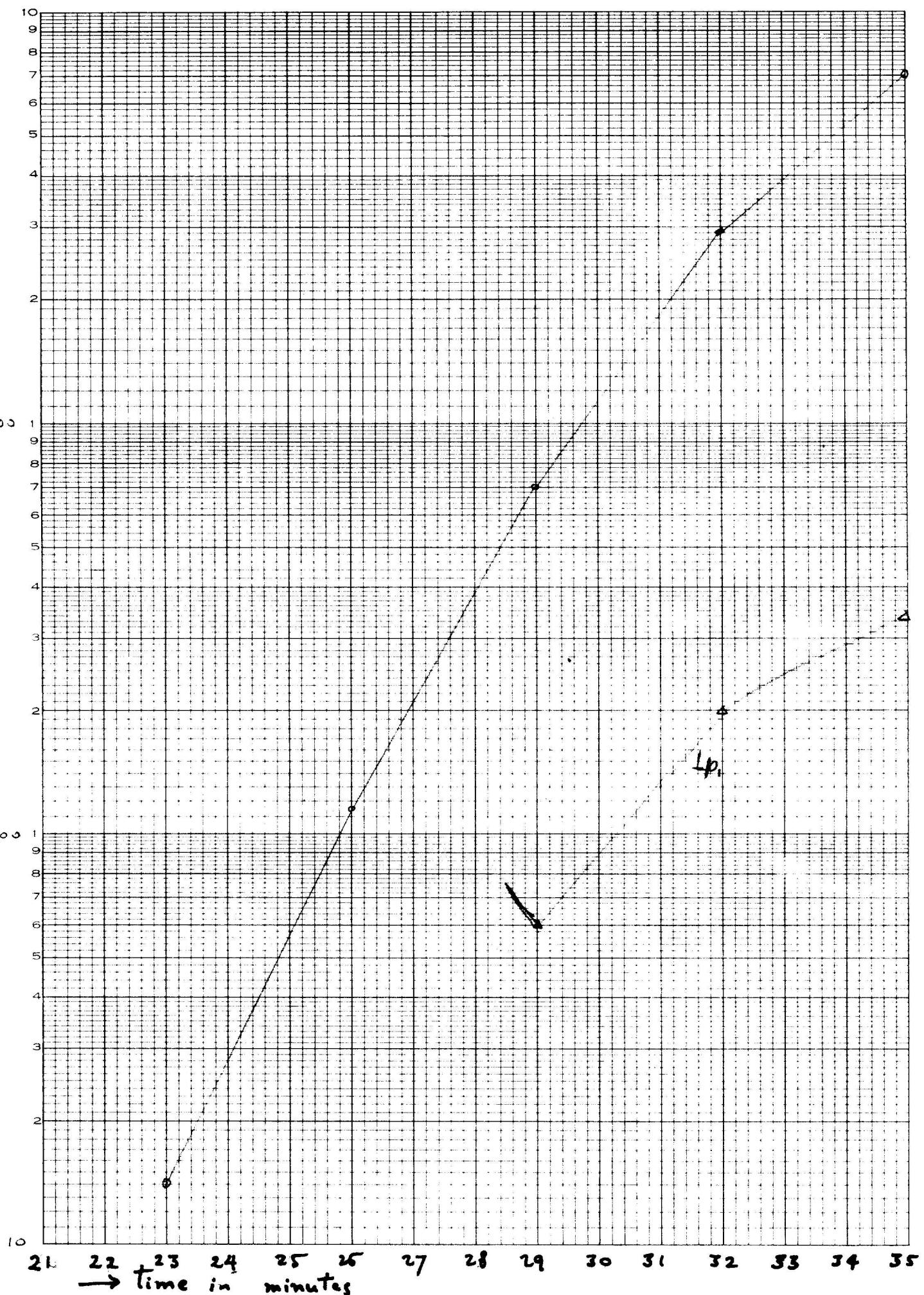


00000 1408/10 gal<sub>2</sub>

(O, 340-L310 DIETZGEN GRAPH PAPER  
ALGARITHMIC-3 CYCLES X 10 DIVISIONS

EUGENE DIETZGEN CO.  
PRINTED IN U.S.A.

