Genetics 107 - Ag. Bact. 107
Heredity in microorganisms

Selected references

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Bact 108

Heredity.
2 concepts
1 likeness of offspring to parents.
2 this constantcy is incomplete.

There must be some influence within bacteria which determines their behavior. - give rise to intrinsic differences between cultures.

Useful because of short life of each generation can be watched over thousands of generations.

The material responsible for heredity does not have complete control. - genotype. - the intrinsic character of cell which is responsible for behavior and which is transmitted to offspring.

phenotype - present behavior under specific conditions of experiment. i.e. its present appearance under set conditions.

eg Ser. marcescens pigment produced only at low T. but colourless cells will again produce pigment if returned to lower T.
This ∴ does not affect potentiality of cell i.e the genotype cf cycle from egg to adult animal.
Retains ~~ps~~ intrinsic potentialities under conditions where they don't show.

Can control ^vary^ environment of bacterial cells much than those of higher animals.
Genes regulate activities of cells under conditions that prevail. ∴ Genotype less distinct from phenotype in higher animals.

In higher organisms then genotype is used for formula which describes genetic content of cells.
In higher organisms different genetic types may give rise to similar appearance - "phenotype."

What is basis of genotype.
Theory: - the whole cell is responsible. - parent organs deter nature of daughter organs. - theory of holocellular determination. - doesn't work well with cycles of different phenotypes
- eg would have to say that whole flagellum is not necessary as there may be flagellate or non flagellate phenotypes. ∴ there must be certain units which perpetuate genotype - ie genes.

genes - that part of cell which has as a function the property of the propagation of the cell throughout a culture.

Cannot separate identity of individual genes without use of genetic variation.

genetic variation must be distinguished from physiological
The change which appears to be incited by a specific environment must persist through a long period of absence of the inciting conditions if it is genetic variation.

S → R variation is genetic because change will persist. produce different colonies under same conditions.

monomorphism - doctrine that there is no genetic variation - attacked S-R as genetic. but due to impurity. - There are variations however - necessary for evolution

LiCl. increases number of S → R mutations

Colorless mutants (at 25°C) of ~~B~~ Ser ~~Ma~~ marcescens occur sporadically in a similar manner.

If sporadic variation is rare.

~~Additional Refs.~~
~~Lea & Cou~~

Refs. - Luria in 4, 11, 19, 20, 13
Newcombe & Hawriko, J bact, 57: 565-572. (1949)
Streptomycin & E coli.

If sporadic mutation is rare it is impossible to find them without selective technique which might actually be causing mutation.

eg if in 10^9 ~~Strep~~ phage sensitive bacteria there are 100 phage Rs. these could only be found by adding phage and killing sensitive. But maybe phage has caused production of resistants - ie does the mutation occur before or after application of selective agent.

To settle ~~it~~ this Newcombe. - if variant is produced at time of addition of agent there will not be any clones of variants at time of application as there are no descendants of mutants.

sporadic — would give 1 colony unspread + spread

directed — would give 4 colonies spread or unspread

He applied phage to plate on which cells have grown. if sporadic mutation there will be clones of resistants.

If directed each resistant colony will have started from a single cell at the same time

If they were clones and the plate is mixed up and respread you will get more colonies than if not respread.

respreading single mutant cells would have no effect on number of colonies.

It is the former.

Respreading done just prior to adding phage.

Not absolute ~~proof~~ proof as spreading itself might increase mutation but very probable.

Also does not show that phage does not produce some of the mutations. but there is no evidence for this.

Unspread gives number of mutations.

Spread gives number of mutants.

Seminar Mon & Thur 3:30. 2007

Assumptions for L + D's method.

(a) every cell divides at regular intervals (actually not very regular. - uniform synchronous growth.)
(b) mutation occurs at time of division one daughter starting out as a mutant.
(c) every descendant of a mutant is a mutant. reversion is at too low a rate to be significant.
(b) constant probability of mutation per division.
(c) immediate expression of resistance to phage in new mutant. - this is the chief weakness of theory

sometimes mutants have disadvantage then tends towards an equilibrium.

a mutant can arise by mutation or by subsequent growth of mutants. ∴ mutant cells must be equal to or more than the number of mutations.

Decendants of one in n generation are 2^{n-1}

Because of increase in numbers most mutations occur in latter generations. but the earlier ~~mutatd~~ mutation contribute an equal number of mutants because of their more descendants. Each generation contributes the same number of mutants to final culture.

L & D tried to detd bacterial mutation rates but got very inconsistent results.

values 3 - 125 but most at 18 cant take average of these as the odd high one would throw it out.

This variablity it to be expected of sporadic mutation not of directed mutation.

Couldnt get value for mutation rat until Lea & Coulson mathematical treatment was developed.

This however is not good proof of sporadic mutation as the different numbers might be due to difference in ~~variance~~ environment between cultures.

This test is more applicable to different mutations than Newcombes.

"No such thing as an average mutation rate."

Mutations for several mutants occur 10^{-6} - 10^{-9} but this is just the conv~~enient~~ range to study. higher ones are not found. lower rates would be regarded as physiological change.

see Shapiro & Bunting in CSHSQB. 11
mention some 10^{-3} & 10^{-2} - selective methods
not needed ∴ must be sporadic.

Null tube method. detn dilution at which
half the tubes have a mutant in them. - a medium
in which only mutants grow - gives value for
number of mutants present.

Phage resistance
notation B/1 strain B of E coli which is resistant
to T_1 phage.
Sometimes resistance to T_1 also accompanies
resistance to others eg T_5 = B/1,5
phage immediately after bar is the one to which
which bacterium is exposed.
All those resistant to T_5 are resistant to T_1 not
vice versa.
B/1,t = B/1 which is tryptophaneless.
B/r = radiation resistant mutant of B.
[see Witkin ~~ref in Lwoff article~~ (1947)
Genetics, 32, 221-228 "Genetics of resistance to radiation
May alternatively use. V_1^r resistant to T_1 V_1^s = sensitive
gives a designation for alternative mutant.

[Demerec & Fano (1945) - Genetics 30, 119 -
"Bacteriophage resistance mutants in E. coli."

Above got resistant mutants to each of the phages
then tested them for cross resistances.

B/1	B/1 + B/1,5	
B/2 rare.		
B/3	B/3,4.	B/3,4,7.
B/4	"	"
B/5		B/1,5.
B/6	B/6	
B/7	B/3,4,7.	

∴ at least six different mutants.
then detn whether these are due to mutations in

parts of genotype - got all rates
Measured B/6 → B/6/1.
got same rate as for B → B/1
also rates of B/1 → B/1/6 same as B → B/6.

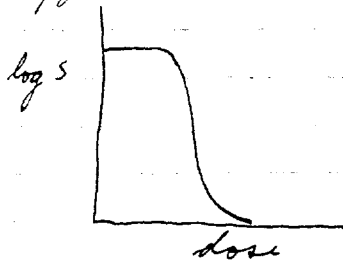
Supports idea that independent elements of the genotype are responsible for the two character because you can get subsequent mutations at same rate.
Actually rate measurements are not accurate.
These independent elements will be called genes.

They did find some B/1,6 to frequently to be completely independent.
maybe it is different from B/1/6 - may involve other factors C as well.
2 different genotypes may give the same phenotype.

