Oct 31, 1954.

Memorandum on conversation 10/30 with Clifford Grobstein

He finds induction through thin membranes provided donor and recipient are both present. Ama Caves trace (protein + polysaccharide) on filters if fixed first, but not if stripped off first). Supernates, etc. are ineffective. Discussed in terms of "working organizer."

Obvious idea - to try similar setup with F^+/F^-. (use ultrathin filters).

He brought up aneuploid mutants. Do spinal cord from these patients? [He took for granted that mesenchyme would be competent].

Briggs was interested in suggestion to use Zeuthen's method to "phase" development.

Had long discussion about tumor antigenities. (Should refer on this later).

OFFICE MEMORANDUM • STANFORD UNIVERSITY

DATE: 4/1/59.

TO : J. L.

FROM :

SUBJECT: Conversation Date With Clifford Grobstein

(1) Use vibrating wire to sever connections between donor and recipient cells.

(2) Equivalence of inducer with T antigen.

(3) Use of specific sero types and iso-antibodies as means of discriminating action of donor and recipient cells. This is something to discuss with Nossal or Makela.

10/4/58. Program for receptor analysis.

(a) assay of receptor — competition by extracts?
(b) periodate effect.
(c) improve & futility with periodate?
(d) other enzymes? — select some? lysozyme presumably does not destroy receptor. (Is it serve functioning as a monovalent cation? rather than chelater)
(e) chemical analysis of ♂, ♀ cells.

7/26/53.

Summarize hang-overs
(over trip West ca 7/28 – 8/25)

A. Salmonella. (?? Brunstein).

1. Abortus-equi
[2. Para A (write to Spilsee re I).]
3. Other φ's, groups : kempudorf/22 ; Cherry phage to tetra ; B805. (Andy?) double lysogenic.
4. H(H1) Fla, Flb, ... linkage problems.
5. Phase variability
   a. UV on N97 ph2.   b. phase "exhaustion"?   ✓1050. LI-2
   c. SW1061 (cf. 1051 Q, R.) –
6. Gal pseudo-alleles to screen for Gal? LT7: 481-4 492-5 503-6 LT2: 950 LT22: 307,8; 485-6.
7. O crosses.

B. E. coli

1! Cytology (assistant?)
2. Hfr Gal   SR+ tests; construct diploids. Review Tom's stuff.
3. misc. Gal Lp....   Nod^R other markers not yet located? Stl?
4. Review data on Gal elimin; correlate c Mal elimin? W583 line 945?
5. B mutants to Cavalli (for x Hfr?)
6. Weed stuff.
7. (W284 x)
8. Secondary diploid → hemizygotes?
9. Transduction of Hfr.
10. Recover Mal- W595, etc from lyophil. Use Stl-?
11. Summaries of pedigree segregation data!

Oct 4 1958. 1428-30. Diploid program and stocks.

Wanted well defined Ara, Gal Hfr stocks. But, as shown by HFT tests and Hfr crosses, the only well-typed culture maybe W4270 = Gal$_2$.

1430 A shows some preliminary crosses of Hfr Ara$_3$ Gal$_2$ × Ara$_2$ Gal$_1$

W4270 × W4308 on MAra, MGal.

a rather low yield of Ara$^+$ and Gal$^+$ have been selected. But none of these are vv.

1430 C is strep of W4276 = Ara$_4$ Hfr — purportedly Gal$_2$.

But in crossing tests (cf. × W4265, W4308) it behaves as Gal$_1$.

It is also rather poorly fertile. (maybe no longer Hfr.) Aim line was to isolate an Ara/Ara heterozygote for proof of position effect. Review of 1430A this seems feasible. Maybe better to use Lac selection for this and forget about Gal. Poor fertility provoked chelsea nutrition and uncovered ~~susceptible~~ unsuspected M mutation in the line.

