Nobley, DCW PSEBM 60: 225 - 1945 Observations on the antimicrobial action of 7,3 dichloro-1-4 naphthoquinone & its reversal by vitamin K.

Shive, W. + J. Macow, JBC 162:451–462 (1946.)
Biochemical transformations...
I Aspartic Ac.

dl hydroxyaspartic ac. is inh. to E coli, reversed by glutamic ac. or by aspartic ac. (c.) pantothenic ac. raises antibacterial index.

An E coli strain initially non-prototrophic was adapted by serial transfer for use in these expts (!!). (Desolated?).

COOH
CHOH
CHNH$_2$
COOH . I
dl.

Antibact index ca 10–15. index. in E coli. In Leuconostoc 60–100.

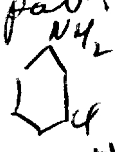
II tried on coli. similar, but index 100–200.

also
COOH
CHNH$_2$
CHNH$_2$
COOH (II)
meso.

At low levels of I, 1γ pant ≐ 10γ aspart in reversal. do. β-alanine. Asparagine less effective. Pant. increased antibact index from 3–20. e.g. Thr. q. + had no effect.

At higher (I) glut. decreases in activity. Oxalac, malic, succ, + fumaric ineffective. Isoserine had no effect at 1mg/cc!

Interprets eff. of pant as indicating shift of limiting reaction from β alanine synthesis to another one. Interprets glut. effect as precursor of aspart by transamin.

II ~~SA~~ pab. 463.
also 
COOH.
II.

II reversed completely by methionine.

Series of antib. indices in addition of different substrates. 1. Methionine 2. adenine 3. . ?

SA:pab
3000 no meth.
10000 meth.
30000 purines.

Presumably II is effective only at a certain locus of pab action.

Madinaveitia, J. Biochem. J. 39:85-91 (1945). Antibacterial substances related to pantothenic ac.

"pantamides". Reference inh. PT.: P-$NHCH_2CH_2SO_3Na$.

L. casei ē used.

pant-hydrazide was active, but not highly so: P-$NHNH_2$. No ther. act.

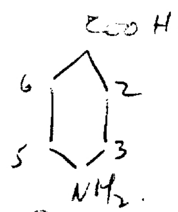
Also, pantoyl-N-2 aminoethyl-(p-aminophenyl)-1 sulfone.

P-$NHCH_2CH_2SO_2\Phi NH_2$. Not reversed by pab; " by pthers.

Sl. therapeutic activity in rats ē S pyogenes.

Martin, AR + FL Rose, 39:91- 1945. Antibacterial substances related to pab.

(overlap Wyss et al; Seem, Johnson + Paul.).



	2.	3.	5.	6.
1.		Cl		
2.	Cl			
3.		I		
4.		Me		
5	Me			
6		HO		
7		MeO		
8	MeO			
9		EtO		
10		NH_2		
11		COOH		
12		MeS		
13		EtS		
14		$MeSO_2$		
		$EtSO_2$		
		Cl	Cl	
		Cl		Cl

	2.	3.	5.	6.
15		~~[illegible]~~ SO_2Et		~~[illegible]~~
16		Cl	Cl	~~[illegible]~~
17		Cl		Cl
18		Cl		NH_2
19		Cl		NHAc
20		Br	Br	
21		Me		Me
22		MeO		MeO
23	MeO		Me	
24.		MeO		Me.

" S. pyogenes; Wright's broth + blood.

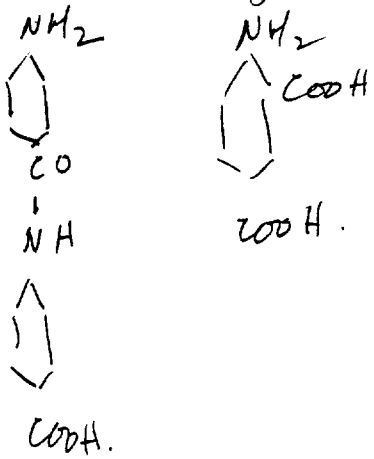
I: ½ eff. as SA. | 2 + 4 anti SA.