These mutations are all all or nothing phenomena.
Physiological basis is that cells of B absorb the phage not the resistant ones.

Others are not all or none - depend on concentration of disinfectant etc. May be several genetic factors. see. Demerec M. (1945) PNAS 31 16-24

Even in the absence of genetic variation the response of a genotype will vary in contact with different dose.



even for a homogeous genotype. their progeny behave as did original culture.

in case of penicillin it effects only actively growing cells others are persistors.
some colonies formed on brilliant green though progeny are no different.

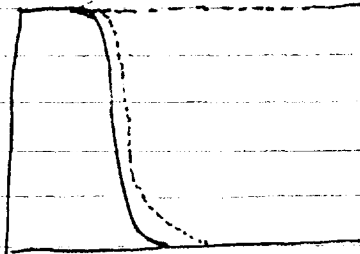
Possible proposition - study the chem of these steps.

This stepwise change is not common outside antibiotics.

May find "leaky" mutants. mutations don't completely block a synthesis - eg. not completely histidine requiring.

May be that for full resistance it is necessary to have cooperation of several genes.

In streptomycin E coli frequently shows complete one step resistance. also a very small step may appear.



Some streptomycin resistant cells require ~~strept~~ streptomycin for growth. If plated on Stmy free media you only get sensitive cells.

		-S	+S
streptomy.	sensitive	+	-
	resistant.	+	+
	dependent.	-	+

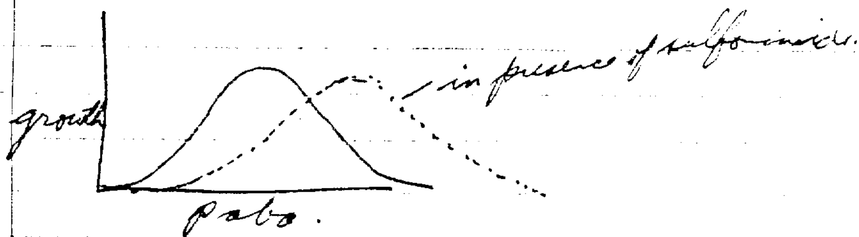
He suggests that in dependent the residual inhibition by streptomy. keeps cell in balance. Otherwise the changes due to mutation upset the cell.

Not likely that it is actually used in its protoplasm.

Another case in Neurospora. - PABA inhibited in some function ~~[illegible]~~ by sulfonimide - some mutants, resistant to sulfonimide are dependent on it. However it does not replace it. resistance to sulfonimide by this is ~~[illegible]~~ inhibited by PABA. wild Neurospora can synthesize PABA. but some mutants are PABA dependent.

cross $PABA^-$ with sfd^-, $\to PABA, sfd^-$.
found it needed some, not too much PABA

[Zelokar (1948) PNAS 34: 32-36



sulf. dependant makes enough paba to inhibit itself when not inhibited by sulfs.

No derivatives of streptomy supply strep dependant without inhibiting strep dependance.

Can use these dependant bact as test for presence of streptomycin.

resistant & dependant mutations occurs at approx equal rate but until strepmy is added the dependant cells do not multiply ∴ there are fewer mutants dependent. This gives indirect evidence of spontaneity. Same number on agar different in both.

— Wednesday 9 August.

properties of penicillin resistant staff.

[Hinck et al 1948 J Bact 35, ~~139~~ 139
~~Gols~~ & Rodwell ibid 55, 161

Phenogenetics. Possible variations - properties of genetic variants.

Penicillin resistant ~~mutants~~ are said not grossly different from wild in Demerec's work

Above workers found staff ~~aureus~~ variant to be gram-, rods, pleomophic. no longer ~~required~~ amino acids, grows more slowly, strict aerobe, ~~moderate degree of resistance to pyocyanin and gliotoxin~~, minor degree of resistance to streptomycin. Produces penicillinase. Cannot ~~ferment~~ ferment several sugars. Cannot grow in presence of salt. Does not reduce NO_3^-

These changes appeared progressively. It is possible to get reversion

He explains discrepancy of results by
1 Complete physiological independance of distinct genetic changes.
2 The metabolic inter organization of all functions

Klinck & Gale did not carry out a series of platings. They inoculated a series of tubes with increasing penicillin conc. took from highest conc showing growth to further series. This picked up a large number of mutations which have only small effect and would not appear in Demerec's method. This method gives a great number of small mutations. This gives continuous selection - selects non specific effects of mutations that increase growth in any manner.

By selection of organisms with some of these other properties you find they grow better in penicillin.

One function of p aba is in synthesis of methionine at least adding methionine reduces need for PABA. However sfo^R need methionine.
Other case CN resistant yeast has no cytochrom.
F " bact has no F sensitive enzymes of glycolysis.
Explanation unknown

Other associated changes.
eg B/1,t - requires tryptophane.
[Wollman 1947 Ann Inst Pasteur 73, 1082

Organism cannot use indol as substitute for tryp. Usually it will couple with serine.
∴ this reaction is one probably blocked.
Indole does inhibit absorbtion of phage.
∴ its accumulation is ~~difficult~~ responsible for ~~effe~~ effect.
Cannot find back mutation direct which does not need t and is resistant. but some can get along on indol - from this a second step can lead to t independent resistant mutant.

Experimental Modification of Mutation Rates.

Not much different from higher organisms.

Auerbach : Biol Rev.
*Demerec + Latarjet CSHSQB XI 1946.
Radiation effect CSH IX 1941
Witkin CSH XII (1947) - Chemical of bacteria.
J. Cellular + Comp. Physiol. Vol 35 suppl. 1 June 1950
Radiation biology - esp paper by Muller.
Lea - Ref 5 - somewhat biased.

Witkin Genetics 32 221

Ref 7 - Lederberg
Early found more mutations at high T. ∴ concluded they involved a local chemical change. Suggested if it were possible to deliver energy in highly localized packages mutation rates would increase. Found X rays did it. - First in Drosophila.

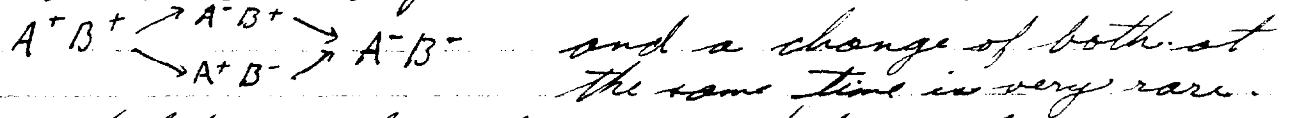
D + L investigated B → B/1 mutations - this includes several different mutations. - B/1, B/1,t B/1,5.

Easy to count the number of mutants.
Could increase number of ~~ant of~~ survivors significantly.
All agents which produce mutations also kill many cells. - can detect effect only when dose is large enough to kill many.
Assume there is no differential survival of mutants previously present.

Assay a prep for previous mutants. Suppose one mutant in 10^7
If the killing is indiscriminate and kills 99%. Starting with 10^9 cells would have only one resistant left.
If there is no mutational effect but selective effect then there might be 100 resistants left in 10^7 cells.
Would give the same result if no selection but 100 fold induction of mutations.

