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New Ways in Anti-Aging with Folic Acid

Cosmetic benefits and formulation guidelines

HEALTH · NUTRITION · MATERIALS

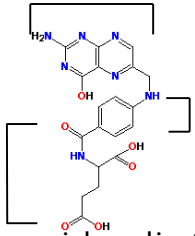
1. Folic acid – the substance
2. First market products
3. Benefits for the skin
4. Formulations guidelines

What is folic acid? - structure and properties

Name and structure

- **Folic acid or Folate** (the salt) are also known as Vitamin B9, Vitamin M, folacin
- Comprise a group of substances that differ in number of glutamic acid units
- Natural occurence (polyglutamic acid forms): vegetables, green leaves

Pteridine



L-Glutamic acid

- **Folic acid**
  - synthetic form used for commercial application
  - Pteroylglutamic acid, the most stable monoglutamate form

Properties

- Yellow crystalline powder
- Practically odorless
- Very slightly soluble in water
- Insoluble in alcohols,
- As a powder it is sensitive to heat and light (especially to UV)

- Involved in many biochemical and physiological processes,
- Long history of food application and foodstuff enrichment but **almost untapped in Personal Care until……**

First Cosmetic Products

- Beiersdorf has succesfully launched DNAge range with folic acid as a key ingredient.
- Claims: anti-ageing (DNA repair, increased cell turnover)

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Folic Acid Efficacy

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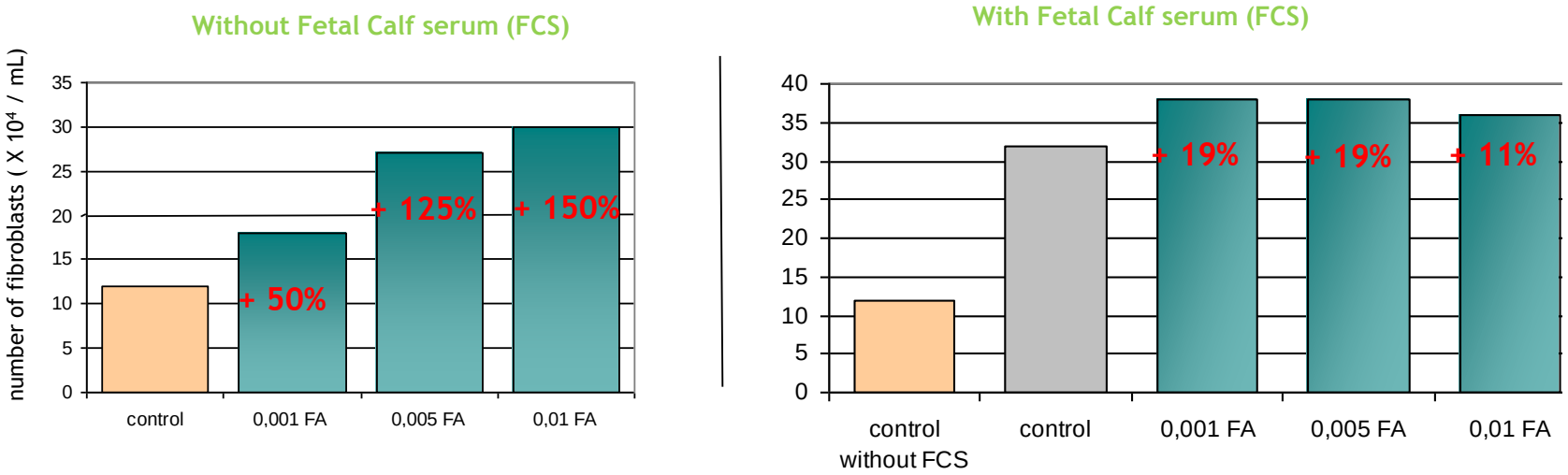
Folic Acid to improve fibroblast turnover

Mature human fibroblasts cultured for 7 days with different Folic Acid (FA) concentrations



Folic Acid stimulates division of fibroblasts growing either in standard medium or under nutrient deficiency

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Fibroblast cytoskeleton under confocal microscopy

Fibroblasts cultivated 14 days without Folic Acid (Control)

- **Fibroblasts:** broad cells, attached to each other, irregular shape. Mitotic divisions sporadically observed.