1430B — Miscellaneous crossing tests. Did also isolate 1430 B3e:

W4308 × W4273 : Gal$^+$ Ara^v (Ara$_2$/Ara$_3$)

W 4283 × 4069, 4062 } both Ara^v ∴ $\frac{c}{f}$ Ara 5 = 1, Ara 4 = 2.

Some confusion due to W4178 really having Hfr.

10/3./58 (comm/EML) ☐ JL priorities

(SAL)

Salmonella problems:

spontaneous appearance of arg⁻ among prototrophs parents?

homology of Ara mutants. (use Hfr Ara testers)

homology of Gal mutants - of Gal_6, Gal_2 differences: and diploid productions

colicins.

[2] W1895 x SW1231 ⟶ Hfr? and Gal^+ Lac^-.

{2345 Gal_1 / 3013 Gal_2} ⟶ Lac^+ Ara^+ $\lambda 2^s$ $P22^R$ Gal^+ can't test for

[1] production of more markers in SW1214 = (TM9). Can best use multimarker as F⁻ to allow better testing of ♂ character.

Ara - Gal - Arg.

odd ideas:

periodated cells as F⁻; acridine orange-treated.

select maximum reactivity Hfr types.

infective factors controlling Salmonella fertility (+ or -).

SS — Scientific Journal.

Stat Sci

4/14/06. Resume analyses of chain pedigrees. Interrupted last July.

Where are former précis? Where is summary index to Salmonella?

Monday April 23, 1956. I have long felt the need for more systematic interpretive summaries of current work. Rather than leave general notes scattered or under subject headings, they can be listed here and indexed by subject. For system, I intend to write on these Monday. The following headings are proposed.

(LAB: Current Expts. & Immediate plans; writing; etc.; visits & travel.
Other activity: notices books home & personal.)

L] Main preoccupation is again assimilating notes on "chemics" for paper. Bruce had sent me his near final draft. This has been hanging fire since c. Jan 1955 and has been almost a millstone (cf. corresp.) I don't have any real difference i Bruce exc. my data do not exclude alternative hypotheses of E-particle. It is a horror trying to collate the various pedigrees, each is an individual experiment. I had started this before, but decided to do it over again to be better organised. It is a little hard to get down to this every day because of ridiculously small details to attend to. E.G.: today

talked to electrician to get buzzer installed
also reminded me to write to Kilalaeva re P/Co.
letters to Edwards (re his trip); Pontecorvo, do., Barnes (Harvell, re set/noise chemics)
Davis re errors in our mss; Bradley to see vegetative → aerial hyphae;
Larry Mace directed me to Railway Exps. i me to help cart home bureaux for our home office.
Lewis i Cotham join Crow; repair thermometer for electrician to use; cf. Cassidy re spectrophotometer cuvettes; c. 2 hours on wording for Bertoli; + DCG re recurrent isolations + preparing some cultures to ship out; Newton re interacting alleles in ; phone calls to student employment (for home help!) to Woods re air conditioning lab; from Harold Deutsch re Cousins + Torrian visit; ..... Boris brought in his ms. on sonic effects. See today I have not actually gotten to my own notes. Johnson re penicillinase.

Experimentally. a) S-forms. Penicillin in 20% sucrose broth induces rod → sphere transformation in K-12 strains. Guess c. ⅔ lysed ⅓ spheres. See 1310 for current status + what needs to be done. Prospects are: development of L-cycle (use protein in agar media?); purify of DNA for —x; spheres highly permeable? x—; (for Boris — effects of enzymes on the spheres, à la protoplasts — Brenner, Spiegelman); (for Johnson — mechanism of action of penicillin.

This work has been a casual development, largely motivated by Dienes last paper (J Bact) and a hunch that protoplasts are related to L-forms. Weibull showed stability of protoplasts in sucrose; Dienes et al that penicillin induced protoplasts + he often uses high salt to maintain them! Should check his paper for improvement of medium.

b) — see 1292 — Analysis of Hfrs. Taking up for test of possible non eliminators and to set these up in pair analysis c W2401 ("g").