5. Inact.

4-amino isophthalic

4-(4'-amino benzamido) benzoic ac. } Sl. anti SA activity

& Et. 4-aminobenzoate



McIlwain H. Biochem. J. 39: 329-33 (1945) Biochemical characterization of actions of chemotherapeutic agents. 5. lack of gross displacement of pantothenate and pabate from microorganisms by pantoyltaurine & sulphanilamide.

Strep. haemolyticus. Limiting pantothenate medium → pantothenate poor cells. Not all exc pnt in heavy pnt medium growth removed by mere washing.

Suspensions contg 15-60 mg (dry) of cells in 2-5 ml M/18 PO_4^-

pnt determined by digesting + Proteus growth.

Wet cells (lg. batches) exposed to SA. No release of anti SA occurred on exposure to buffer, saline or SA.

Pnt. content of bugs grown in initially 2×10^{-6} m was 30 mμ mol/g (dry) Growth for shorter periods - more pnt, the contemporary level being important. The cells inactivate pnt.

Cells c̄ up to 700 mμ mol/g were obtained

No pnt was liberated on exposure to pnt·taurine of the poor pnt cells. Nor did washing. ptaurine inh. pnt inactivation.

In pnt rich cells, pnt stable at R.T. was released into saline at 37°. the quantity remaining being ca that of pnt poor. Large exc pntaurine had no effect on quantity removed.

The amt of SA-antagonists present is not altered by large amts of SA.

"It is suggested that although pab + pnt function in resting bacteria these activities, when the resp. substances are incorporated are not influenced by SA + PT but the reactions involved are the assimilation of the substrates." These are stably bound.
Therefore expect a lag in action for dilution of ~~[illegible]~~ substrate.

McIlwain, H + DE Hughes, Biochem J. 39:133-139 (1945). 3. Relationships between metabolic and growth inhibitions by pantothenate analogues: their structural and spp. specificity.

Assay = Proteus.

Several analogues tested for (1) effects on growth, reversed by Pthen

Some comps. inh. growth but not pthen inactivation: bis nor desoxy pantothenate. These were not reversed by pantothenate!

All analogues which competed = pthen, inhibited the inactivation of pant.

order of activity of different analogues

+ of pant T. in different species is the same for growth + pant metabolism.

McIlwain, H., Biochem. J. 39:279- (1945) 4. Time-relationships between metabolic and growth inhibitions by pantoylataurine.

1. pnt + streptococci ⇒ slow inactivation of pnt at uniform rate.
2. not occur at 0°.
3. Inhibited by pantoylataurine immediately.
4. Growth inhibition has lag ca. 1 hour.; recovery also lags.
5. Reversible on washing & removal of pnt. occurs very quickly.

∴ assumes action of ~~pnt~~ PT is to inh. the synthesis of a pnt derivative essential for growth, which can be produced in excess.

Field, J.B., EG Jansen, J Spero, and KP Link, JBC 156:725-737(1944)

Studies on the ~~hemorrhagic~~ hemorrhagic sweet clover disease.

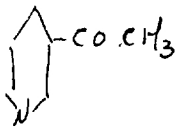
XL Hypoprothrombinemia induced by methyl coumarins and its effect on the action of 3-3' methylene bis (4-hydroxycoumarin).

Caffeine, theobromine + theophylline stimulate liver to production of prothrombin + fibrinogen; reversing dicoumarol.

NICOTINIC AC. analogues (Acetylpyridine)

Woolley D. W. JBC 162:179-80 (1946) Reversal by tryptophane of the biological effects of 3-acetylpyridine.

Tryptophane was as effective as nic in reversing effect of 3-AP on mice (pellagra).



Rockefeller.

RIBOFLAVIN, analogues
L. casei

Lareth, H.P. JBC 162:87–97 (1946) The effect of riboflavin analogues upon the ~~use~~ utilization of riboflavin and FADD by L. casei

Review: isoriboflavin has < .5% activity of B_6 for L. casei
inhibits rat growth at low B_6

Shows: in presence of suboptimal B_6 or FAD, stimulates ac. prod.

Diaminophenazine competitively inhibits utilization of B_6.

Lumiflavin competes ≈ low B_6, stimulates ≈ high.
inhibits FAD utilization at lower conc.

