<< 1940

Research Ideas.

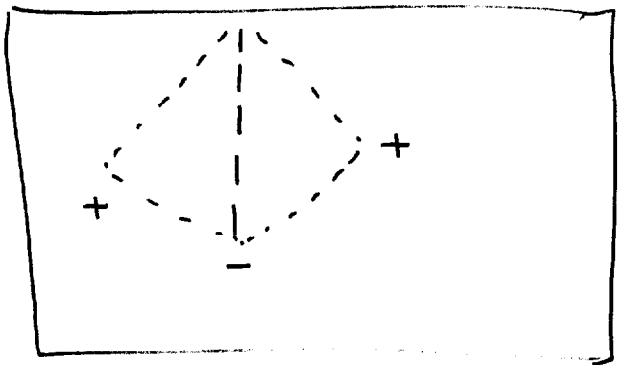
1. Implant an egge or emryos of frog in abdomen or onto omentum. Determine influence of 'old' environment on growth of embryonic material, and vice versa. 1. Look for rates of development of the embryos, and possible development of teratomata.

2. Tissue culture of egg cell: in Arbacia, using Ca free seawater if necessary to induce separation of blastomeres, attempt to induce indefinite proliferation of the egg cell by using various media. This should be wothout differentiation or organization.

3. Culture of grasshopper testes--- whatvinduces the spermatogonium to start its cycle of spermatocyte and differentiation into spermatid Can sperm-formation be induced in vitro? Egg?? Serious practical and bio-philosophical consquences:: the perpetuation of the germ without the Soma.

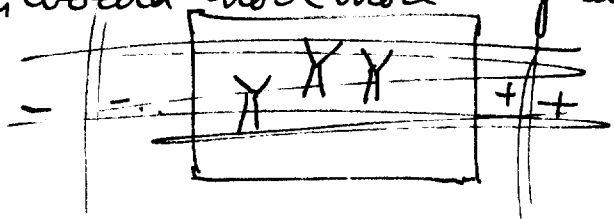
The influnece of respiratory inhibitors on the mitotic figureL Look for a material in which the mitotic funetion is pronounced, and which is amenable to reppiratory and chemical analysis. Even the old one of cyanide on onion root tip would be intersting. Work out a case here even now???Why bother to introduce the complication of colchicine.

Repeat the electridynamic experiments of McClendon etc, and determine whether the root s really remain alive. One presumptive test might be the assumption of the c-figure on subsequent treatment with colchicine, Reversibility is the impirtant factor. If there is a fairly simple electrical polarization in the root tip it should be demonstrab as follows

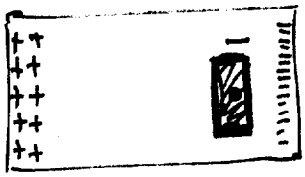


and there is a rigid framework.

If charges balance, there should be no movement of the figure as a whole. But catch the chromosomes themselves - how? Used colchicinized cells. e.g. The presence of smaller mobile ions which would move more rapidly would polarize and neutralize the imposed field.



What does the anodic movement of chromosomes mean? That they bear a net charge.



A significant movement also implies a considerable permeability of the cell walls to all the small, mobile ions in the cells. The electrical apparatus should not be difficult to construct. Exact impedance measurements would be difficult. A high capacitance term implies a low permeability to ions. Still the exps. have to be repeated.

The Mechanism of the Production by Radiation of Chromosome Aberrations in Tradescantia.

DE Lea and DG Catcheside, J. Gen. 44:216-245 (1943)

1. Thesis (Sax). "The primary breaks formed by the radiation is considerably in excess of the total number observed in aberrations of all kinds, and that the majority recombine in the original formation after existing free for a limited period during which interchanges are possible."

a. Assumes that radiation is administered in a short dose at high intensity. Then simultaneous production of breaks. Let n_0 = number of initial breaks, and in that case time. If the number of reformations is large, the computations of n from these is accurate. Then $n = n_0 \cdot f(t)$ where $f(t)$ does not depend on n_0 since reunion is unaffected (relatively) by other breaks.