→ Indicates stationnary phase cells

Fibroblasts cultivated 14 days with 0.01% Folic Acid

- **Fibroblasts:** regular and spindle-shaped cells with small cytoplasma volume

→ Indicates high cell division rate

Folic Acid rejuvenates fibroblasts

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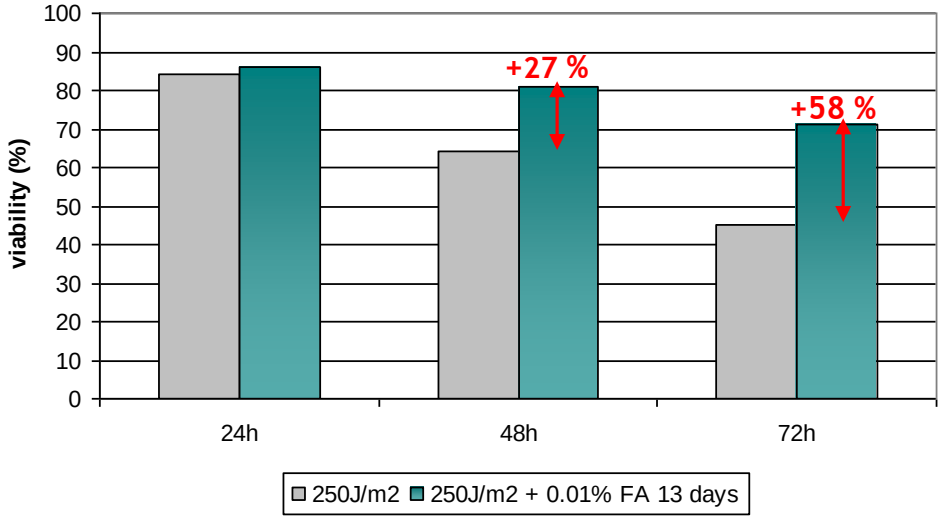
Folic Acid – to protect skin cells from UV damage

Viability of primary human fibroblasts after UV-B radiation



Folic Acid increases viability of fibroblasts after UV radiation

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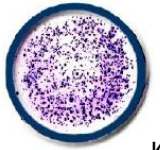
Influence of Folic Acid on UV-induced apoptosis of keratinocytes

Visual evidence of keratinocytes‘ protection by Folic Acid

Untreated cells

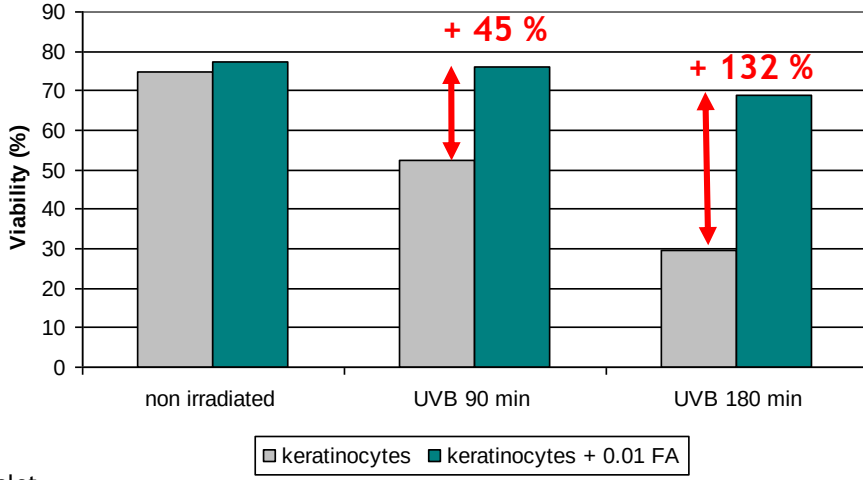
UVB 250J/m²



UVB+0.1% FA

Keratinocyte colonies stained with crystal violet

Viability of keratinocytes incubated (16h) with/ without folic acid after UVB (1W/m²) irradiation



Folic Acid protects keratinocytes from UV-induced apoptosis

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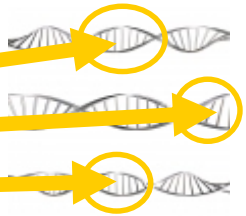
Folic Acid – Repair of damaged DNA

- Background level of damaged DNA is elevated under Folic Folic acid deficiency. Folic Acid is critical for the synthesis of S-adenosylmethionine and prevention of aberrant DNA methylation. Kim Y, 1997
- Folic Acid deficiency causes wrong incorporation of uracil into human DNA and chromosome breaks. Blount BC et al, 1997
- Folic Acid treatment is suggested to increase the repair rate of UV-damaged DNA by the NER (Nuclear Excision Repair) mechanism.

Folic Acid plays an essential role in DNA synthesis and repair

Repair of DNA damage by NER mechanism

1. DNA damage (chemical, UV)



If no repair mechanism →

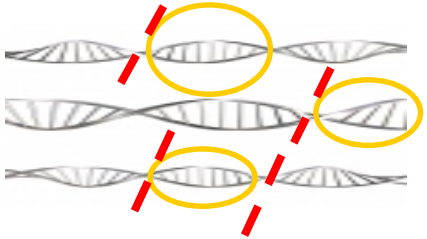
No excision

Long DNA chains

Limited number of breaks

Repair mechanism ↓

2. Excision of damaged bases



3. Increase of small DNA breaks

The high number of DNA breaks (DNA damage) is indicative of an ongoing NER repair mechanism (Graph)