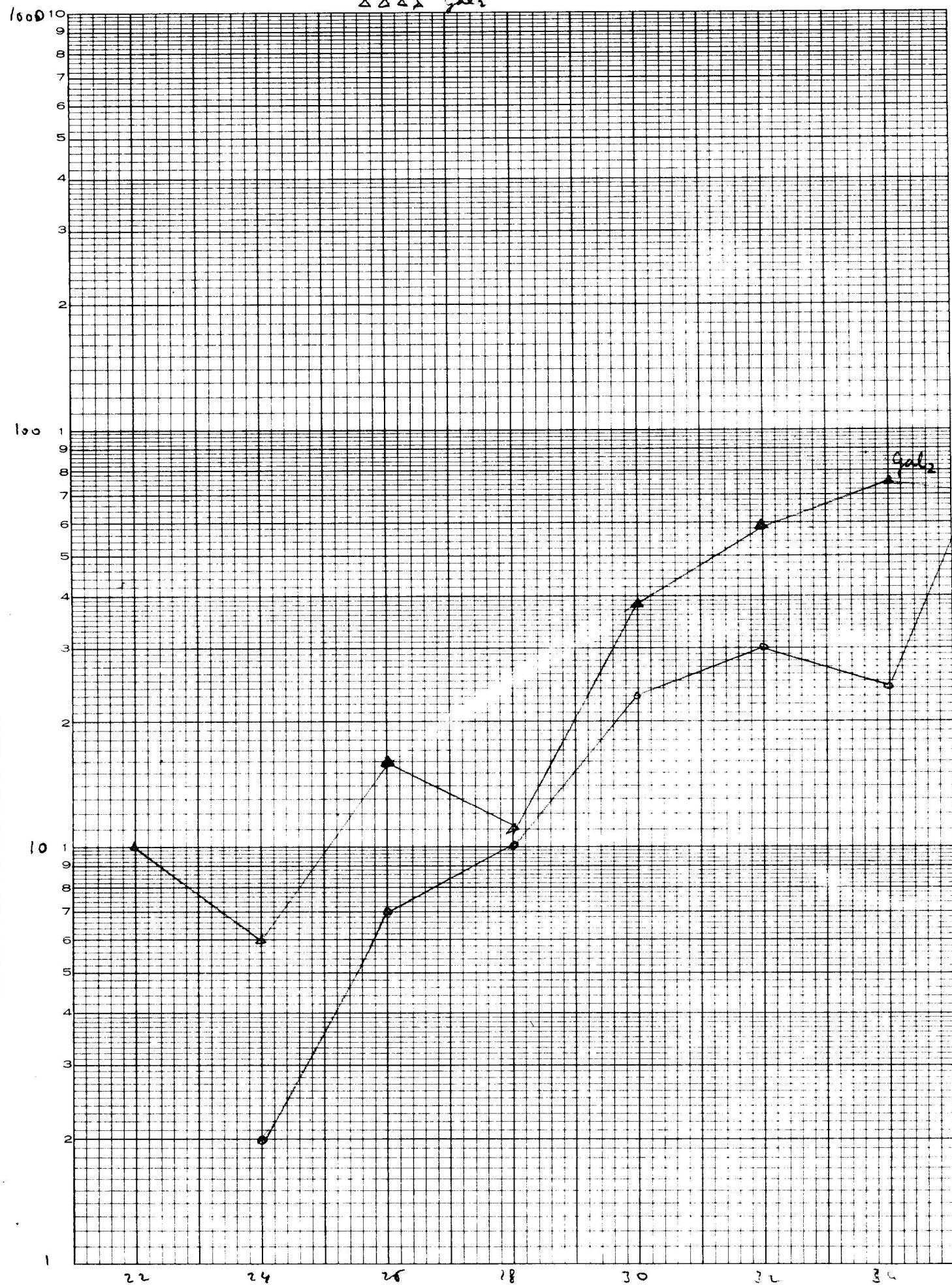


21 22 23 24 25 26 27 28 29 30 31 32 33 34 35  
→ time in minutes

1408/13

0000 Gal,  
△△△△ gal<sub>2</sub>

partial reversion & f+ of 3870.





1

19

REF-

1408/14

( ) between branching: values used for calculation  
of empirical factor; total plate count given in A

19

June 18, 1957.

REF:

1408/15.

1      2      <sup>3</sup>  
<sup>4</sup> After timing of Gal<sub>4</sub>, Gal<sub>2</sub>, Gal<sub>3</sub>.