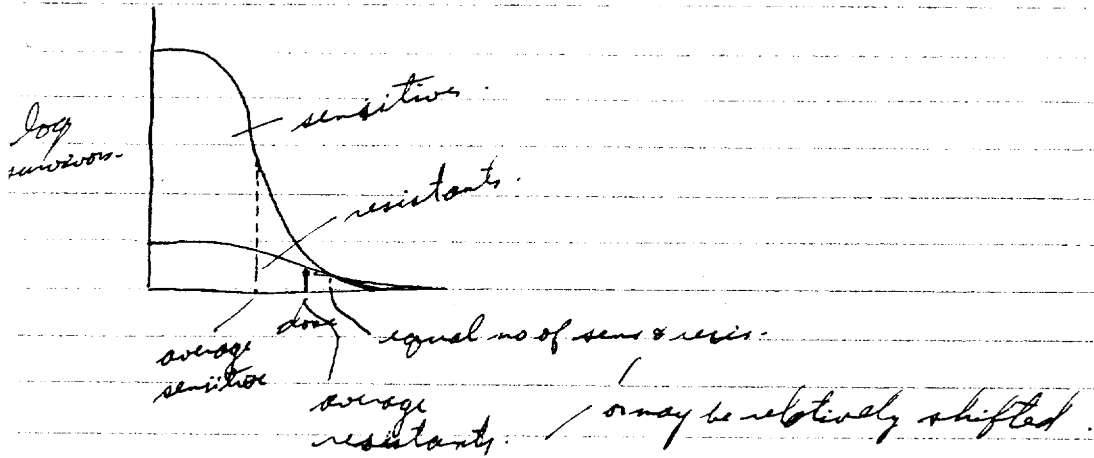
Criteria for Separating Genes.

1 Qualitative independance in variation.



2. Quantitative independance in mutation rates.
$A^+ \rightarrow A^-$ rate is the same regardless of state of B.

But hard to maintain homogeneous genotype.
∴ in cultures usually get some resistant cells - can be selected by growing in penicillin.
To get rid of most of sensitives have to kill some resistants.



∴ always difficult to get a true count of the resistants.
over all curve simply has a longer tail - not much difference.
There may be several different kind of resistant cells.
Has to be sufficient differential in the survivorship to compensate for difference in concentration
Survivors of first resistant strain in high dose may show a further shift.
Generally the families of curves from first order resistants are separate from second order.
Demerec took this for 5 steps to get complete resistance.
Probably represent changes in more than one distinct factors.
In this it seems there are 5 factors of approximately equal effect. This keeps each family of curves together

cant be sure unless there are actually more mutants afterward in spite of killing.

This is different from induction to Penicillin resistance by penicillin if that occurs because the mutations induced are not specific here. There are a great number of different mutations produced by N mustard.

Can check this by:
(a) getting cultures with no mutants present to start with.
(b) sometimes can adjust the dose to get an absolute increase in mutants present. must use rather small dose of agent. survival should be e^{-1} (about $\frac{1}{3}$ surviving.)
This has been found in neurospora under X-rays, u-v, mustard for inositolless ~~mutants~~ to inos.+

Difficulty is verification of assay methods. check it by not giving dose to blank.

There are mutants B/r resistant to mustard & radiation. But this mutation cannot express itself during course of experiment ∴ they don't affect this work. This makes even ~~[illegible]~~ relative increases probably valid.

Check for selective survival of mutants by detn ratio before & after treatment in ~~[illegible]~~ mixed culture. Adding mutant culture to wild in conc to outweigh mutants generated.
~~[illegible]~~ Still possible that in this higher concentration the effect would be different.

Radiation.

X rays very penetrating - react with electrons of about 2nd shell in. - expells electron - primary ionization - this at high velocity can knock out other peripheral electrons. Density of ionization is greatest near end of track.

Can assume that energy of rays going through bacteria are undiminished.

Ultraviolet - energy corresponds more to chemical bonds than intra atomic bonds - may cause rupture of bond in a molecule. There are characteristic absorption spectra of different chemical entities. 2600 A° peak in nucleic acid due to purines & pyrimidines. Not penetrating ∴ cannot use a thick suspension. You can either shake the dish to get even exposure - assume all energy absorbed ∴ total dose divided among total cells - water absorbs negligibly.

Or use thin suspension. Express results in terms of incident energy.

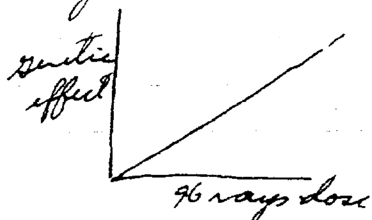
May calibrate dose by bactericidal effect.

Can deter structures which absorb by taking uv. photographs. Sometimes nothing apparent sometimes

These granules are DNA. RNA distributed - under some cultural conditions there is much RNA which covers everything.

Hit theory. (Treffer theory).

The genetic effects could be understood in terms of a local hit. 1 quantum absorption leading to effect on genetic material. No intermediates.



Seemed to be independant of Temp.

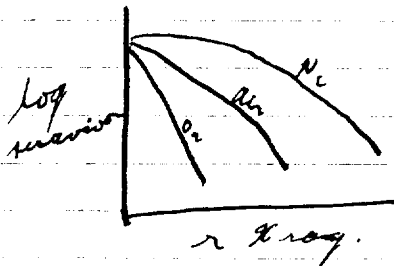
Effect pptnl to number of roentgens.

Roentgens = a certain number of ion pairs / cc of material

Over a wide range of X-ray wavelengths there was still a pptnality between dose & effect.
Assumed therefore that these secondary electrons were directly responsible for the effect.
Two kinds of effect - lethal & mutation.

However.
Kauffman, Hollaender & Swanson independently showed genetic & lethal effects can be affected - cells sensitized by infra red.
infra red has almost not chemical effect or genetic by itself.
but they increase effects of other rays 50% - 100%.
Some of this effect is just in improving chance of recombination of fragments.

Giles & Riley. - Effects of a given dose of X radiation are very much influenced by atmosphere. O_2 has much greater effect than others.



Only the atmosphere at the time of radiation had any effect.

It might be that all effects are mediated by chemically active molecules in neighborhood of genetic material.
Peroxide (OOH) radicals may be the immediate agent. instead of peroxide molecules. If it is radical it would have to be formed near point of effect as it is unstable
- There is still ~~some~~ 10% effect in completely anaerobic conditions.

Ultra Violet.
No ~~st~~ good data on kinetics. Just preliminary of Dem & Zatany in CSH.
Was difficult to ~~to~~ get u.v. to testes of flys for work with them - Corn fly eggs & pollen absorb heavily.
Therefore mostly confined to microorganisms.