4. Replacement of damaged parts Ligation – rejoining of strand breaks → **Repaired DNA**

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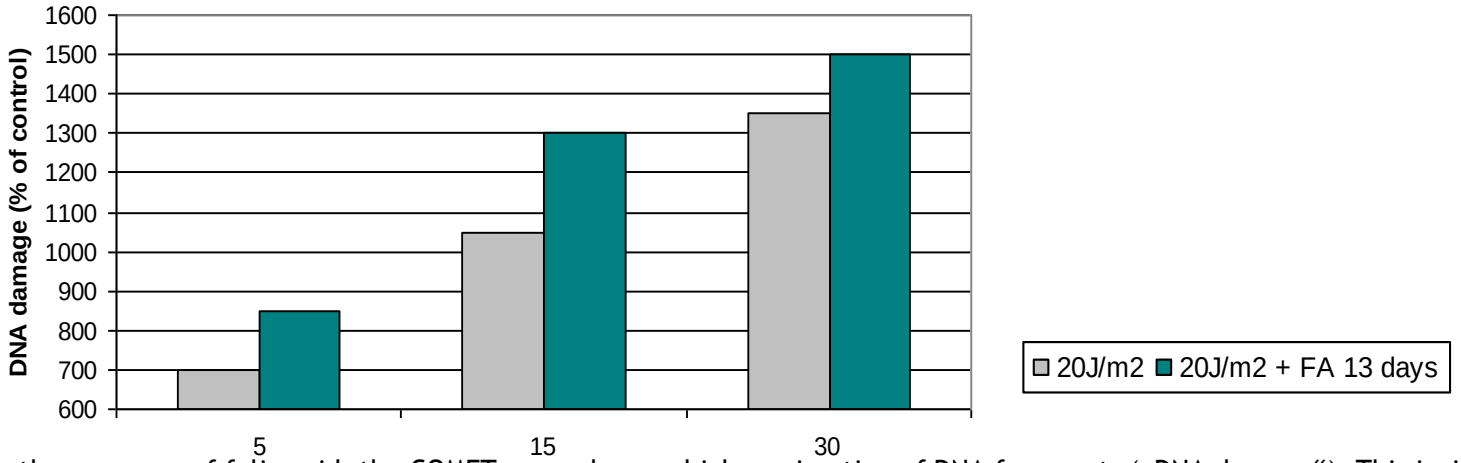
Folic Acid to improve repair of damaged DNA



In the presence of folic acid, the COMET assay shows a higher migration of DNA fragments („DNA damage“). This is indicative of the NER (Nuclear Ecxision Repair) process promoted by Folic Acid.

Repair of damaged DNA increased by Folic Acid

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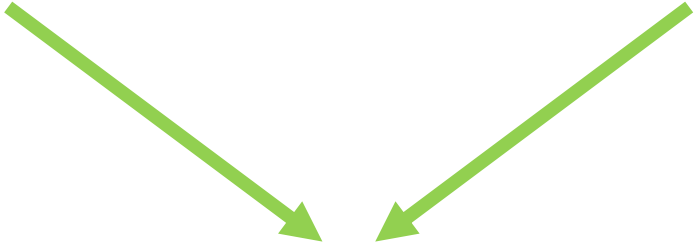
Folic Acid – New Ways in Anti-Aging

- Intrinsic aging
  - Partly due to reduced turnover in skin cells
- Photo-aging
  - UV light triggers cutaneous photo-aging by a pathway that starts in dermal fibroblasts with alterations of (mitochondrial) DNA*.



Folic acid can slow-down premature skin-aging

- by stimulation of skin cells such as fibroblasts
- while repairing DNA damages at the same time

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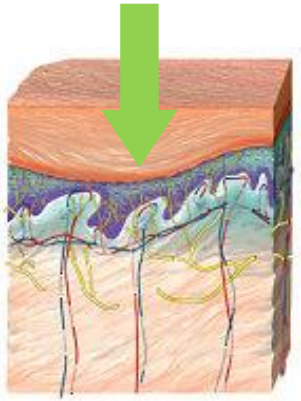
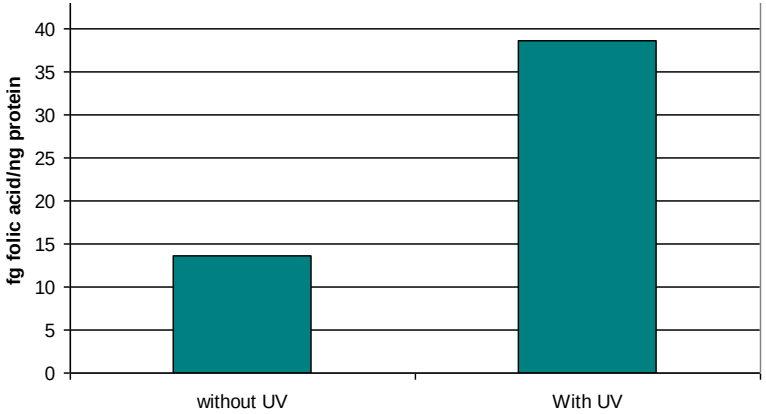
Folic Acid – Availibility in the skin

- Topical application: Folic Acid penetrates the skin
- Cell culture: Increased Folic Acid uptake by fibroblasts under UV-light irradiation.





Folic Acid is available in dermal cells

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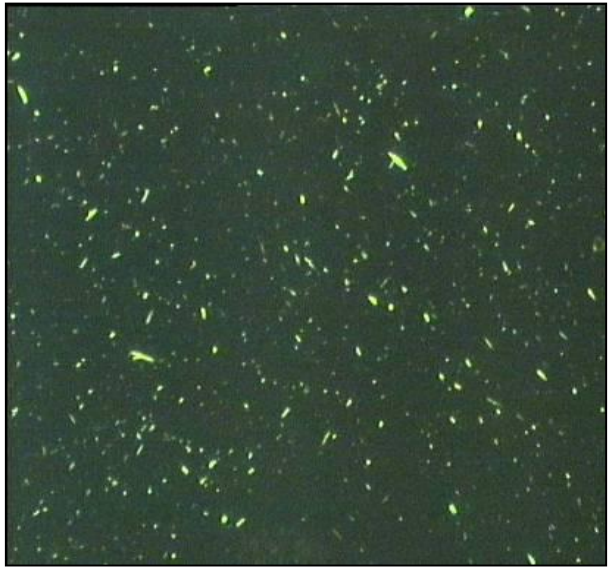
Formulating with Folic Acid



Solubility in water

0.2% Folic Acid stirred into water

How to overcome these obstacles?