The rate of interchange formation $\frac{dI_n}{dt} = k \cdot n^2$. As is a bimolecular reaction.

$$\therefore I_n = \int \beta n^2 dt = \beta n_0^2 \int f(t)^2 dt. \qquad (1)$$

Over the entire period, $I_n = \beta n_0^2$; since $n_0 \propto$ dose, $I \propto \text{dose}^2$

We assume that the average time τ elapses between break and reformation. considering the rate of reunion as a first order reaction. (which is ill understood anyhow. Assume, however, that $\frac{n}{n_0} = f(t) = e^{-t/\tau}$. If the dose is given at I r per min. the rate of formation of primary breaks is kI

$$\text{then } \frac{dn}{dt} = kI - \frac{n}{\tau} \qquad \text{if } I = 0 \text{ at } t = T$$

$$n = kI\left(1 - e^{-t/\tau}\right)$$

$$n = kI\left(1 - e^{-t/\tau}\right) e^{-\frac{(t-T)}{\tau}} \qquad \text{then in (1)}$$

$$\int \beta n^2 dt = \frac{1}{2} k^2 \beta \tau (IT)^2 G \qquad G = 2\left(\frac{\tau}{T}\right)^2 \left[T/\tau - 1 + e^{-T/\tau}\right)$$

for for a dose $IT = D$, $I_n = \text{dose}^2 G\left(\frac{T}{\tau}\right)$

By fitting various data to these curves, the values for
τ of 3.3 min. (Cambridge) and 4 min (Harvard) are obtained.

Strauss & Goldstein — Zone Behavior of Enzymes. — J. G. P. 26:559-583 (1943)

Consider:

X = substance combining with

E = enzyme.

and Sp = products of dissociation yielding E. $E + X \underset{k_2}{\overset{k_1}{\rightleftarrows}} EX \xrightarrow{k_3} E + Sp$

If $k_3 \ll k_2$, X is a reversible inhibitor.

Consider cases where $k_3 \to 0$.

Then let X = I for inhibitor and X = S for substrate. If v is velocity of substrate breakdown

$v = k_3(ES)$. If S is in large excess, $ES \doteq E$ and $v_{max} = k_3 E$.

By the presence of I, $v = k_3(E - EI)$. $\frac{v}{v_{max}} = 1 - \frac{EI}{E}$ if $i = \frac{EI}{E}$,

$i = \frac{v_{max} - v}{v_{max}}$ = percentage inhibition. If $k_3 = 0$, $E + I \underset{k_2}{\overset{k_1}{\rightleftarrows}} EI \overset{k_3}{\underset{R_1}{\rightleftarrows}} P$ $K = \frac{(E - EI)(I - EI)}{EI}$

$EI = iE$, $I = \frac{Ki}{1-i} + iE$. (3B) since iE is combined inhibitor, $K\frac{i}{1-i}$ = free inhibitor.

For simplicity, express (E) as $\frac{E}{K} = E'$ $I/K = I'$

$I' = \frac{i}{1-i} + iE'$ (3A) For E' small, $I' \doteq \frac{i}{1-i}$ and inhibition is a function of (I) only. For E' large, $i = \frac{I'}{E'}$ (3C) .... These are defined as Zones of enzyme behavior.

Curves are given showing the ranges where, for given fractional error, eq. 3A or 3C may be used within Zone C, dilution has no effect, in Zone A only amount of dilution. The dilution effect is investigated in a simple algebraic manner. There is apparently a close confirmation in the exp. results

$I' - iI' = i$ $i + iI' = I'$ $i = \frac{I'}{1+I'}$ Do this approximation valid for large i yes. i ≠ 1

Marshak, A. The Effect of X-Rays on Chromosomes in Mitosis. PNAS 23:362-369 (1937.) (Deaconess, Boston)