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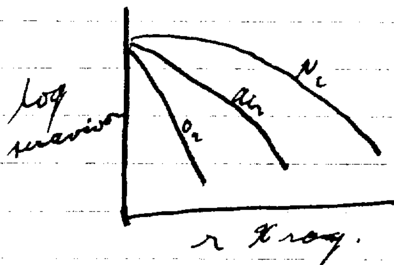

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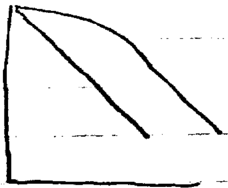
In bacteria
2 kinds of curves



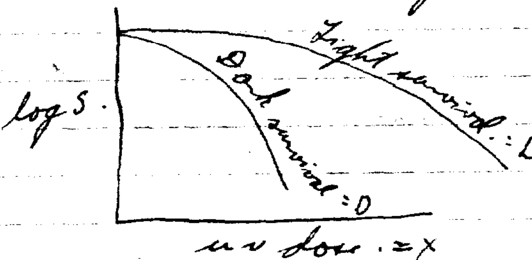
Interpret that with threshold value as requiring more than one hit.

[Norman & Atwood - PNAS 1950 March - kinetics + math of killing curves.

If there is on the average one hit per organism e^{-1} organisms will have no hits - i.e. about 37%.
If 5 hits number of survivors = $(\frac{1}{e})^5$

Photoreactivation.

Kelner: Found variation in viable count after treatment according to amount of light received.



The effect of increased light reaches a maximum. irradiate - u.v. illuminate visible.

May be that illumination prevents inactivated cells from progressing to death.
Novick & Szilard suggest illumination destroys toxic material produced by u.v.
Photoreactivation must be done fairly soon. up to 24 hrs in refrig.
Suggest :. that the toxic substance can be converted into irreversibly toxic substance - i.e. reac with cell - or in light revert to its original form.

dose reduction principle.

$D(x) = L(\gamma x)$ [const.] — curve D vs x

i.e curves are same except in one x has greater effect.

This does not always hold.

Dulbecco in J Bact May 1950 - tested effects of different λ for reactivation - the very short visible are most effective. Suggested very complex mechanism.

It takes 10-15 min of sunlight to saturate with effect.

Phage inactivation by u.v. is st ln. - It can be reactivated after days by light if illuminated just after absorbtion on bact. ∴ illumination & absorption must be at same time. May be because phage absorbs light poorly. Even when only one phage per cell

Mutational effects are reversed as are lethal.

Photoreac in yeasts, fungi, actinomyces as well.

[Haas F.L. et al. Am. Natur. 84 261 (1950) - brief review.

Other Evidence for indirect effect of Ultra Violet.

1947 Stone Wyss & Haas PNAS 33, 59

Found irradiated medium had effect on inducing mutations resistant to penicillin. Difficulty in penicillin conc has big effect on number that can survive in it. ∴ results very sensitive to penicillin stability.

Adding H_2O_2 to broth has same effect as u.v.

Addition of large amounts of catalase protects from both u.v. & H_2O_2 - H_2O_2 no effect direct on bugs.

Dickey F H et al PNAS 35 581 (1949).

Treatment of neurospora spores with organic peroxides not H_2O_2 produced mutations.

Used adenineless neurospora, ~~~~ studied reversions.

Wagner thinks pure H_2O_2 is active in producing mutants in neurospora. Lederberg agrees.

There is no report that ~~H_2O_2~~ peroxide treated bacteria can be reactivated.

Different groups used diff wave lengths - may explain some discrepancies

May be H_2O_2 effect is only the non photoreactivable effect.

Radiation effects. killed most with uv or 96 rays.
Demerec & Latarjet found an increase in number of phage resistant colonies after growth. Meant that some mutants appeared after a period of growth up to 13 generati needed to get full effect of irradiation. Beyond 13 spontaneous mutations obscure and further small numbers.
zero point mutations - those that appear immedi after irradiation.
end point " those after 13 generation.
ratio of zero to end point 1:100-1000.
Most appear after 2-3 generations.
Explanation
① A true delay in effects of radiation: mutation process delayed.
② May be phenotypic lag.
③ Genetic complication up segregation.

First is least likely.
It is actually hard to understand how you could get any zero point ie immediate loss of receptors. Would expect ②- May actually be no zero point: may be some bact are not killed till they have had time for a few generations and to show the new phenotype. dead bact may absorb phage and hold it long enough to protect some cells. In this work there was little excess of phage. Phage can grow on killed cells.
③. The genetic model assumed may be over simplified ie that there is just one unit involved in control of this factor. but there are several nuclei like bodies in bacteri
(83 83) if one has mutated the new form might be recessive to sensitivity. Would not show till separated by fission. If four genomes nucleus single it would take 2 divisions & then phenotypic lag. This does not explain lag up to 13 generations unless some cells remain inhibited in growth for a long period. If some ~~all~~ mutated cells are amongst those inhibited there will be mutants appearing latter.
To check this if true you will get two types of cells from one parent. Separate clones from each

irradiated cells and see if they are pure or mixed
do it by streaking culture across streak of
phage. ~~Concompart~~ Sensitive will show break in
streak. Newcombe got 2 mutant clones which
were pure.. but these may have by chance come from bacteria
with one nucleus. Or the other genome my have been
killed by a ~~lethal~~ lethal mutations.
Can use EMB lactose agar. fermenters give black
colonies. non-fermenters give pink. - mixed give variegated
He finds half the mutants occur in mixed clones.

(1) - May be that unstable intermediate forms
of genes occur. - sometimes get different mutant
forms of the same gene - some stable some unstable.
from the unstable form one might get some wild type
progeny. That the mutation itself may be delayed
is hard to investigate.

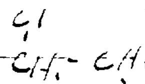
Davis:- Studied Tryp - → Tr+.
He got occasional mutant without any thing
but many more if he added just a little tryptophan
This would indicate some growth needed to allow
showing of mutant not time. May need growth
to produce missing enzyme. (Can't break down some tissue
to yield this he thinks)

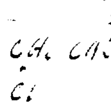
Suppl #1 Vol 35 J. Cell. Comp. Physiol.

Suppl to Vol 22 Publ. Zool. Sta Naples. QL1 N28
Mutagenic agents. - English summaries.

Chemical Mutagens.

First definite report by Auerbach on mustard gas but
it was secret during war.
mustard oil (different cmpd) was next. - $CH_2=CH \cdot S-CN$
allyl iso-thiocyanate.
mustard gases





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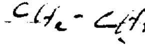
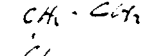
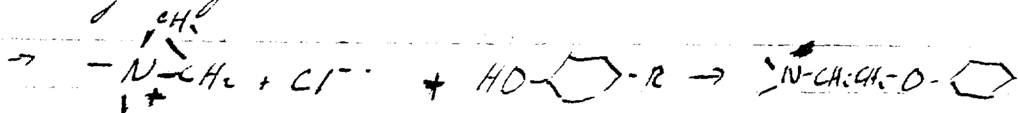


Nitrogen mustard



Think it forms a cyclic cmpd in H_2O + acts as an alkylating agent.



will react with carboxyl, phenol, imidazol, amino Hydrogens.

Most effective vesicants have two side arms may cause cross links. However one arm mustard do have mutagenic effects.

N mustards are solid, inactive in acid ∴ much easier to use.

Other compounds have not been studied so widely eg peroxides, formaldehyde.

formaldehyde has two OH groups (hydrated) able to react with active H's of bakelite.

diazomethane in Hereditas by W——

ketene by Rappaport in neurospora.

acetic anhydride, acetyl chloride, ethylene oxide

He thinks any alkylating agent does it.

Carcinogens - Hydrocarbon derivatives can cause tumors

Radiations can cause cancer as well as mutation but might be due to non specific irritation.