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Solubility of Folic Acid in water at different pH

pH adjustment with: Solubility of folic acid in %

	pH 6	pH 7	pH 8
KOH	0.06	>2.0	>2.0
NaOH	0.08	>2.0	>2.0
Triethanolamin	0.1	>2.0	>2.0

- Folic Acid, an organic acid, can be neutralized with different, commonly used bases. The salt has a significantly higher water solubility
- After neutralization the solution is clear

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Folic acid - Formulation Examples

- O/W emulsions with 0.1 and 0.5% Folic Acid
  - With AMPHISOL®K
  - With Steareth-2/ Steareth- 20

- Carbopol Gel with 0.1 and 0.5% Folic Acid

Amphisol® K emulsion (O/W)

0.1% Folic Acid was pre-neutralized before addition to the emulsion

Microscopic view

Day 0, RT

After 3 months, RT

⇒ No differences of the microscopic view at day 0 and after 3 months

⇒ no crystals in the emulsions after 3 months at 5°C, RT and 43°C

⇒ No influence on the pH and viscosity

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Steareth-2 / Steareth-20 emulsion (O/W)

After 2 months

Analytical results after 3 months from storage sample

Content of Folic Acid		
	RT	43°C
Start	0.09	/
After 12 weeks	0.10	0.0855

- No decrease of Folic Acid content
- Slight discoloration observed for the 43°C sample

Carbopol Gel

- Pre-neutralization of Folic Acid with potassium hydroxide
- Folic Acid was then added with the water phase to a 0.5% Carbopol gel
- Viscosity: 0.1% Folic Acid no influence, 0.5% reduced viscosity

Recommendation: max 0.2% Folic Acid in gels

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Influence of Folic Acid content on color of emulsion

Steareth-2 / Steareth-20 emulsion

- Color intensifies with increasing Folic Acid concentrations
- With 0.1% only little color impact in all tested formulations
- Minor discoloration at 43°C with 0.1% but rather intensive with 0.5%

Recommended use concentration: 0.05- 0.2%

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Formulation Guidelines

- It is recommended to neutralize Folic Acid :
  - Pre-neutraliFolic Acid can also be easily added to existing emulsions as a powder. Then adjust the pH of the emulsion to 6.5 or higher and homogenize the emulsion intensively
  - ze Folic Acid in a separate water phase to increase its solubility
- pH value of formulation has to be 6.5 or higher to get stable formulations
- Add Folic Acid below 40°C
- Add antioxidants agents to the formulation
- Avoid exposure of formulations to direct sun light. Use opaque packaging.

Folic acid – high freedom to operate

Patent situation

Folic Acid – state of the art

- Skin cream and dodorant containing folic acid (Koehler – 1974)
- Folic acid against seborrhea and for skin tanning preparation (Koehler – 1974)

Combinations with additional ingredients

- A few patent documents claiming combinations with other actives for specific applications need to be checked but are not considered as a hurdle

→ High degree of freedom for applications with Folic Acid

Regulatory situation

- Folic acid can be used in all countries for any kind of cosmetic products
- There are no local particular restrictions known

→ Allows development of global products

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Folic Acid – new ways in anti-aging

- Folic Acid stimulates the proliferation of fibroblasts
- Folic Acid modulates and increases DNA repair* of damages after UV exposure.
- Topically apllied incorporated in cosmetic products Folic Acid helps to ensure sufficient supply

Folic acid can slow-down premature skin-ageing

- by stimulation of skin cells
- by prevention of DNA damage at the same time

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Interested?
Just order your
Folic Acid sample now

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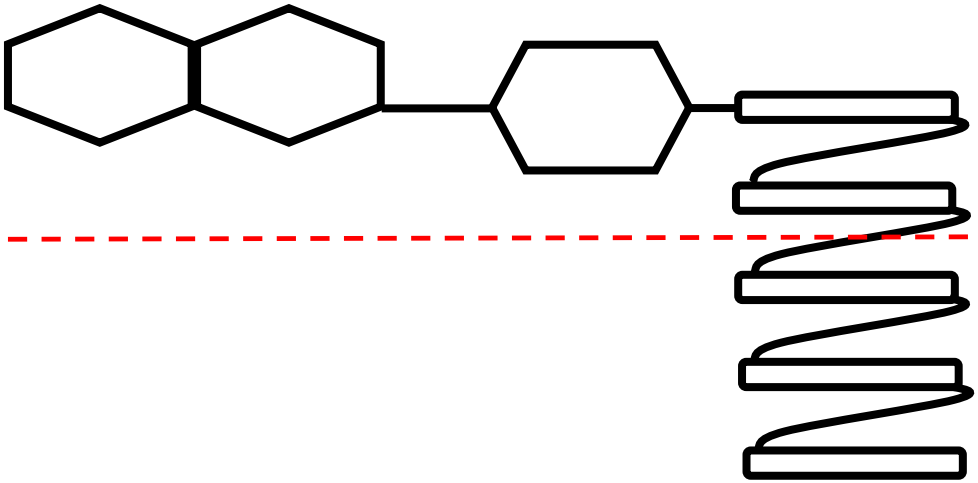
BACK UP SLIDES