L. casei in alkali-treated peptone, or Casamino (Sandy + Dielon)

main effects on B_6 enzymes, and not on B_6 → FAD reaction

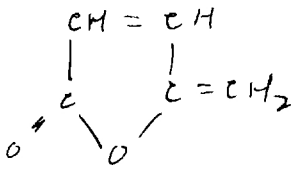
Tulane, New Orleans

ANTIBIOTIC: Buttercup juice

Baer, Harold, M. Holden and BC Seegal, JBC 162(1):65-68 1946

The nature of the antibacterial agent from Anemone pulsatilla.

~~Anemonin~~ ANEMONIN obtained, a polymer of proto-A.



proto-anemonin.

Activity measured against E coli, Staph and Candida albicans.

Acetylacrylic ac., nor vinylacrylic had no antibacterial effect.

Kimball, R.F., Genetics 24:49-58 (1939). A delayed change of phenotype following a change of genotype in Paramecium aurelia.

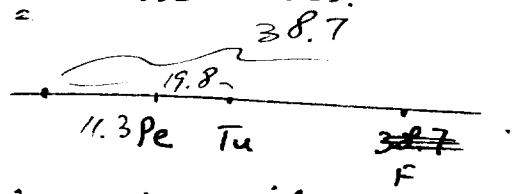
Following endomixis there is a delay in the expression of change of mating type that may occur.

Lindegren, C.C. + G., Genetics 24:1-7 (1939) Non-random crossing over in the 2d chromosome of Neurospora crassa.

See L. '36. Genetics 32: 243-56.

9 chromosomes.

knot-tow, peach, tuft + fluffy.



1. Excess of 2-strand exchanges. Deficiency of multiple exchanges.

Severens + Tanner, J Bact 49:383- 1945.

The inheritance of environmentally induced characters in bacteria.

Graded conc.

(Selection favoring wild type in mixed cultures in absence adaptive agent.)

Inoculate mass populations into Agar.

Changes of critical conc.

NaCl - from 3 to 8%
$CuSO_4$ - 1:4000 to 1:800
$HgCl_2$ 1:300,000 to 1:50,000

∴ use 6% salt agar

Horowitz, N JBC 162:413 1946.

The isolation & identification of a natural precursor of choline.

$CH_3-NH-CH_2CH_2OH$ isolated from 47904, active on 34486

Appears only after 7 dq. more conc. in mold than medium;

47904 must synthesize type. choline. methylation of diMe EtOHNH$_2$ also affected.

$NH_2EtOH \rightarrow MeNHEtOH \rightarrow Me_2NEtOH \rightarrow Me_3N^+EtOH.$

↑ 34486.   ↑ 47904

Fries, Nils. Svensk Botanisk Tidskrift, 39:270-8 (1945)
Two X-Ray induced auxo-heterotrophies.

Ophiostana (Ceratostomella) multiannulatum.

wild type requires: B_1 + B_6. Mutants for Biotin (225) and pab (617) obtained by X-Ray. Isolated by special selection technique.

Ark. för Botanick, 32:1-9 (1945) Über Röntgen-induzierte physiologische Mutationen bei Ophiostoma multiannulatum.

50kv. 2-3ma. 100cm. Plated irradiated spore suspension onto minimal "Fries agar + B_1+B_6. Mutants "durchsichtlich schlechteres Wachstum abweichen wurden." Von den die auswachsenden Ansporenzellen wurden deshalb nur solche isoliert, die sich in dieser Beziehung von - des Meisten - normalen Typpflein unterscheiden.

1. Temporary radiation effects (back mutations?)
2. Morphologicals.
3. Mutants.

627 isolated. 30 mutants - 6 biochemicals.
None from unirradiated material.

\# 225 Biotin
358. Reduced S. (parathiotogh - cystine etc. or Y valent S. ($SO_3^=$)
446 Parathiotogh - can not use tetravalent S.
460 - ~~yet~~ Uracil
513 Adenine ? Low activity
617 pab.
848 Guanine.

Naturw. 30:44/5 - 1942. Adenin als Wachstumsfaktor für Ophiostoma ulmi (Buisman) Nannf.

Requires only B_6.

Nature, No. 3947 : 757 (June 23 1945) X-ray induced mutations in the physiology of Ophiostoma.