Action of chemical carcinogens may be indirect through a derivative ∴ hard to study.

Might be specificity of action.

Mustards do produce cancers if sufficient dose without death.

Leukemia can be treated with mustard as it especially attacks rapidly growing cells - fear possibility of mutant descendants

Phenol - dissect out ovaries of Drosophila and dip them in phenol then implant them but no definite known effects in bacteria.

No desoxycholate, acriflavin (esp good example), Pyronine B (complicated by clumping effects, stains RNA) by Witkin. but methyl green which stains DNA does not.

NaCl claimed but believed selective.

Interclonal variation.

A. One clone may modify or suppress growth of another clone. - Antibioses - not genetic.

[Gratia & Fredericq. Antagonism in cultures of enteric bacteria. Suppl. #4 Rev. Belge path & med exp. V 19 1948

colicins - antibiotics against one species of bact produced by another - not phages. - there are mutants to resistance to colicins. - there are about 12 distinct colicins. There is cross resistance between some colicins and some phages. Coating bact with antigens protects ~~for~~ from colicins. Often filtrates of killer strains show known effect.

Phage - may be considered an always fatal external parasite. - at least the lytic phages are always fatal; but there are others which do not cause death of all cells attacked - in these virus and cell can grow together - lysogenic phages. - phages transmitted intracellularly during fission. Salmonella and Staph are particularly often contaminated with phage. May be that every Salmonella culture from natural sources is contaminated by at least one phage. This amounts to something similar to a change in genotype or a carrier state.

[Williams-Smith. J Hygiene 46 82-89 (1948)

On some Staph the resistance to phage is detd by phages already present not mutations

A ~~mixt~~ mixed culture ~~==~~ each resistant to a different virus will become resistance to both by cross infection. Some cells are lysed but those that survive are predominantly doubly resistance. Terms this infectious transmission.

~~Pneumococcal~~ Pneumococcal transformations.

Mucoid → Smooth → Rough.
with capsule or R or Extreme R.

Occasionally you get unstable S form which will revert back to the M type from which it came.
Some strains however have completely lost this property.
Griffith found that S strains that did not revert would give mucoid types when grown with killed M vaccines. Did it in vivo only.
Main diff from phage is in chemical simplicity. MW of this about 500,000.

Davis

Syntrophism - feeding of one strain of bacteria by another growing near it.

Trying to get penicillin resis. mutants but found that they would not grow in the presence of high conc. of wild type or killed wild cells. (by irradiation)
Took $10^{4.5}$-10^{7} cells of wild type / cc to have effect.
Could be 'syntrophism'.

5 True delayed mutation
4 Phenomic lag.
2 Segregation from normal nuclei.
3 Segregation from lethal nuclei.

Did get mutants if the irradiated culture was grown a while first. These mutants were not masked by adding culture irradiated but not grown. Therefore it could not be synthophism exerted by ~~not~~ non mutated cells.

He seems to like phenomic lag. i.e. takes time for new genotype to express new phenotype.

He doesn't think it is segregation because he gets pure colonies of mutants growing immediately after irradiation ~~then~~ on limiting enrichment.

This does not rule out ~~the~~ segregation from lethals. And may still be a considerable number where normal has overgrown mutant.

This idea of trypt. required to allow establishment of new phenotype.

Figures it may be same effect as effect of large inoculum. Need accumulation of substance in media. But growth of other cells nearby doesn't help.

This rules out segregation from lethal mutation.

He says these may not be in protein equil as higher animals as they have no sig't limit; they may not have free trypt in cells.

(eg coli trypt. requir. a good one for ave work)
2-3 always in 10^8 mutants in without irrad.
If irradiated to give 10% survivors get twice as many mutants and even more if grown in trace trypt.

Infective transmission again.

Sometimes bacterial virus causes very little damage even on first infection - eg Bruce White found virus attacking Rough vibrio cholera.
Can be found
1 Secretes virus which can be detected on lyso-sensitive indicator strain.
2 Resistant to lysogenic viruses and to related strains after infection.

Staff infected with virus (C) behaves exactly like mutant to resistance to virus B. There is a little lysis during infection with C - the only distinguishing factor. Virus C would appear like a transforming factor except for the small amount of lysis.

Maybe viruses are wild cytoplasmic genes or genes tame viruses.

K (killer factor) in paramecium was thought to be a plasmagene. It could be passed only by copulation. it kills other nearby cells.
But kappa can now be seen as granules. and other paramecia can now be infected by crude mass of killer cells.

Harry Taylor. mutations of transforming principle

plasmagene.
cytogene.
virus.
transforming principle.
viroid
genoid
plasmid

} not distinguishable
infectious hereditary agent.
transmission independent of genes
show some effect on phenotype.
regularly transmitted to offspring

Some bacteria are almost similar. in cockroaches they are necessary to insect and cannot grow alone.

Genetic Recombination in Bacteria - Sexual Cycle.

~~Here he does not refer to~~
Complex mutations affect more than one factor by one step
Multiple " " " " " ~~more~~ more than one step.

Bacteria might fuse but morphological differences would be slight.
Used E coli K-12.
M+ T- or M- T+ remained pure if kept separate.
In neurospora if these were crossed you got parent types plus two other combinations in variable frequency
Easy to find M+ T+ in presence of others - very sensitive
(Prototrophic - nutritionally like wild type strain)
He did get prototrophs (They could be result of exchange or by reverse mutation. Do this by using stable strains or use double mutant parents which require two substances.

M- is stable
the others revert
about one in 10^7

eg B+ M+ T- L-
B- M- T+ L+.

Mutations governing these factors are independent. Possibility of double mutation is 10^{-14} ie one in 100 l. of dense cultures - insignificant. Have not been observed in pure clones.

Other possible mechanisms of prototroph formation.
1 Fusion followed by gene exchange.
2 Secretion of infective particles from A to infect B.
3 Mere contact without material exchange - too metaphysical

He hunted for infective particles smaller than bacteria cell. He & others could not find any.
Can transmit entire genotype ∴ this infective unit would have to be very complex
Would also expect the other ~~three~~ kinds of recombinations and it is possible to find these.

$\begin{matrix} B- \\ C+ \end{matrix} > M+T+ < \begin{matrix} L- \\ L+ \end{matrix}$, $\begin{matrix} D- \\ B+ \end{matrix} > M+L+ < \begin{matrix} T- \\ T+ \end{matrix}$ etc.
4 kinds 4 kinds.

. These are all found but can come from reversion.

B- M+T+L- would require 2 spontaneous mutations

Uses extra characters to study - eg phage resistance Lactose - . Can see if these are exchanged along with ones we are selecting for. - get four kinds with B+M+T+L+ two of which are parents. i.e V_1^R Lac-, V_1^S Lac+. These are unselected marker. Look for changes in those cells that are selected for possible recombinants

Also used Streptomycin^R and azide^R as character for selection for recombinants. - select for $S^R Az^R$ and look for other recombinants - this may include some spontaneous mutation which will have markers of the ~~host~~ parents. - in practice out of 100 isolation 93 were recombinants 6 resembled Az^R parent. most of these will be simple mutations. one could have been by mutation of $S^S \to S^R$.