DCG is doing most routine work to ⓐ secure Lp's, Gal⁻ derivatives and ⓑ F⁻ and mot-Hfr. The F⁻ will be tested × Hfr-1, and after F+ × F⁻ for other evidence of chromosome rearrangement.

Have suspended diploid studies, cf. H406. — EML is screening this one. May prefer to use new Hfr's in further work.

When writing obligations reserve, review angles of 1290 ff on Hfr selection techniques etc. But current resolution: do one thing at a time, at least till routine is fully settled + new assts. are broken in.

— Note nis — see Bristol record re phlorizin, antiglucose. Any literature?

At present time, Marie Wright has retired; two undergraduates (Milae and Kitty von Dubrelzig have been working part time in making up media.) DCG still supervises them + spends a little time on my own program.

NM is working on Lac allelism, following up lines of EML's thesis with help of new techniques (Hfr crossing; later on, phage-transduction). Latest finding today: intransitive allelism of W3133; W3134; W1941. Possible meaning? are the "+" recombinants or diploids?

Home - major preoccupation is planting in garden: anticipate major job on trees this Friday. Finally this summer 2 + some shade. Yesterday uprooted one old black currant stump. My aching back! Trying second one by burning out. Hasn't worked so far!

Bks: Tilsley: Russ. Revol.
Priestley: Hollow where sunlight bent.
Blumenthal - Bookman's Bedlam
Decinique - Arenzo.

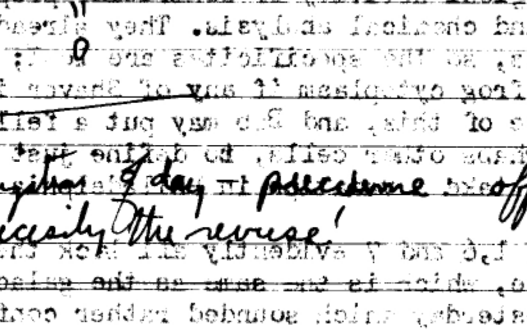
Theater: Morgenstern Salzburg.
Pajama Game (NY road co.)

APR 24 1956 "Bacterial Anatomy" just came in mail. Weibull especially seems to realize the correspondence of protoplasts with L-forms; most people are concentrating on gram positives. After checking Dienes' (2/53 J Bact) and Lavallencie A(P) it is obvious they have hit on penicillin + conc. salt which is counterpart of p + sucrose. So I will compare a balanced conc salt (at c. M/2 which > Lavillencie) with sucrose pen assay. Obvious trick for further cultivation is use of protein-agar. (Dienes anaerobic may mean humid?). Note both Dienes + Lav. emphasize cultivation of L-forms, rather than initial R→S transformation, but Dienes must have this in mind also.

Strategy: what first. Main pending issues are ① Chang's paper ② Hfr types ③ S-forms. Must do one thing at a time. Only reason for pressure on ③ is "the competition" which is poor tactics (to waste your own time to scoop another fellow rather than have him do your work.) ∴ continue only casual study on ③ & focus on ②, complete ①!

Phlorizin: checked some literature (esp McKee Physiol Rev 1945) — on mechanism all is chaos: should be very productive avenue for Bristol but not obvious where bacteria come in.

phloroglucinol. what about phloroglucinyl glucoside



actual organization of day is precisely office routine > lab > thinking & should do precisely the reverse!

APR 2 6 1956

Trees MAY 1 1956 Net (+) last week practically nil. A negligible amount of work on paper (19); was much preoccupied with planting trees + shrubs this last week. EC: put in 6 red pines all yesterday AM as it rained heavily over the weekend & still waiting for birches and honey locust for shade in front. After a very drought[illegible] last year + winter, we suddenly had 3" rain last week.

Also a lot of time on arrangements for Pontecorvo lecture next week, with calls from TMS and him re airfare and Bloomington timing.