1. In A. cepa, P. sativum, V. faba, after irradiation there is a peak about 3 hours after irradiation, with a gradual return to normal. (how desirable?) - non-viability following [illegible]

2. The % normal anaphases are simple exponential functions of the x-ray dose. The slopes are identical for 120, 180 and 400 × 10^3 volts. ∴ The ion-pair rather than the quantum is significant. I.E. it is not the energy of the quantum, or its products that is significant, but the number.
[Compare response to fluorescence]. This is essentially all that is stated

3. Curves for the inhibition of mitosis as a function of time after irradiation parallel those for chromosome abnormalities. "Mitosis is inhibited in or at the end of the resting stage since division stages disappear and there is no immediate killing of cells. Therefore cells in anaphase at the peak of → inhibition and abnormality curves were in early prophase at irradiation.

4. Inhibition of mitosis does not depend on chromonemata length. (~~Source~~?)

5. Slope of ~~inhibition~~ "survival" curve varies with total metaphase chromonema lengths. JH 27:459 (1936)
In Vicia & Pisum, ratio of slopes is 2.5, of lengths 2.3 with [illegible]

6. ∴ Cross-section area of sensitive volume is same.

7. Sensitive volume is of order of size of x × 10^{-21} cm. and diam: 10×10^{-8}

See JGP 19:179-198 (1935)

Fano, U. On the Interpretation of Radiation Experiments in Genetics
QRB 17: 244-252

1. Hit-Theory, or Target Hypothesis:

a gene-mutation is produced by ionizing radiations whenever some primary phenomenon, a hit, occurs within a certain region of space, the target.

2. Ionizations normally distributed are considered the primary phys. phen. Assuming random distribution of ionizations, the probability of producing an ionization within the target, and therefore the frequency of mutations is proportional to total ionizations. Therefore the number N, of mutations in N_0 Drosophila specimens is; by the "dosage" D.

$$N = k N_0 D$$

6. If D is measured as the ionization produced per unit volume, N should be independent of γ and of time distribution.

3. Freq. of ionizations within target is given by $V_{target} \cdot D$ where $D \propto \frac{\text{ionizations}}{\text{unit volume}}$. From experimentally determined k, therefore, target size can be estimated, and has been found as a single mutation, as 1 mgm $\simeq$ 1000 atoms.

4. When distance of successive ionizations is less than the target distance two ionizations occur in the same target, but yield adjacent mutations. This is called saturation effect. In a few cases this has been found (or claimed!) with mutations, a saturation effect for sex-linked lethals is found

The total volume of targets can be found from 3. and from 4 the size. The total number is thus determinable. For Sea, this was ca 1860 splines.

If each gene is capable of mutation, this represents number of genes.

The targets thus found are of same order of magnitude as those for individual visible mutations. Sea's estimate is ratio of frequency of all sex-linked lethals to that of individual mutations.

"The broadest principle of hit theory is that
$N = k N_0 D$ is determined inherently by the biophysical process.

That this has meaning requires: (1) all or none primary phenomenon. (2) genetic material of different species is identical (3) the law is valid down to low dosage and long time. k then is the probability that unit dose mutates a particular spermatozoon. k is manifestly the effective cross-section or volume. Let us use

5×10^{-13} roentgen. = 1 ionization/cc.

The hit theory assumes that k is the actual volume. It probably may represent also a spatial and a physical probability factor. Latter is quantum-mechanical.

Bibliography
Sea JGen 39: 181-188 (1940)
Muller JGen 40: 1-66 (1940)
Fano CSH IX (1941)
Fraikel + Demerec
PNAS 23: 320-7 (1937)

Then $\frac{\text{ion. vol.}}{\text{vol.}} = \rho V_{sens.}$ The sat. effect may lead to evaluation of V_s

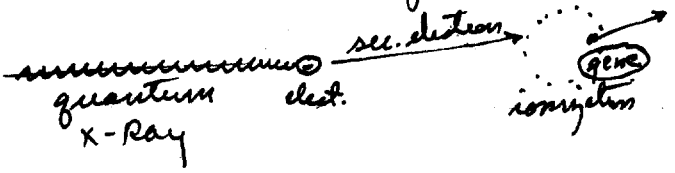
Nature of hit:

a. Absorption or scattering of a quantum and liberation of e
b. Passage of sec. electron across target
c. Production of ionization by a sec. electron in a target.