S^R - 10^{-10}/cell division
Az^R more frequent

All the possible recombinations occur but not with equal frequencies. - Mal+ & Xyl+ stay together most of the ~~type~~ time. - i.e if you combine Maltose+ Xylose - & Mal - Xyl+ the recombinants are mostly ? Mal+, Xyl+ or Mal - Xyl- - Evidence for linear arrangements of genes. This however does not seem to hold for all markers. None behaves independently.

Since you select for certain factors you may find that some other factors very near will be recombined in all cells selected.

Check it with opposite markers in parents. In selecting for M+ you usually get ~~also~~ recombinant with same S character as occurs on the M+ parent regardless of whether it is S^R or S^S. Therefore it does not depend on difference of mobility between S^R + S^S

The fact that the entire genotype is involved and that units can go in blocks one must assume if infectious particles are the method the particles must carry whole genome. i.e would be gametes these may be round cells or specialized ~~str~~ structure. There has never been any example of infection with nutritional characters.

If there are three types of cells in culture the

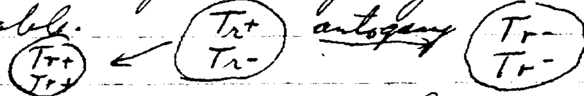
recombinants will have character of only two parents. Recombinations are too rare to find double successive recombinations. therefore infective units cannot be in small parts of genome.

Davis separated two strains on opposite sides of sintered glass filter and washed fluid back and forth. Small particles would have swished back and forth. No prototrophs were found ∴ particles must be several times larger than phage.

In many microorganisms there are + & − mating types but there doesn't seem to be any diff here.

About one recombinant in a million. But this is one a few thousand collisions by Brownian Movement.

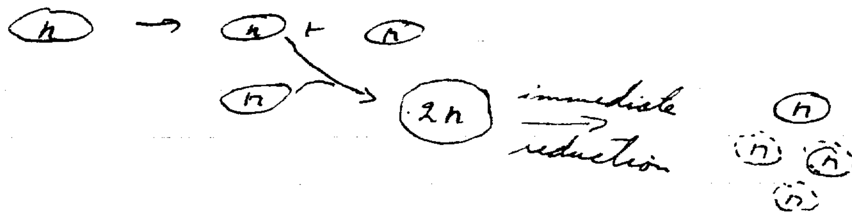
If E coli were diploid it would be necessary to have two mutations where new form is ~~sti~~ recessive.
In some bacteria it is hard to get mutants these might be diploid.
It may be that by "autogamy" one allele would take over not probable.



However if you cross A⁻ B+ lac− and select prototrophs
A+ B− lac+
each colony may be lac−, or lac+ but will be pure one or the other.
If they were diploid on fusion you would expect one genome to exchange. Then all ~~diploids~~ 1st generation recombinants would be heterozygous but you never get a recessive showing up in lac+. If diploid you would get mixed colonies. There is not even time probably for a split before plating.

Probable system - common to algae etc - "haplobiontic"
Vegetative cells are haploid and can reproduce by fission all haploid.
During fusion a diploid is formed but this does not persist for any length of time. Usually reduction gives 4 cells but we don't know if more than one survives

209 D Hilgard
3 PM



No evidence has been found for sexuality in E coli; B.

Only evidence for more than one cell formed from diploid is very rare mixed colony.

Frequency of prototroph formation is nearly that of zygote formation.

Yeasts - some mostly haploid some mostly diploid. The strains mostly diploid can be maintained haploid as it is heterophthalic, when you have a pure strain of one sex.

E coli can be obtained diploid. - If you get mixed prototrophs in one colony it is probably from diploid. - This is from diploid that does not segregate immediately - this can be found by lactose negative thrown off if it is unstable - if stable it would not show so easily. This was once found - gives variegated colonies. Most cells from this give pure colonies but some give variegated. This could arise from extreme mutability. But in this ~~if~~ it was found that the pure descendant colonies were not all prototrophic. It is not a case of two nuclei as these would not recombine and would give only two kinds of descendants.

but all possible 2 recombinants

Phenomenon is repeated if you use descendants of this colony in subsequent crosses. If crossed with another colony 10-15% of ~~progeny are~~ prototrophs formed are variegated.

Similar effects have been noted in yeast.

There are several different forms of Lac⁻
Lac_1^- & Lac_4^- are very closely linked very seldom cross. Can pick crossovers as Lac^+ when parents are Lac_1^- Lac_4^+ and Lac_1^+ Lac_4^-. The resulting Lac^+ may be diploid or crossovers. They occur about one in a thousand and about half are diploid.

In this colony has center of diploid Lac^+ outer part

is too. These occur 1000 instead of 10 with those that have the Het factor - ie mutants that form more mutants.

There are complications.

Viral Recombinations.

Lederberg: methods of preparing mutants. Methods of Medical Research vol. 3

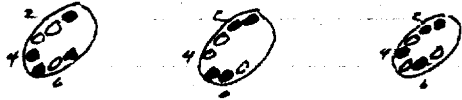
2 groups Luria & Dulbecco.
Hershey & Rotman-Sussman.

Luria gave u.v. to T_2 ~~you~~ got more plaques if mixed with bacteria in high conc. than if phage is dilute. He ~~x~~ interpreted this as the presence of particles no longer able to multiply singly.
Suspension more active if multiplicity of infection is high.
Assume phage composed of many genetic units each of which is essential to action. If two particles each with a different unit inactivated attack one bacterium ~~the combin~~ they fall apart into units and a complete set is present.
Confirmed by kinetics. - Compute the fraction of bacteria doubly infected or more, fraction of phage with one inactivated units - compare to number of active plaques.
There is no inactive phage released : not just a crossing over

Hershey got similar evidence with visible marker.

~~x~~ Aug 28
Different members of T_2, T_4 & T_6 series can reactivate each other as above. - Indicates that they are closely related.
More than two particles can participate.
This is different from bacteria. - 8-10 units may be reorganized to give one of viable.
This is evidence either for disaggregation of units in viral particles or very many recombination. More likely the former & this is evidence for the disaggregation theory of multiplication.

These multiple recombinations done with heavily irradiated particles with several "genes" knocked out.
There is no decrease in efficiency of reactivation though one would expect it if it were several successive recombinations.



eg no pair of these could ~~alo~~ alone form an active particle.

<u>Hershey</u>.
A much more direct method.
Recombination between visible markers. The number of possible markers is rather small - only characters having to do with nature of plaque & lysis.
One such marker is m which gives a very small plaque. - another is h which can lyse a normally resistant bacterium., r - rapid lysis - lysis not inhibited by other phage outside particle as in wild r^+ type. - another mutant is heat resistant, another does not require ~~s~~ tryptophan for adsorption, serological mutants are another possibility
Can get recombination from hr^+ and h^+r pairs got hr and h^+r^+ progeny as well. He found the h factors closely linked to certain r factors there are ~~sev~~ several different r mutants. Can cross $r_1^+r_2$ with $r_1r_2^+$ and get wild type and $r_1^+r_2^+$ Identify the latter because it gives no wild type when crossed with single r mutant.

done by isolating phage released from single bacteria

20-25 r mutants have been found. - Never yet found and recurrences in r's from different sources. By probability this means there are a total of at least 200 possible r mutants which seems very unlikely.
Most of the yields contained only parental types. there were many more hr crossovers than h^+r^+ as would be expected from ordinary type of crossover.
This may mean that breakdown on reproduction into groups of characters not into individual characters. The difference in hr or h^+r^+ ~~no~~ numbers

may be due to lack of survival of h⁺r⁻

Sturtevant

or zipper theory - growth of new units proceeds linearly along a "chromosome chain. the partially synthesized "chromosome" may then move over to another pattern. This would allow one recombinant type and not the other. Would to allow synthesis of h gene on one pattern r on another. - Similar to Bellings theory of crossovers. - No evidence. This does account for linearity and for hanging together of certain units

Yields of recombinants in T_1 have been very small but number of markers is much less.