Bob Briggs was visiting from Wednesday P.M. which also took some time but was well worth it. He gave 2 talks on his nuclear transplantations, and we also spent a lot of time together, as he stayed over with us through Saturday P.M. Talked a lot about the R→S effects with penicillin + the stabilization by Ca, which seemed to interest him particularly for analogy with pneumococcal nuclear function. One of the more exciting co-stimuli was about centrioles considered for plasmids, which came up obliquely in discussing various experimental designs in re nuclear transplantations. I had not realized how sharply dependent the frog egg was on a sperm "or center", having thought that pricking would activate cleavage as well as the initial fertilization reaction. But Bob had seen that experiments involving, e.g., blood as a reagent did not exclude centrioles; this is of course the answer to Shaver's problem of the "second factor" in parthenogenesis; Briggs was sceptical about the extent of any of the activations with ill-defined material, as Shaver did not score his material very late, and his yields were very low. What excited me was that the activated unfertilized egg would constitute a simple assay for the biological activity of centriolar preparations, qua plasmid, with all the possibilites for genetic and chemical analysis. They already know that Tritarus irradiated sperm will activate frog eggs, so the specificites are low; it is even conceivable that mouse centrioles will function in frog cytoplasm if any of Shaver is to be relevant here. Anyhow we did comverge on the importance of this, and Bob may put a fellow "Subtelny" to the problem of pulling apart sperm, and perhaps other cells, to define just what the activating center is. I would almost be tempted to take a vacation in Philadelphia myself.

Note from Kalckar and Kurahashi: Gal 1,6 and 7 evidently all lack the second enzyme, galactose-1-phosphate-uridyl transferase, which is the same as the galactosemic infants. Esther took a phone call from Herman yesterday which sounded rather confused; I don't understand the constitutivity of the enzyme sequence myself. Anyhow, these mutants form one position-effect group; we have to send them Gal_2 and Gal_8 to see if these are in any way different.

In the lab only one new point: the effect of Ca in stabilizing spheres of K-12 was confirmed; it now remains to 1) review viability of these preps.; 2) biochemical properties— per Boris, and 3) try to grow them out. So far, nothing promising on non-lambda transduction. as L forms.

MAY 1 1956

Ctd Brink has had some remarkable findings lately with R and R^{st}. In crosses of rr x R-, the Rr progeny differ depending on whether the - was R^{st}. The differences persist another generation of selfing! There are similar differences in R^{st} x R-! Brink evidently concludes that R is modified by contact with R^{st} at meiosis, but the modification is maintained in Rr, lost in RR. (How about R'R', where ' stands for the modification). Until the heritability had been shown, I had argued that there was some sort of paternal carryover effect on the endosperm phenotype, not necessarily at the R locus itself. As a further alternative, R^{st} may carry an plasmid which cannot be maintained in RR, but is transmitted through both sexes. The test of this is R^{st}r x RR, examining the Rr's for an affect via the r. This may have to be carried another generation too; this does not necessarily prove cytoplasm, only that ' can be carried in series from Rst to r to R.

Current irritations: the degradation of "locus" from a precise recombinational definition to a loose "functional" unit (per Demerec, Pritchard & Pontecorvo, Winge). Also note that Hotchkiss has turned tables, and discusses "Transduction- a phage mediated transformation" in "Nucleic Acids". This is not so bad; the main point is the taxonomy, not the nomenclature. But suppose Griffith's observation had not been a typical transduction (which was not known or realized for 20 years)?