O. multiannulatum. strains mentioned above.

Parathiotrophs ♀ crosses lost ability to reduce tetravalent S. (#358). Other features inherited as 1 gene ♂ crosses

Needed large quantities of adenine.

Uracil-less used cytidine or cytidylic ac. but not cytosine (blue 1298).

Nature #3847 : 105 July 24, 1943. Vitamin B_1, Vitamin B_6 + Biotin as growth substances for some ascomycetes.

Ophiostoma:

	Needed	Stimulated
O. piceae	Pyr	
ulmi	Pyr	Biotin
coeruleum	Pyr	B_6 "
quercus	Pyr	" "
piini	Pyr · Biotin	B_1
ulmi	B_6	Pyr
fagi	B_6	Biotin
pilliferum	B_6	Biotin
multiannulatum	$B_1 + B_6$	—

"Artificial symbiosis" tested + worked. (Heterocaryon?)

Mutants needs biotin ± NH_4 for N; desperate ± NO_3 + acid!

Hollaender, A. Effect of long uv & short visible radiation on E coli
J. Bact 46: 531-41 1943.

Saline = NaCl 3g KCl .2g $CaCl_2$ .2g/100 ml H_2O. Protected by beef broth somewhat.

1. Growth delaying effect before appr. lethality (plate counts)
2. Survival in saline: (incubation).
   controls survival quite well 10 hours. (98%).
   irradiated died much more rapidly

Longer wavelengths much less efficient (10^5 energy req.).

Wickerham '45

8 ascospores/ascus. after copulation. Relatively anaerobic. Bottom fermentation 3 pellicle.

Under slide conditions, hyphae are formed. (rel. anaerobic). Nucleus visible in terminal hyphae, ca. 8-10μ, particularly anaerobically.

glucose, maltose & sucrose rapidly fermented. Also melibiose.
Not galactose & lactose

Sporulation did not occur from hyphae, or was diminished temporarily.

Trypan blue in agar leads to dark pigm. in aggl. phase (obt. from normal). Growth rapid 30-37°. Colonies develop slowly - 4-6 days. Copulation occurs readily at 20-33°. Asci ruptures before completing development.

Wickerham, L.J., & Enrique Duprat.
J. Bact 50: 597- 1945.
A remarkable tetrad yeast, Debaryomyces nyces variabilis, n.s.,

Lwoff, A. + A. Audureau, Ann. Inst Pasteur — ? 1941.

Sur une mutation de Moraxella lwoffi apte a se développer dans les milieux à l'acide succinique.

pp 1-2 missing

Typical strain will not utilize succinate. Rarely mutations appear, influenced by succ. from S− to S+. In presence of EtOH S+ outgrows S+. S+ → S− not found. Rate S− to S+ ~~Retest mutation~~ ca 10^{-8}.

——— 70:51- 1944. Recherches expérimentales sur les mutations bactériennes.

Succinoxidase is present in both strains. Oxaloacetic ac. is decarbox. spontaneously but not rapidly enough for growth.

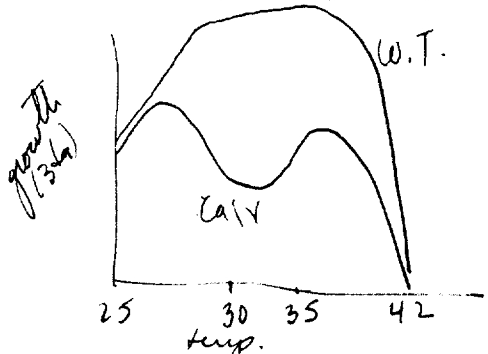
Hydroxy fumaric acid studied (enolic form of $\begin{matrix} COOH \\ | \\ C{=}O \\ | \\ CH_2 \\ | \\ COOH \end{matrix}$). Rate of decarboxylation studied. Rapid at first as ~~by CO2~~ S+, but slows down to spont. rate (almost as rapid)

Prove there is an enzyme present? in S−. which is not present in S+?

Mitchell, HK and MB Houlahan   AJB 33:31-   1946.

Neurospora IV. A temperature sensitive Neurospora mutant.

51602.   At 31° or above, requires riboflavin absolutely.
S-shaped response curve .1 - 2.5 µg.   At high B_2, growth curves like wild, at low levels, bimodal temperature response.