1      2      Exactly as 1408/14, but:

1      2      3      4      5      6      7      8      9      10  
 1) ♀ parents were Gal<sub>3</sub>, Gal<sub>2</sub>, Gal<sub>3</sub>, and they were  
 2      3      4      5      6      7      8      9      10  
 seeded in this order.

1      2      3      4      5      6      7      8      9      10  
 1) ♀ 3 was grown after Gal<sub>4</sub> and Gal<sub>2</sub> were isolated, as  
 2      3      4      5      6      7      8      9      10  
 0      had been  
 a substitute for Gal<sub>3</sub> which was supposed to be tested  
 1      2      3      4      5      6      7      8      9      10  
 today and was found to be fit. ♀ 3 was found to be  
 1      2      3      4      5      6      7      8      9      10  
 slightly less concentrated than the other two females and  
 1      2      3      4      5      6      7      8      9      10  
 three tubs were collected into two, thus reaching 1.5 x conc.  
 1      2      3      4      5      6      7      8      9      10  
 for this ♀.

3) Mating mixtures: 5 ml ♂ + 12 ml ♀.

Plate counts. (48<sup>hr</sup>) (72<sup>hr</sup>)

| Time | Gal <sub>4</sub> | Gal <sub>3</sub> | Gal <sub>2</sub> |
|------|------------------|------------------|------------------|
| 0    | (35), 7          | 2, 0             | 13, 0, 0         |
| 15   | 7, 1             | 0, 3             | 11, 0, 0         |
| 18   | 2, 9             | 3, 27            | 27, 27 0, 1      |
| 20   | 3, 8             | 1, 0             | 23, 15 5, 2      |
| 22   | 18, 13           | 2, 3             | 18, 1, 10        |
| 24   | 138, 112         | 1, 3             | 16, 17 32, 24    |
| 26   | 451, 348         | 1, 1             | 8, 17 68, 51     |
| 28   | 410, 448         | 0, 1             | 11, 17 160, 110  |
| 30   | t.m.t.b.c.       | 3, 12            | 13, 27 420,      |
| 35   |                  | 28, 25           | 38, 34           |
| 40   |                  | 55, 69           | 60,              |
| 50   |                  | 205, 190         |                  |
| 60   |                  | 391, 356         |                  |

Note: Gal<sub>3</sub>, all large colonies; in addition, few fine, minute, small ones and 1 or extremely small.

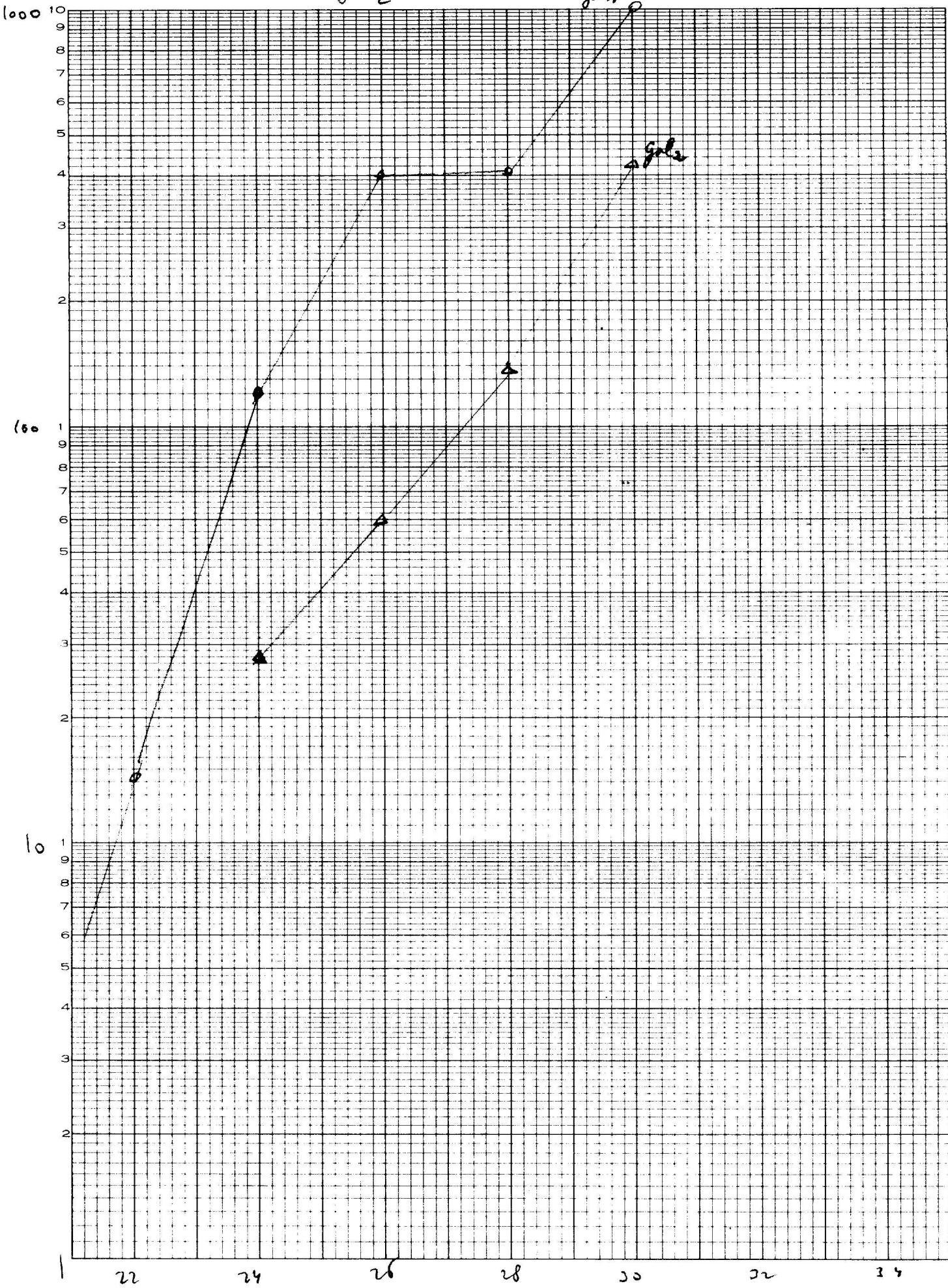
Gal<sub>2</sub>, Gal<sub>4</sub>: all colonies small, as in former except.