When folate is taken up by food folate (1/2)

- Folate is the natural (complex) form found in foods such as dark-green leafy vegetables, broccoli, asparagus, lentils, beans, peanuts, strawberries, kiwi, orange juice, liver.
- The structural difference between folic acid and food folate accounts for differences in bioavailability, with folic acid being more readily absorbed.

- Folate is absorbed as monoglutamate (free folate = Folic Acid)
- Efficacy of folate absorption is estimated at 50%.
- It requires the action of an enzyme to break it down to the folic acid form for absorption (in the proximal jejunum via active and passive transport)



Folic acid
“monoglutamate”

Food folates“polyglutamate”

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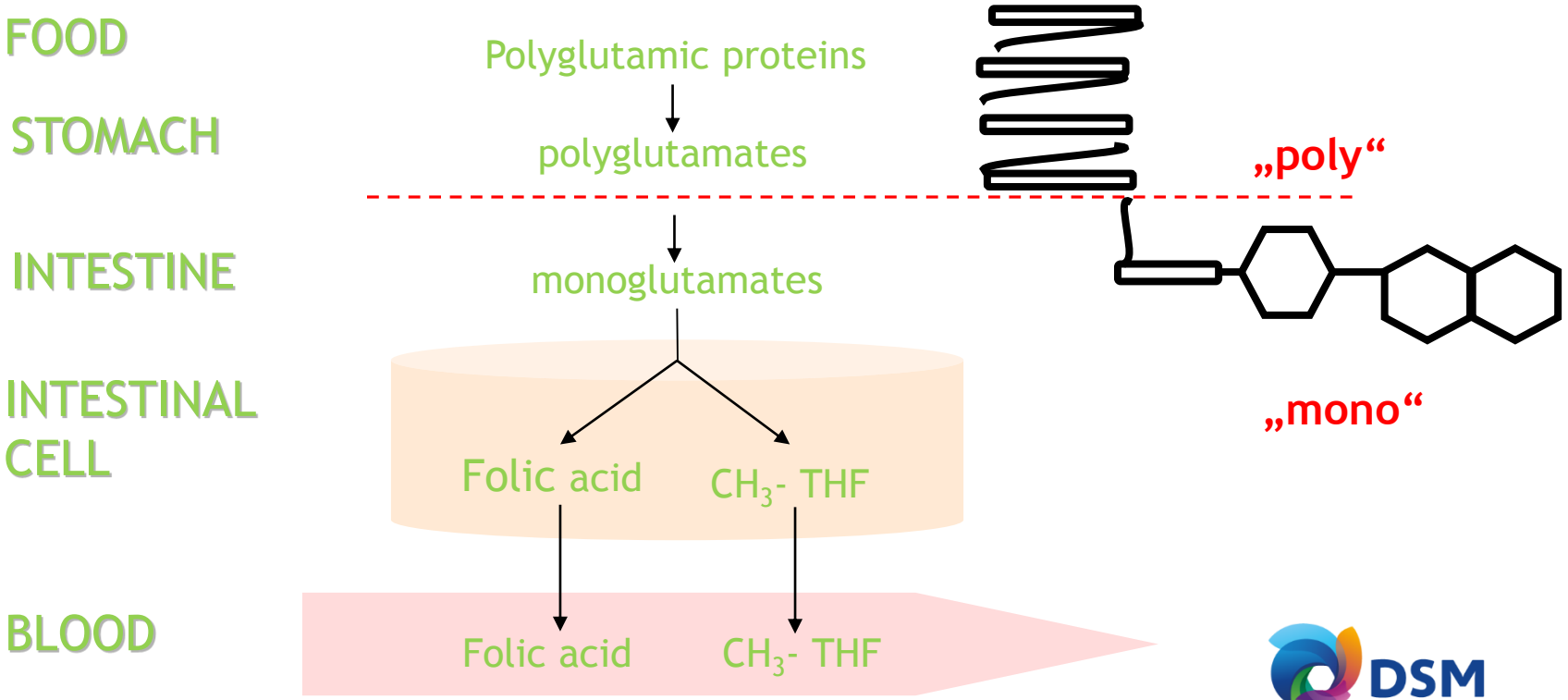
When folate is taken up by food folate (2/2)