Grows on 20 ml

At higher temperatures, c̄ a small B_2 supplement, (ca. .3 γ) full wt. can eventually be obtained (200 hours = 8 days.) containing full B_2 content by L. casei.

For B_2 detm., autoclave cultures in medium & analyze filtrate. F. wt.

ca 6 - 9 γ/100 mg. Mutant grows intermittently, rising up + stops -

Uncoupling vitamin. Not tested on Neurospora.

Inhibited by lumichrome; reversed by B_2. R_{50} = 1.2 - 2.5.

Some inhibitors in tissue extracts.

Neurospora may contain a doubly functioning set of genes for different temperatures.

Abb 4/A x Chia.

Tatum, E.L. + T.T. Bell. A.J.B. 33(1): 15-21 (1946)

Neurospora III. Biosynthesis of thiamin.

			Distance from centromere
1090	(sitophila).	45 asci	23
9185		24 "	8.3
18558		8 "	0
17084.		33 "	35

No interspecific heterocaryosis.

3 da growth, 20 ml / 125 ml flask.

18558 requires thiazole
9185 intact thiamine

When grown on limiting thiamin, accumulation of pyrimidine was established by 18558 (tested on 17084, + Phycomyces)
Analogues of thiazole had activity very similar to Phycomyces, except that 5th ethyl may have ca. 1% activity of B_1 for 18558.

2-methyl deriv. was also app. active

Factor 5 did not influence 9185 response.

17084, 1090 (and 56501) require both pyr and thz. Mixture had same activity as thiamin. Filtrates have a 9185 active compound, which loses activity on sulfite treatment. It is also active for 18558 and Phycomyces Not active for 17084.

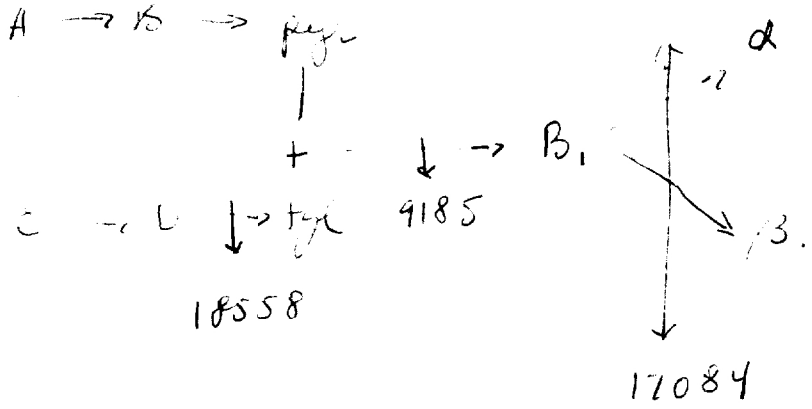
299 is low B_6 responds only to B_1 or pyr + thz.

Wooley's conclusions on pyrithiamine not confirmed. 17084 and 1090 can use pyrithiamine for pyrimidine.

A thiamin metabolism enzyme exist in 1090 + 1008 y.

These strains have a high requirement.

i.e.



Carrel, A. Pr. Am Phil Soc 68: 129-32 (1929) The nutritional properties of malignant cells.

Numerology

Kellogg, W.N., et al S 103:49. 1946. Spinal conditioning in dogs.

RADIATION: Cathode

Wyckoff, RWG + T.M. Rivers, ~~JExp~~ JEM 51: 921- 1930.

The effect of cathode rays upon certain bacteria.

1.5×10^5 volts

The <u>absorption</u> of a single electron will kill a cell.

Concluded that only .008 of the incident electrons are absorbed from phantom expts.

"Only 85% of the single hits were effective, but when death occurred, a single hit sufficed.."

(data from dose / response curve, and calculated absorption by the bacteria.)

[How can this be compared c the production of β rays by radioactive P, etc?]

RADIATION: u-v

Hollaender, A + RM Duggar, J. Bact 36:17 1938

The effects of sublethal doses of monochromatic u-v radiation on the growth properties of bacteria

2650 Å

Giles, N.H., Genetics 28: 398- 1943. Comparative studies of the cytogenetical effects of neutrons and X-Rays.