1408/15

0000 Gal 4  
△△△ Gal 2

Gal 4

Gal 2





19

June 20, 1950.

REF: 1408/16

|  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|--|---|---|---|---|---|---|---|---|---|----|
|--|---|---|---|---|---|---|---|---|---|----|

1 Hfr<sub>2</sub> timing of Gal<sub>5,2,1,3</sub>.  
2  
3  
4

5 Same as expts 13, 14, 15.  
6

7 Mating mixtures: ♂ 4 ml + ♀ 12 ml.  
8

9 Order of seeding flasks: Gal<sub>5</sub>, Gal<sub>2</sub>, Gal<sub>1</sub>, Gal<sub>3</sub>.  
0

1 Times: 0', 18', 22', 25', 26', 28', 30', 32', 34', 40'.  
2

3 22'

4

5

6

7

8

9

0

1

2

3

4

5

6

7

8

9

0

1

2

3

4

5

6

7

8

9

0

1408/16

19

June 22, 1958.

REF:

1408 / 17

1            2            3            4            5            6            7            8            9            10

# Hfr<sub>2</sub> timing of Gal<sub>1,2,8</sub>

Same as crypto 13, 14, 15, 16.

Mating mixtures: ♂ 4 ml + ♀ 1/2 ml.

Order of feeding flasks: Gal<sub>2</sub>, Gal<sub>1</sub>, Gal.

Turing 0° 18' 22' 24' 26' 28' 30' 32' 35'

Note: gal. at 22' is actually 23'.



19

June 23, 1958.

REF:

1408/18

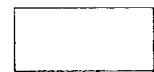
1      2      3      4      5      6      7      8      9      10  
1      H<sub>2</sub> mating of Gal<sub>2</sub>, Gal<sub>3</sub>, Gal<sub>6</sub>, Gal<sub>9</sub>  
2  
3      same as expts 13, → 17.  
4  
5  
6

Mating mixtures : 15 ml ♀ + 5 ml ♂,  
7      jarrings at times : 0', 20', 22', 24', 25', 28', 30',  
8  
9      for Gal<sub>9</sub> also 35', 40', 50'.  
0

Order of seeding : 2, 4, 6, 9

Note : Suspension of Gal<sub>9</sub> is granular, and slightly  
less conc. than others in spite of using 4 tubes  
resup. to 24 ml. (4/3 conc.)