In crosses between T_2 & T_4 etc the recombinations go much more in blocks than with one species.

Burnett Recombination has been found with influenza virus - using neurotropic markers - ability to grow in mouse brain - and also serological markers - not yet published.

Virus Mutation - Luria No 25.

- Host range phage mutants. - showed they were not just induced.

Can also study mutants in single bacterial cells - gives evidence on method of reproduction: Get Poisson instead of clonal distribution - not by binary fission.

Beadle & Cumming No 34

Neurospora crassa.

- fungus produces ascospore covered with fine lines.
- an ascospore has two identical nuclei - which are entirely equivalent. - to activate spore you have to heat to 60° which also kills all vegetative cells. - simplifies matters. Can grow at 4 mm an hour. Aerial mycelia become pinched off into small round cells - (macro) conidia. - they can reproduce - no genetic change. - microconidia also but less frequent. - come out singly from a special structure. - smaller spores. - these contain only one nucleus - macro have several.

In hyphae the septae are perforated and nuclei can pass through.

mycelial anastomosis — If two hyphae of of different genetic types come together they will fuse and nuclei mix. - hetero-caryons.

You may find mutant nuclei present with others in hyphae. These may sort out on sporulation and look like mutation - Dual phenomenon.

Aug 29
Viruses 1950
Caltech Book store
$2.50

Coenocyte - any structure with multiple nuclei.

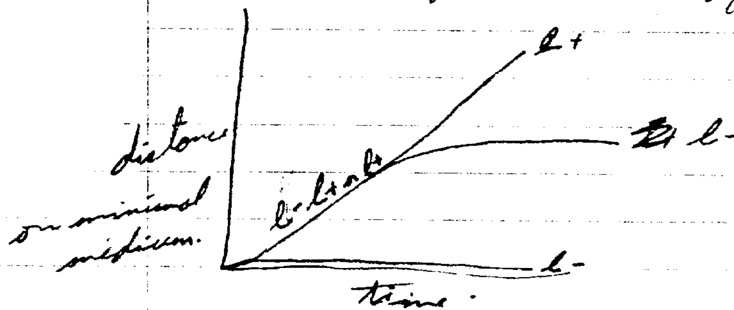
A heterocaryon between two different biochemical mutants has positive properties of both.

Can use this to find out whether mutations for requirement of A are at same step or not.

Can put two mutants together on medium and only the heterocaryons will grow if mutation are for different points.

A heterocaryon with L- + L+ on medium with leucine will result in repression of L+ mutant.

Can compare rate of growth of different mutants.



This would seem to be a disadvantageous type of evolution.

In most biochemical mutants both nuclei of heterocaryon get along together the one unable to synthesize something living of that produced by the other.

One factor heterosis.

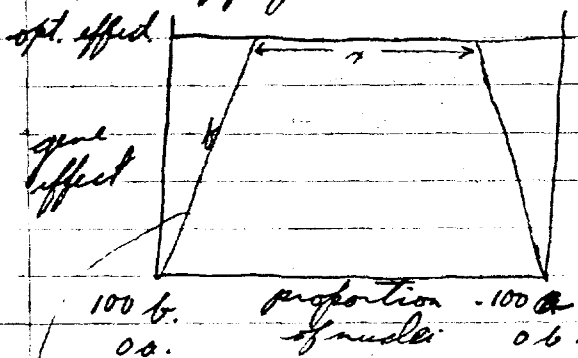
strains pab+ sfo- (requiring sulfonimide).
pab- sfo- (mutant of above unable to synthe. pab.

A heterocaryon of the two above grow without either added. The concentrations of the two nuclei will adjust themselves to produce optimal amount of pab. Mechanism of adjustment of nuclear ratios not well understood - may be simple selection of hyphal tips with best ...

Alternatively there might be some controlling mechanism. - may be by localization of effect close to nucleus. i.e. if inositol is less if inositol synthesizing nuclei exert most effect locally then they will grow better than the inositol - but he doesn't like this idea.

In a heterocaryon $A^- B^+$
$A^+ B^-$

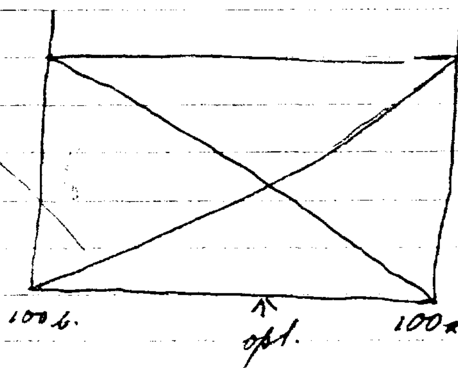
If both factors are very effecient - say 10% of A^+ or B^+ can supply the rest.



in range x of nuclear composition you get optimum ∴ there will be considerable fluctuation of ratio.

actual curves are not known.

If factors are inefficient.

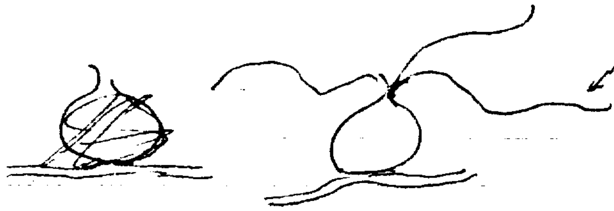


A single optimum point but much less than that of wild type.
Ratio will be stabilized.

Can conclude that factors are inefficient if best growth of heterocaryon is less than wild type.

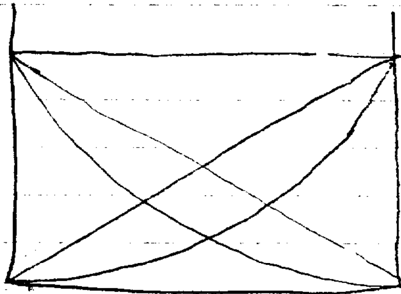
Homocaryons from hetero.
1 By cutting off small tips in which all nuclei are one type.
2 By conidia esp micro conidia. - can get ratio from these.
3 By sexual stage.

Sexual part of life cycle.
- zygote only between the two opposite mating types. - either type can form protoperithecia.



if these trichogynes contacts any part of vegetative part of other type it ~~bo~~ becomes fertilized. → 4 diploid ascus initial cells. one of these undergo meiosis → four ~~~~ haploid cells. These by mitosis → 8 ascospores. They appear arranged linearly in ascus.
When mature nucleus divides again → 2 nuclei in each spore.
- Even if from heterocaryon the protoperithecia are usually genetically pure.
May be used in analyzing heterocaryons - not used as much now as microconidia.
~~So~~
Sorbose in medium. induces compact colony growth - can do plate test, with it present.
tergitol-7 has same effect - called "paramorphogen"

Aug 30



Heterocaryon effect common in ~~~~ biochemical mutants. When there are different degrees of dominance. If wild type is ~~dominant~~ completely dominant its biochemical effect will be apparent in heterocaryon.