In case (a) the unit of D. is 1 sec. electron/cc
(b) flow of 1 secondary e./sq. cm.
(c) prod. of 1 ionization/cc. = submultiple of r.

Only (c) is independent of the wavelength, as found in biol. exp. and in chemical exp. It can be considered as a working model, but does not necessarily represent the actual hit.

As $N = N_0 k D$, k is the efficiency of a unit of radiation. "The biological action depends only on the total number of ions produced by all secondary electrons.



Each ionization is an independent cause. Here "excitation" must be considered as well as ionization. One may also distinguish the effects of tertiary electrons.
Concentration effect: cooperative effect when single ionizations are ineffective
Saturation effect: density of ionization tracks. When ion density is small, the effectiveness of particle flows or energy delivered to matter per unit path. The slow onset of saturation may be ascribed to the heterogeneity of the particles produced.

Bacterial genetics

date:
pres '45 ~~45~~ 46

1. Segregation after X-irradiation. Compare mutant frequency ~ plate counts as a function of time. [Correlate ~ cytological studies, particularly ~ treatments such as colchicine, acenaphthene, etc. ?? Hyp. Vegetative bacterial cells are binucleate; haploid. segregating by cell division at each fission. Problem: effective duplicity in the nuclei at time of irradiation. Problem: selection during growth.

2. Statistical correlation in reverse mutations.

3. Sexual recombination genetically studied.

4. Induction of mutations by radioactive labelled atoms in various compounds.

5. Localization of the radio-isotope: Drosophila material containing relatively high activity of radioisotope in a fluorescent solution (e.g. eosin), and water for scintillation. Test ~ a PO_4 ppt. (Look up radiographic technique.) Also, in considering mutations, isolate those cells which have had a discharge. Study lethality of discharge by direct observation.

~~PNAS~~

~~La Nat. Acad.~~ Ed.

1/20/46

Plasmagenic Inheritance

The plasmagene concept refers to the production by nuclear genes of ~~independently~~ self-reproducing units which are topographically and physiologically independent of the nuclei from which they are derived. For the study of plasmagenes, it is necessary to find situations in which (1) nuclei of a particular genotype can be separated from cytoplasm that has been under their influence and (2) the plasmagenes are in some stage of the organism's life cycle eliminated. These conditions have been met with in two conditions: in the maturation of male gametes of higher plants and animals, cytoplasmic elements are largely eliminated, while they are retained in the maturation of the egg; the comparison of the progeny of reciprocal crosses allows this study. In another instance, reported by Lindegren & Spiegelman, plasmagenes have been studied in yeasts. Here, genes are separated from plasmagenes by hybridization, and isolation of segregants. The plasmagene is eliminated by virtue of its instability in the absence of substrate (~~galactose~~ melibiose). These investigators have shown that a plasmagene for the production of melibiozymase is initiated by a nuclear gene, but is propagated in its absence so long as melibiose is provided. The plasmagene is, however, unstable in the absence of melibiose, and disappears. Its reinitiation depends on the presence of the nuclear gene.

The formation of heterocaryons in Neurospora provides material for the study of plasmagenesis. The diffusion of nuclear products across

nuclear membranes is well shown by the growth on minimal media of heterocaryons between different biochemical mutants. It is not now known what these products are: plasmagenes, enzymes, or enzyme products (while) it is possible to segregate nuclei by isolation of (presumably) uninucleate microconidia; however, the failure by this procedure to obtain evidence favorable to the plasmagene hypothesis can readily be interpreted on the basis of an elimination of plasmagenes during sporulation (analogous to sperm maturation.)