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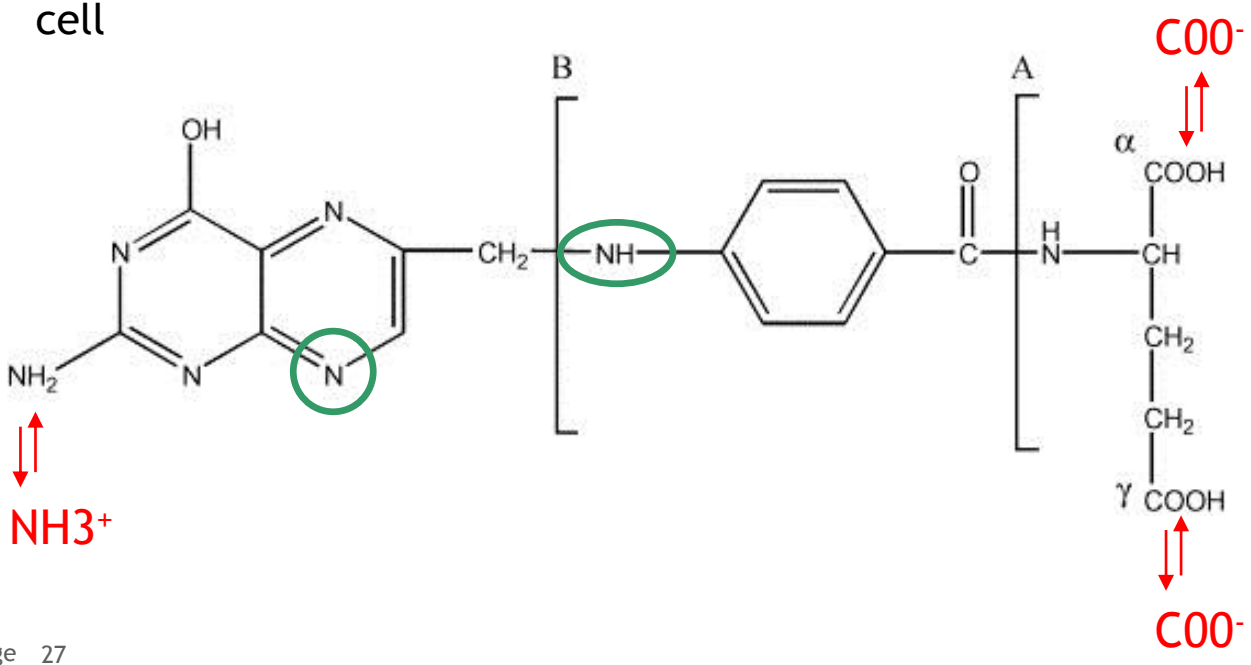
Folic Acid – physico chemical properties

- pH varations involves acido-basic reactions (red)
- The active biological sites (green) are engaged in reduction-oxidation reactions in the cell



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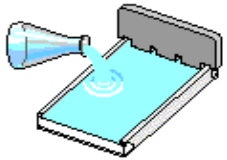
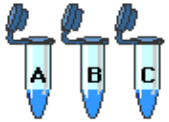
Electrophoresis (1/2)

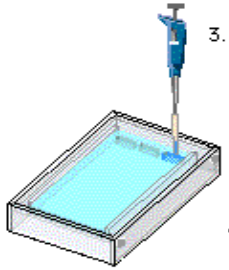
1. Make gel.



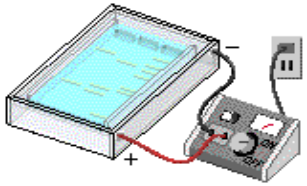
2. Obtain prepared DNA samples.



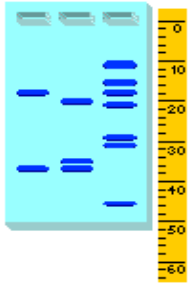
3. Load samples into gel.



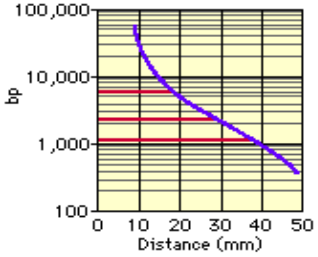
4. Separate fragments by electrophoresis.



5. Stain DNA fragments and measure distances.



6. Prepare a standard curve. Determine fragment sizes.



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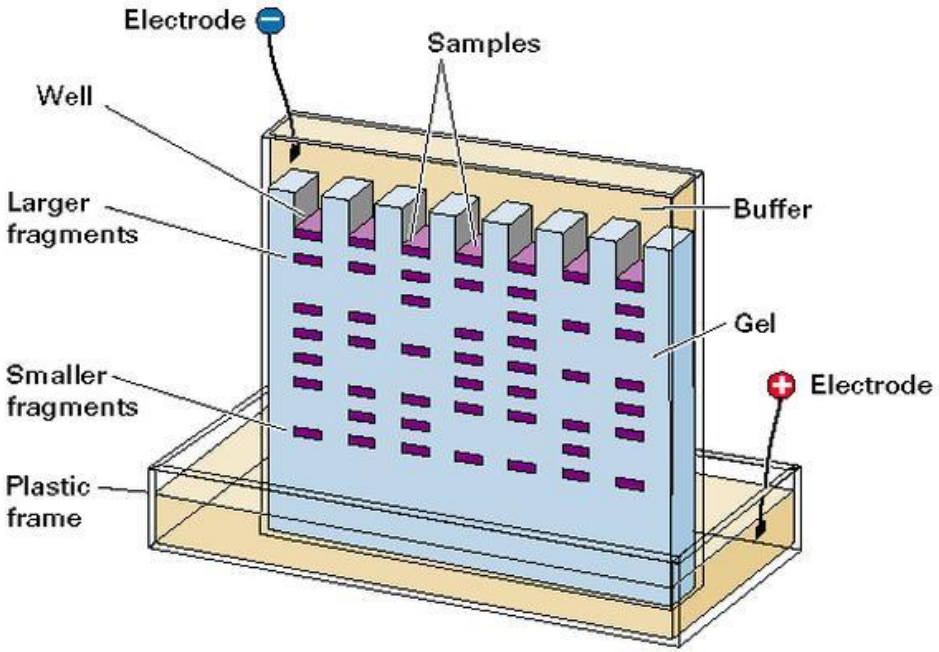
Electrophoresis (2/2)