Neurospora tetrasperma - has four spores in ascus. each of which has four nuclei - single spore cultures can produce fruiting bodies - crassa cannot - must have 2 sexes. - tetraspora is not homophthalic but ascospores usually have both + & - in spores. - that is they are regularly heterocaryotic for mating type.
Can get homocaryons from microconidia.
Occasionally find five instead of 4 spores - 2 of which are small. These dwarf spores are usually homocaryotic.

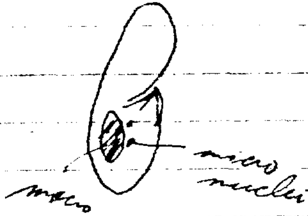
possible mechanism of spore ...tion

Each spore starts from two different nuclei
- each nucleus divides again

Dodge Proc. Am. Phil. Soc. 94: 38 (1950)
You can get hybrids + heterocaryons between crassa and tetrasperma.

<u>Paramecia</u>. by Sonneborn.

Paramecium aurelia.



The micro nuclei are each diploid but equivalent to each other.

In fission macronucleus divide by what appears to by ~~meiosis~~ amitoses.
Micro divide by mitosis → 4
Any part of a macro nucleus cut out by surgery can maintain its function.
∴ Removal of micronuclei from cell does not affect its vegetative growth + reproduction.
i.e. all that is needed is a piece of the macro nucleus.

In conjugation -.
Must be two mating types. - ~~a~~ attachment but not fusion.
Macronucleus degenerates. - each micro undergoes meiotic segregation each → 4 haploid.
7 of these degenerate. - remainder divides into 2.
one of these is exchanged for one from other cell.
Now both ~~of~~ cells have identical nuclear constitution and they fuse to give one diploid nucleus
this → 4 diploid. - two of this give rise to macro nuclei.
Micro nuclei divide → 4. Then fission to give normal vegetative cells. - There seem to be some differences between ~~the~~ the macronucleus may cause differences in descendants. - still not understood
Also no exchange of ~~ct~~ cytoplasm ∴
the two parents may still have differences though nuclei are identical.

factors there may be some cytoplasmic exchange.
Can sometimes keep old macronucleus and then phenotype is like the old.
Sometimes in autogamy the whole process is carried through by one cell but without exchange - this gives rise to a homozygous cell. May bring out a recessive mating type from a heterozygous parent.
clone the ~~p~~ descendant of one of the fusing parents.
karyonide - half a clone - the two ~~half~~ halves differ according to the differences of the macronuclei of F1.
If autogamy occurs in a karyonide there will be differences brought out.

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Any differences between two exconjugants must be due to inheritance through cytoplasm. Difficulty is that so far relatively few genetically determined characters are known.
The killer factor - paramecin is determined in part by the cytoplasm. - Must have necessary gene as well. If you cross homozygous killer & non killer parents both exconjugants will be heterozygous but only if ~~a~~ cytoplasmic factors is present will it be a killer.
In autogamy half will become homozygous killers half will loose killer gene completely and cytoplasmic factor disappears.
If some kappa is passed in prolonged conjugation it takes several generations for sufficient to appear to have effect. If the paramecium are grown rapidly some become non killers. If through autogamy fission is held ~~up~~ up those with nuclear factors will become killers ~~at~~ quickly.

Kappa can be stained & seen. - DNA

kappa my be destroyed by X rays, heat treatment.
X rays knock out micronucleus so animals die when macro is lost in autogamy or conjugation but it can still multiply by fission.
Antigenic types are also extranuclear.
If animal is put with corresponding antigen it will change to another type. This is too common for selection

but is inheritable. Theory type A also has some B C etc antigens in antisera A is absorbed then the other components grow out & become dominant.
He doesn't consider this specific mutation since nucleus is not involved.
Sonneborne now postulates initial product of gene may go in several paths - final product of one path stimulates production of intermediates on its path. This instead of cytoplasmic genes. But no evidence for it.

Yeast.

Normal vegetative phase is diploid.
On some media these ~~dip~~ will sporulate → 4 spores in an ascus - each is haploid. Sometimes these germinate & fuse in ascus. but it is possible to get haploid cultures.
Usually two haploid cells fuse quite quickly.
~~Cells of one ascus are of one mating type~~
It is possible to get heterophthalic strains of yeast.
You may have mutations from one type to another.
In cerevisiae the inheritance of mutations is regular Mendelian type.
Harder to work with because spores are so small.
If you get hybrid of cerevisiae with carlsbergensis you get very irregular behavior - all spores in one ascus may be of one type. Some, therefore, discard the regular theory here - he doesn't - just aberrant behavior.

Lindegren & Spiegelman: Me - able to ferment melibiose after adaptation. me - not able.
from a heterozygous parent you would expect two of each in one ascus.
If the parent diploid was actively fermenting melibiose there would be some of the enzyme present. Perhaps this would keep replacing itself without gene if melibiose was continuously present. They found this so in a number of cases. On removing melibiose those without gene should lose abilility to ferment.

(mc Mc) → melibiose (mc) (Mc) (mc) (mc)   glucose   (Mc) (Mc) (mc) (mc)   melibiose   + + − −

This was taken as evidence for cytoplasmic inheritance in yeast.

However it was found that the ~~mutable~~ number unable to readapt varied with time on glucose, glucose depended on lack of ~~(certain nutrients)~~? N in medium with

If you did cross in presence of glucose you got an irregular number of those able to ferment.

All in a mess at present.

Ephrussi ~~found~~ studied small colony formation in presence of acriflavine - many large colony cells become small colony - continued in absence of acriflavine. May be specific induction of mutation.

Small colonies have no cytoplasmic ~~[illegible]~~ granules necessary for aerobic respiration. (similar to mitochondria)

Evidence but not proof for cytoplasmic system.

Might mean that mitochondria of higher animals may also be self perpetuating.

This about completes the course. Tomorrow is just recapitulation.

Microbial genetics.

Questions.

(Margin notes above "genotype": hereditary / intrinsic / permanent; above "phenotype": outward appearance / transient.)

a. distinguish between genotype and phenotype

b. What is a mutation?

c. Distinguish (i) sporadic mutation.

(ii) specific induction of mutation.

(iii) non-specific " " "

and give (hypothetical) examples.

(d). What is the argument for the sporadic nature of phage-resistance mutations as given by:

1 Newcombe (Nature 1948)

2 Luria & Delbruck.

3 Burnet. (quoted in and in lecture)

(e) Distinguish "mutation" and "mutant".

(f) Why is the number of mutants per culture a poor estimate of sporadic mutation rates? Mention a method of which avoids this difficulty.

(Margin note: because is hard to get [illegible] value)

(g) In what units are sporadic mutation rates expressed.

mutations / cell / division.
second.
fission cycle.

(Margin note: use median instead of mean. null tube or use method.)