It sometimes occurs, however, as a statistical accident, that all of the nuclei at the hyphal tip of a heterocaryotic mycelium are of a single type. The fact that this hypha has grown on minimal medium demonstrates that products from the heterologous nuclei have mixed. The isolation of such hyphal tip's to minimal medium allows conclusions to be drawn as to the nature of the diffusible gene products. [A coenocytic uninucleate septate organism might be profitable! - See Phycomyces.] If these products are the diffusible end-metabolites (vitamins or amino acids), the growth of this isolated hypha on minimal medium should be minimal; if a stable enzyme is formed there should be linear (not exponential) growth of the hypha, until the enzyme is too diluted to be active, or disintegrates. If plasmagenes occur, however, there should be considerable growth.

The only methods now available for determining the homo- or hetero-caryotic condition of a hypha are genetic. Since no genes affecting nuclear characteristics are available, the genetic constitution of the hypha must be determined by labels of mold characters.

It is essential, for obvious reasons, to exclude the possibility of plasmagene transmission of the labels. This can be accomplished by passing through a sexual generation, for characters known to be inherited in Mendelian fashion. Thus if a hypha from an arginine + lysineless heterocaryon is crossed ♀ arginineless of the opposite sex, the absence of growth on minimal from a mass inoculation of ascospores is evidence against the presence of any lysineless nuclei in a parental nucleus. Crossing over of lysineless ♀ arginineless will yield a wild type nucleus, which can grow on minimal. Failing this, a lysineless nucleus can form a prototrophic heterocaryon ♀ arginineless. A converse argument applies to lysineless.

This would, however, be a laborious procedure if each hyphal isolate which grew on minimal had to be so isolated. From Beadle and Coonradt's data it is clear that most such hyphal isolates are heterocaryotic. Fortunately, there exist more facile methods. In independent evidence it is probable that plasmagenes are not produced at the different albino loci 4637 and 15300. This is indicated by the disappearance of color from heterocaryons between lysineless-15300 and 4637 heterocaryons when either nucleus is selected for. If the constituent nuclei of a heterocaryon are labelled ♀ these color genes, heterocaryosis is indicated by the formation of colored conidia; homocaryosis by albino conidia. If this is achieved, the conidia should be tested for prototrophism. If the conidia

grow as minimal, the possibility of genetic modification must be considered. If the conidia do not germinate as minimal, there are either 1) no heterocaryotic nuclei 2) an unusual selective phenomenon preventing the manifestation of them. This can be investigated independently. Another heterocaryon possibility is colored wild type + the albino ~~mut~~ - heterokaryon mutant.

Experimental procedure (A). X-4637A. + Y-15300A.

1. Prepare heterocaryon on a minimal slant. Conidia should be colored.
2. Inoculate these conidia on an agar plate (minimal).
3. Isolate hyphal tip and transfer to minimal. (only a few).
4. If conidia are colored, transfer to minimal plates.
5. Isolate numerous hyphal tips and transfer to minimal medium
   (1) to growth tubes. Atypical rates interesting.
   (2) slants. Tools for white conidia.
6. Test conidia on minimal medium. If they do not grow, test on X, Y, complete
7. If they grow, cross with
   (1) X-
   (2) Y-
   (3) X-Y.
8. Test progeny in mass

	X	Y	XY	
1.	+	−	−	Y plasmagenes
2.	−	+	−	X plasmagenes
3.	+	+	+	still a heterocaryon. ~~transmutation~~
4.	+	+	+ −	Transmutation

9. If "still a heterocaryon" or "transmutated", spores must be ~~rede~~ isolated from XY cross.

2/13/45. This is plenty 1946. GP 1/82

1. The detection of nutritional mutants of microorganisms.

The now classical method for the detection of nutritional mutants consists in (1) isolating pure lines and (2) testing these individually for their nutritional characteristics. Since in Neurospora[1] and bacteria[2] only a small proportion of the cells are mutants, it is evident that considerable labor must be done in (1) for the collection of a few strains upon which (2) will be successful.