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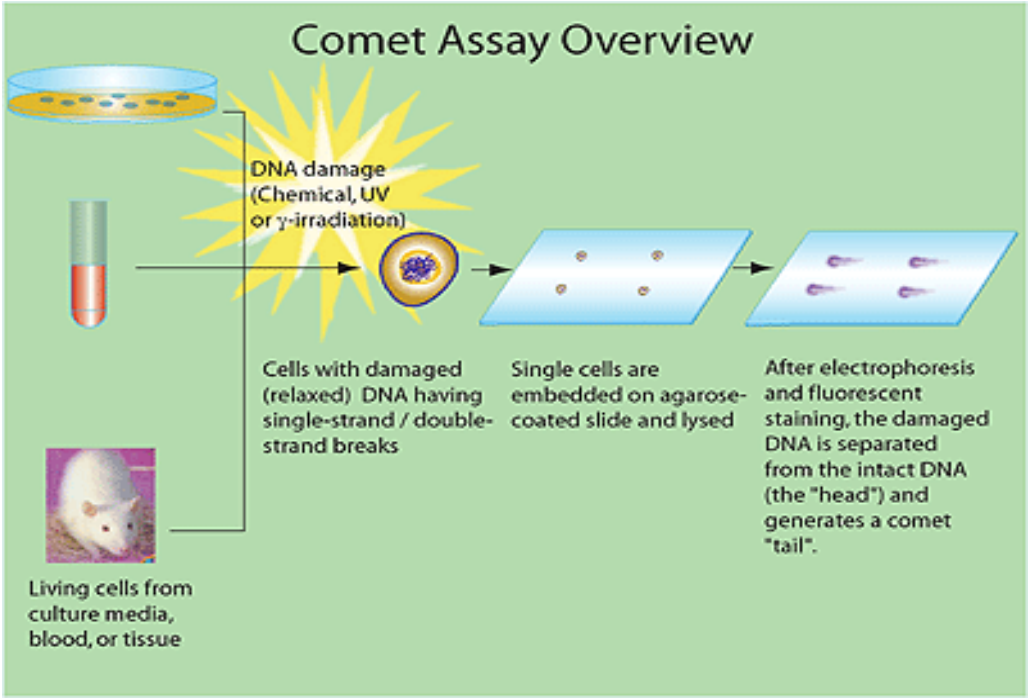
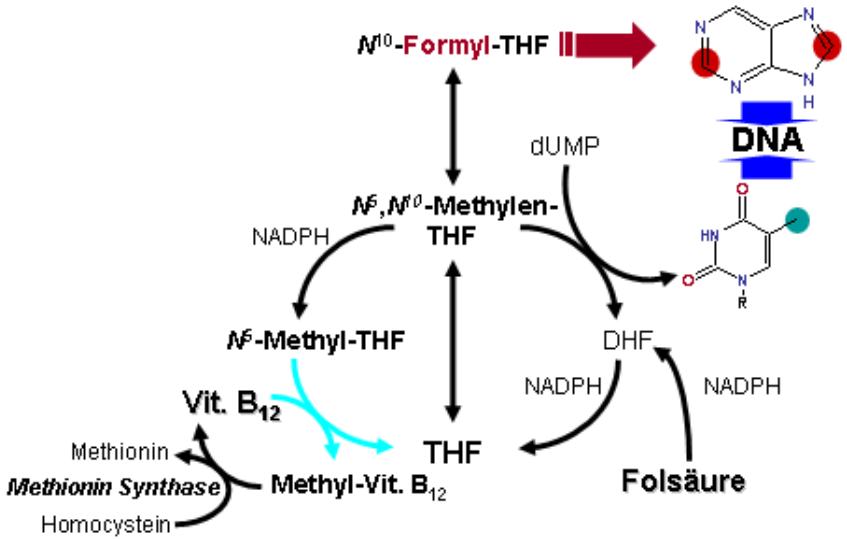
DNA damage repair by NER mechanism



More about folic acid



Author: PD Dr. med. habil. Stephan Gromer Shown is a simplyfied sketch of the metabolism of folic acid and the interaction with vitamin B12 metabolism.

