – 28%



# Inequalities in NTD prevalence

- Grewal J et al., 2008
  - Frequency of NTD in low socio-economic status individuals and neighborhoods
    - About 2-fold increased risk in mothers with less than high school education
- Increased prevalence in Hispanics, as mentioned previously

# Is flour fortification cost effective?

- Yes!
  - Annual cost to fortify approximately **\$3 million**
  - Estimated annual economic benefit in US **\$425 million**
  - Bottom line- although there are costs to fortify, they are less than the costs to care for patients with NTDs

What are some areas for  
future research?

# Future Directions

- Is fortification sufficient to prevent folate-related NTDs?
- Do we need to add a higher level of folate to the food supply?
- What role, if any, does Vitamin B12 play?

# Disclaimer

- The following represents some interesting new research
  - Not yet definitive!
- To all women of childbearing age in the audience:
  - Please keep taking your folic acid supplements!

# Do women still need supplements?

- Mosley et al., 2009
  - No decrease in NTDs in patients using folic acid supplement compared with non-users
  - Reported use of supplements similar between NTD affected and unaffected pregnancies
  - Have we prevented all folate-related NTDs with fortification?

# Arguments for more folic acid in flour

- Some claim that the reduction we have seen in NTDs since fortification is not as great as theoretically possible reduction
- Would increasing fortification to 350  $\mu\text{g}/100\text{g}$  grain prevent more NTDs?
- Would it be harmful to ingest a higher dose of folic acid?

# Vitamin B12

- Molloy et al., 2009
  - Low vitamin B12 level is an independent risk factor for NTD
  - Also seems to have additive effect with folate
    - Women with low B12 and folate had higher risk of NTDs than low level of either alone
  - More work remains
    - Is B12 fortification safe?
    - What dose is needed to offer adequate risk reduction?



# Conclusions

- NTDs result from failure of the neural tube to close during development
- Maternal serum AFP + follow-up testing allows us to screen for NTDs
- Folic acid fortification and supplementation can reduce the number of NTDs
- Further work remains to discover how folic acid prevents NTDs, best dose of folic acid, role of B12, and how to eliminate inequalities



## Folic Acid

### Folic Acid Homepage

- [Facts](#)
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### What Should You Know?

If a woman has enough folic acid in her body ***before and during pregnancy***, it can help prevent major birth defects of the baby's brain and spine. **Women need 400 micrograms (mcg) of folic acid every day.**

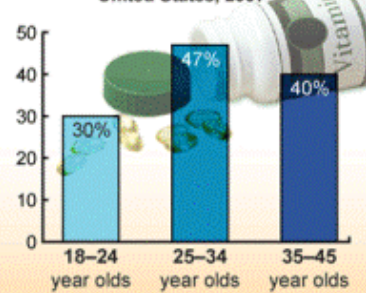
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Use of Supplements Containing Folic Acid  
Among Women of Childbearing Age—  
United States, 2007

[More Data and Statistics »](#)

### Popular Links



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How much do you know about folic acid?



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#### Pregnancy Information

Learn how to be healthy before, during, and after pregnancy.



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- [National Center on Birth Defects and Developmental Disabilities](#)
- [Division of Birth Defects and Developmental](#)

# Acknowledgements

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