May 13, 1956 (Sun)/ Pontecorvo visited last week, arriving several hours late owing to fog, and only just in time to meet us at the Hoffman House for a dinner honoring Jim Neel by a local medical student fraternity. But we had tickets to the Dublin Players, to which Ponte' escorted Ann Crow to see Arms and the Man, rather than Neel's lecture on hemoglobins. Neel himself is in the midst of a changeover to a Genetics Department at U/Mich Medical School. He still construes this rather narrowly as human genetics (my own hopes would be for a department having the same relationship to medicine as ours does to Agriculture.). For example, he had projected some tissue cultures studies on human mutant material, but for biochemical rather than any hope of genetic analysis. We discussed Newton Morton's position at the medical school here; Jim had also talked to Mortenson, and I feat there is a growing misunderstanding on Netwon's role in Anatomy. (I also ran into Phil Cohen whose apprehensions are even stronger, and he is going to reopen the question with Bowers. Newt' himself doesn't seem to be strong enough to stand up to these pressures, and he is especially vulnerable just because his research program is in abeyance while he learns laboratory genetics.) It's too bad we don't have a stronger start (in add. to, not in place of Newton-- I just thought of Mitchison as representative of some of the other hopeful directions. This did not jyst come from the blue, Ponte(' told me that Mitch had just done the experiment on degregation of cells heterozygous for H_2 which I talked about at the Ascites meeting, and which I suppose G Klein had also planned.

We had an intensive going-over with Ponte, after which I feel rather pumped dry though this is largely my own impulse. It must still be barely possible to do some mapping from diploid automixis in coli, but the worst problem is breaking into the cycle of circular reasoning on the location of markers. This has been something I had hoped to find the right student for, but he hasn't materialized, and Alan seems less likely than at first. I don't really have much worthwhile data, but now that Gal_5 is out of the stocks, and we can score Lac_1 vs Lac_4 fairly readily by progeny tests, the problem should be simpler. The best designs at first might be to select for crossing-over of near--linked genes. For example, between $Lac_{1,4}$ and V_6, selecting for V_6^r still Lacv, or between $Lac_{1,4}$, or between Mal and S (+s/-r, selecting for +r/-r) and seeing the ditsribution of homozygosis for other markers. But we need the right marked diploids first, and these msy just now be coming through from Lac_4Hfr x Lac_1 crosses, the stocks for which are a by-product of Newton's wOrk.

Experimentally, last week was only a couple of days between vistors (MTW) which concerned mainly more on "protoplasts" (I like Stahelin's term gymnoplasts better). The experiments were rather messy, probably because of haste, and perhaps because summer started abruptly with some muggy 80+ weather. (At least the planting is essentially done now). Viability has been variable, some preps showing at least 50%; others less than 5. No show so far on an L cycle, but haven't tried hard enough yet. It is certain that the protoplasts do make NPGase, but even the control rods are not too happy about the hypertonic sucrose, and it may take some more fiddling with the medium to perfect that, if at all. No more trials yet on DNA --x; it would be exciting to be able to isolate nuclei cleanly fast (as Sol claims he can with megaterium). I have to review my plans carefully on the whole story, to avoid digging toofar into details that will be done over anyhow by all and sundry. For example, it is probably pointless to include controls on NPGase synthesis by rods, which are hardly comparable even in mass assay. Meanwhile, Dotty is pushing some of the routine preparations for study of the Hfrs, e.g., getting motile, F-, Lp^s and Gal selections.

Occurred to me while distracted during Ponte's lecture yestreday (I think most of my imaginative thinking happens when I am paying fairly strong attention to something else, including conversation, often on seemingly irrelevant subjects): I had already planned to test the fertility reactions of Hfr and of F- protoplasts preformed.

If there is a conjugal bridge, however, pairs might be isolated to penicillin medium, and there show full fusion owing to the derigidification of the cell wall. Then, if the co-spheres are viable, we would have started with a fairly certain dikaryon. This would be almost the opposite of Jacob's experiment.