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Titration of Folic Acid

Titration: 1-wertige Base

'fr

Datum 08.05.2007 Zeit 14:42:07 Nr. 4

Anwender dg TiNet 2.40

Methode 1-wertige Base (HCl 1N)

Bez UT06050068

Name der Base Folsäure

Molgew 441.41

Einmass 463.4 mg

Endpunkte:

DET pH.EP1 1.1058 ml pH 11.299

Resultate:

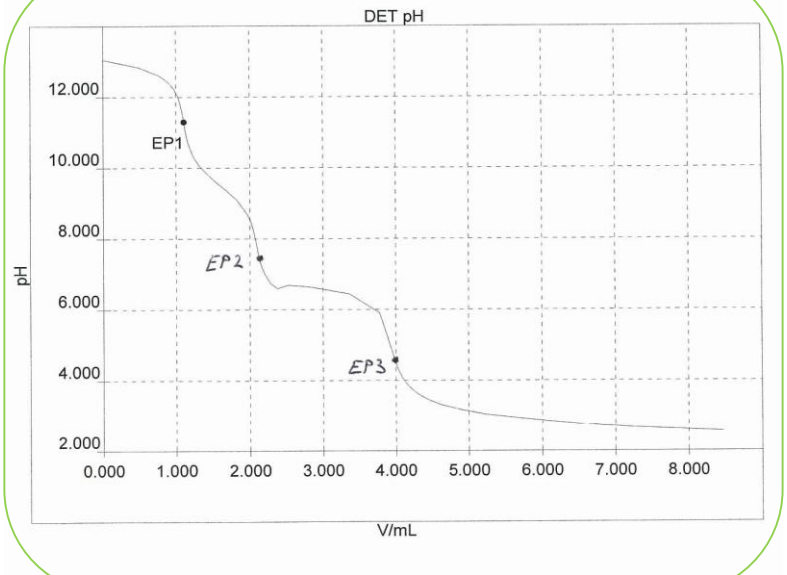
Base-namen Folsäure

Molgewicht 441.41 g/mol

Base ~~105.333 %~~

==========

5ml NaOH 1N zugeben, dann Rücktitration.



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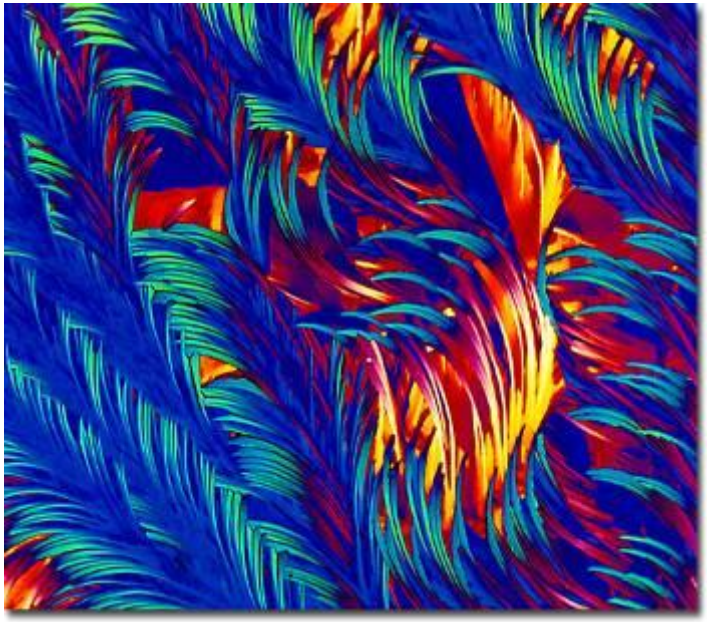
More about folic acid



Folic acid in polarized light

Biochemically, folic acid (or folate) functions as a methyl donor after being enzymatically reduced to tetrahydrofolate by the enzyme dihydrofolate reductase. This biochemical reaction is the target of a number of chemotherapeutic antimetabolites such as methotrexate that bind to the enzyme and prevent the reduction.

Folic acid is found in brewer's yeast, liver, fruits, leafy vegetables, oranges, rice, soybeans, and wheat.

Clinically, folic acid promotes normal red blood cell formation, helps to maintain the central nervous system, and promotes normal growth and development.

Deficiencies in folic acid cause conditions such as anemia, weakness, lack of energy, paleness, mental confusion, and headaches

Folic acid – History

- 1931: Wills in India observes the effect of liver and yeast extracts on tropical macrocytic anaemia
- 1938: Day and coworkers find an antianaemia factor for monkeys in yeast and designate it “vitamin M.”
- 1939: Hogan and Parrott identify an antianaemia factor for chicks in liver extracts, which they name “Vitamin BC”.

- 1941: Mitchell and colleagues suggest the name “folic acid” for the factor responsible for growth stimulation of Streptococcus lactis, which they isolate from spinach and suspect of having vitamin-like properties for animals.

- 1945: Spies demonstrates that folic acid cures megaloblastic anaemia during pregnancy.
- 1962Herbert consumes a folate-deficient diet for several months and records his development of deficiency symptoms. His findings set the criteria for the diagnosis of folate deficiency. In the same year, Herbert estimates the folic acid requirements for adults

- 1991: Wald establishes that folic acid supplementation reduces risk of neural tube defects 1992: Butterworth finds that higher than normal serum levels of folic acid are associated with decreased risk of cervical cancer

- 1993: The US Public Health Service recommends that all women of childbearing potential consume 0.4 mg (400 µg) of folate daily
- 1998: Fortification of all enriched cereal grains (e.g., enriched bread, pasta, flour, rice and breakfast cereals) with folic acid becomes mandatory in the USA and in Canada. In Hungary, wheat flour is fortified with folic acid.

- 2000: Fortification of wheat flour with folic acid is established in Chile.