Time 22' of Gal<sub>2</sub> seems very thin, possibly  
amount measured out of flask was spilled in  
ice bath?



19

June 25, 1958

REF:

1408/19

|   |   |   |   |   |   |   |   |   |    |
|---|---|---|---|---|---|---|---|---|----|
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|----|

1  
2  
3  
4  
5  
6  
7  
8  
9  
10

Hfr<sub>2</sub> timing of Gal<sub>2</sub>, Gal<sub>5</sub>, Gal<sub>7</sub>, Gal<sub>8</sub>.

Same as exp 13-18. 1<sup>h</sup>10' rotation. Gal<sub>5</sub> slightly less turbid than others.

Mating mixtures: 5 ml ♂ + 15 ml ♀.

Sampling at times: 0', 20', 22', 24', 26', 28', 30'.

Order of seeding 2, 5, 7, 8.

22/8 and 24/2 may have been exchanged at mating?

19

June 27

REF:

1408/20

1

2

3

4

5

6

7

8

9

10

### Hfr<sub>2</sub> timing of Gal, Try.

ORC cultures of W 3870, W 3908, W 4066/<sub>1</sub> (Try-S<sup>R</sup>Gal-).

Refreshed, 1 ml + 7.5 ml for <sup>1</sup> hr <sup>1</sup> spcm, resusp. in 2 ml  
<sup>I. index</sup> <sup>T = 37.5°C</sup>  
<sup>(4 x conc.)</sup>. Crosses: 3870 x 3908; 3870 x 4066 - UV Gal-  
<sup>W 4076</sup>

Mating mixtures, in flasks: 2 ml ♂ + 6 ml ♀ = 1:3.

Samples: 0.2 ml + 1.8 chilled H<sub>2</sub>O, <sup>four flasks</sup> at every time: series A

if plated directly (.05 ml), if further diluted: series B

### Schedule:

| Time | D(SmB <sub>1</sub> ) 4, S Gal SmB <sub>1</sub> | M Gal SmB <sub>1</sub> , Try |
|------|--|------------------------------|
|------|--|------------------------------|

♀ Control

A      B

A      B C

0

1/10

1/10

10'

1

20'

1

22

1

24

1

26

1

28

1

30

1

33

1

36

1

39

1/20 = A 1+1

26'

1/20 = A 1+1

42

1/40

42'

1/40 1+3

45

1/100

28'

1/100 1+7

48

1/200

30'

1/100 1+7

51

1/400

33'

1/400 .5+9.5

54

1/800

51'

1/800 .1+9.9

57

1/1600

54'

1/1600 .1+9.9

60'

1/3200

60'