A method has been devised for the more facile detection of some such mutants. The method depends on the fact that the growth of a mutant strain is limited by the concentration of its unique nutrilite in the medium. Thus, by supplying distinctly suboptimal (liminal) amounts of the nutrilite in an agar pour, plating the mixture of prototrophic and mutant cells into such a plate. The mutant colonies are often detectable as they are much smaller than the wild type colonies in such a plate. They can then be picked individually, transferred to an optimal medium, and its requirements determined.

The critical problem to be overcome in the application of this method is the elaboration of a satisfactory liminal medium. This has been accomplished on the basis of the simple, if only roughly accurate, assumption that the needs of an organism are in proportion to their composition. Thus, whole bacteria have been hydrolyzed, and added in such quantity that a single mutant (methionineless) can readily be distinguished from the wild type. It was found this that this quantity was adequate for the detection of other, already available, mutants, so that we may reduce

that it will be satisfactory for other mutants. It has not yet been applied to X-Rayed material.

The advantages of this method for quantitative studies, over the old one of picking and testing at random are obvious. Several studies detailed below depend on this method.

A second method may be of more general application, but has not been studied, and is suggested purely on a priori bases. The mixture of mutants + ~~wild~~ prototrophs is washed and plated into a minimal agar medium. Only the prototrophs will develop colonies. after 24-36 hours, giving these a uniform "head start." At this time, an optimal (or specific) nutrient supplement is added. As this diffuses through the agar, new colonies will appear, which can be ~~detected~~ detected at the appropriate time by their size and recent appearance. When they are sufficiently grown, they can be picked and tested. Since this method does not require a carefully titrated medium, it should be particularly valuable in the detection of mutants requiring particular substances.

These methods should be adaptable to any organism which can be "plated out", in particular bacteria and yeasts. Molds should be more refractory.

Analysis of the growth of mutant bacteria

2. The response of a mutant strain to its specific nutrilite affords unexampled material for the study of growth, inasfar as the "master reaction" controlling its rate is identified. E.g. the utilization of leucine. The analysis of the growth rate — nutrilite response curves can therefore be accomplished on a rational basis. The quantitative extent of the intermediary absorption of the nutrilite can be studied, as well as the efficiency of its utilization. Temperature and pH responses also lend themselves to study, both in Neurospora and bacteria. Sufficient has been done ~ leucineless Neurospora to warrant selection of this organism for more detailed study. One may also inquire why different mutants vary in their requirements for the same substance. The lower limit should be the composition of the organism.

This problem admits into that of the composition of the organism.

A corollary to this study is the development of ordinary microbiologic assay methods. Of more novel interest is the possibility of the use of such organisms under certain conditions, for the quantitative removal of specific substrates, ~ applicability in the isotopic analysis of small samples.

Effect of a "final" product on the intermediary syntheses.

5. Genetics of Bacteria. I. Segregation of Radiation-Induced Mutations.

There is cytological evidence that the vegetative phase of bacterial cells consists of a bicaryon. That is, a single, (haploid) nucleus gives rise to two nuclei in each bacterial cell; when the cell divides, a monocaryon is obtained, which by nuclear division gives rise to the next generation's bicaryon. An implication of this is that the expression of recessive mutations will ~~be~~ ~~not be~~ be delayed until one cell division has occurred in order that the mutated nucleus segregate from the prototrophic one. This can be studied by comparing the ~~effects~~ mutant proportion in plating at varying times after irradiation, and correlating ~~&~~ increase in cell number, and cytological changes. Groups of individual clones can be replated also to show the later appearance of the mutants.

As further extensions, attempts may be made to induce cytological changes ~~&~~ lungs such as acenaphthene, ~~fulvene~~, etc., and determine changes, if any, in response to X Ray. Other organisms may be studied by this genetic method.

Ryan, meanwhile, is working on back mutations in multiple mutants (mutual influence of mutation, etc.) studies such as influence of temperature, mutating agents (ethyl isothiocyanate) are best bare here.