I was beginning to feel fairly relaxed, even with two meetings this summer (Baltimore and Ann Arbor) and the possibility of Esther's leaving for Tokyo, when I had an invitation from Demerec to attend the CSH show. I don't know what to decide about that; I don't particularly want to hear so many papers, and will see most of the people elsewhere. The conceivable pros are : 1) embarrassment at
cf '53 refusing again (with the suspicion that this may be Demerec' intention; 2) possible checkrein on fancies of Demerec, Hayes et al (from a position of disadvantage, and probably futile anyhow 3) it will probably be too damn hot to work here anyhow, and Esther thinks it may be pleasant there 4) chance for sidetrip to ~~Baltimore~~ Bethesda for talks with Law, Kalckar, etc. Contras: too damn many meetings already — talk talk talk; expense and time needed to bridge the meetings, and the composure that goes with it.

MAY 25 1956 As above, eventually decided contra.

We had a delightful experience last weekend at the Illinois SIB in Chicago. We drove down Saturday noon arriving at the Edgewater Beach Hotel at c 3 P.M. For about 10 m. I was helped by the reporter for the Jewish Daily Forward — he wanted to know what bacteria were and why, and where I isolated E. coli etc. I couldn't make him understand what "feces" meant. Some of the other newspaper people have been little better. Previous Thursday a Miss Hamblin from *Life* was in the office but I gathered she wanted to do a "human interest" story on a "typical scientist" — no encouragement! The press agentry in all this has been a pain but it should have blown over by now. We arrived in midst of a notoriously dull business meeting, followed by a "symposium" chaired by Agnes Norris and "dedicated to the Lederbergs" — evidently Luria's idea, partly spoof & partly very touching gesture. Spkrs: Al Koch, Lennox, Lu, Levinthal, Lehninger & Bogard & rather entertaining. I made a point of getting a little closer to Lennox. Carl was silly — along lines of his recent Science article but I had to agree with him in principle: why not, but substrate-induced mutations?!

Dinner covered mostly c Gunsalus who was presiding & turned out to be an excellent M.C. Told none about protoplasts but will have to send Sol a copy of note this week, (just written = 46). Gunny made things rather pleasant: I was rather terrified by a very mixed audience & having to follow the symposium but talked happily briefly about "genes" — i.e. the genes in genetics — bacteria — development & the ambiguity of viruses & genes (transduction of genes; segregation of viruses). Have no idea how it went off. Night & next day at Sussman's & met R C King & wf. for dinner, then drove home Sunday nt.

Thursday visitors night

Sheldon Reaven now at Bethesda

Cell Biology (Carl's accelerated, to learn micromanipulation)

2 1956 (Sat.) Meso ε ND2 Rollins + he called me up Thursday very highly perturbed about the res. on principal[illegible] served. I sent him My correspondence concerning ~~Rollins' preemption~~ preoccupation. The security of his ideas is closely neurotic & Novick seems to have caught the same attitude. My correspondence c. these dates covers the matter, but I lost most of Thursday writing same. I hope Novick comes to his senses: we'll see when he visits here. I really do feel guilty (in a mischievous fashion actually) for not having talked to Sol.

Aaron Novick will have received formal invitation to join Zoology faculty as assoc. professor; he's rather diffident but may come, as I hope & urge.

MAY 25 1956

This week have been completing details on background of proto-hosts & wrote up a survey for PNAS. Now that's over I can concentrate on the genetic hypothesis & let Norton + Sol et al. go into the enzymology of their work. This is now a turning point and important to decide carefully what to do. See analyses of situation infra.

PROGRAMS. 6/27/55.

Salmonella.

Conclude that it is wasteful to spend more time on chemis. Should learn to control wetting of slides + pellicles (more num c methods through dubrosis!

To finish ① Review notes on pedigrees
② " " " H/Fla segregation! More tests for crossover Fla⁻'s?
③ Any more isolations?
④ Pictures.
⑤ Pick up cells for EM? Will need technique for coli pairs anyhow.
⑥ Notebook analysis will doubtless suggest more.

+ Iino - go over extant Fla⁻ for H₂ linkage! of coli/Salmonella mutants microscopically

WRITING! Amherst — Chemis — BOOK. — Bact Rev?
Misc. papers.

MLM!!!.