1/3200 .1+9.9

0/60 #

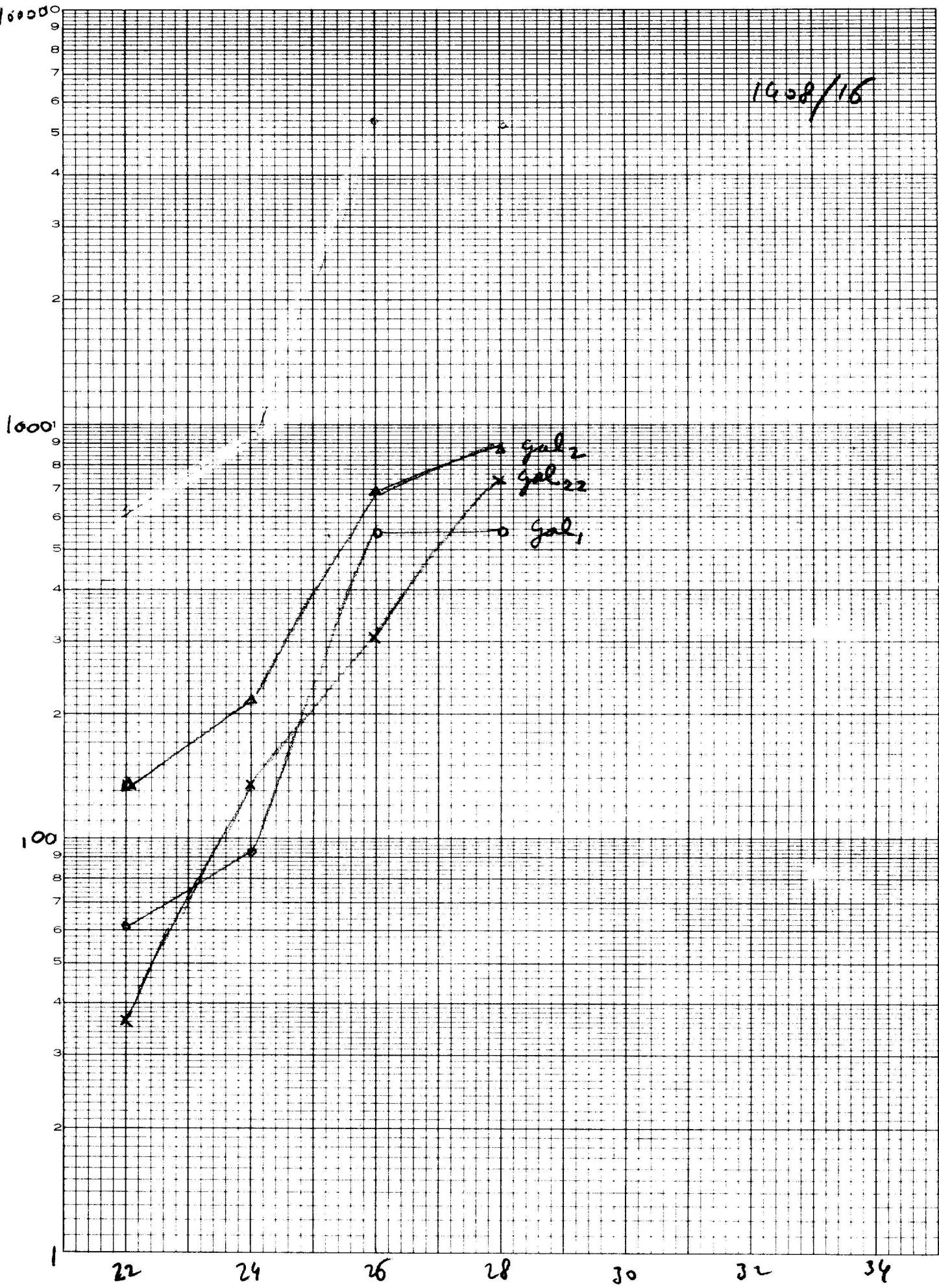
-

0/60

0/60 .1+9.9

0/60 : control of plate recombination with parents kept in waterbath throughout the exp. (60').

Note: 51' killed. 33' was 40" late.



DATE: 4.16.54.

REF: 1409-I.

## ENZYMES ON PROTOPLASTS.

1 and 2 ml of 3060 + 10 ml  $\text{L}_2$  +  $10^4 \mu\text{U}$ /Penicillin

0.5 and 1 ml of 3064 + "

Centrifuged, resuspended in half the amount of  $\text{L}_2$ .  
Mixed in equal volumes with:A RN-ase 2 mg/ml in  $\text{L}_2$ 

B Chymotrypsin "

C Lysozyme "

D DN-ase "

N : = control +  $\text{L}_2$  medium,F Veroene 4% 0.25 ml added, + 0.25 ml  $\text{L}_2$  + 0.25 ml  $\text{S}$ 

G Lysozyme 0.25 ml + Veroene 0.25 + &amp; 0.25.

After 15-30': Protoplast counts in Petriff chamber. (millions/ml).  
(20 small squares: sum, multiply  $\times 10^6$ ).

| N  | A  | B  | C  | D  | F  | G   |
|----|----|----|----|----|----|-----|
| 63 | 70 | 60 | 54 | 80 | 82 | 59. |

After 2 hours:

|    |     |    |    |    |    |           |
|----|-----|----|----|----|----|-----------|
| 44 | 27* | 41 | 49 | 42 | 63 | not done. |
|----|-----|----|----|----|----|-----------|

\* many empty.

Conclusion:

Only RNA-ase seems to affect, and only to a moderate degree, the protoplast count -

This is test of lysis of protoplasts by total count.

DATE: 4/18/58.

REF: 1409-2

## ACTION OF ENZYMES ON MATING ♂♂ X ♀.

3060 ♂ (2 ml + 10 ml L<sub>2</sub>, 2°30' incub.), centrifuged, concentrated  
10x in L<sub>2</sub> + 3064 ♀ conc. 36x in L<sub>2</sub>.

0.3 + 0.3 ml in waterbath for 20', then 0.1 ml. added to  
1 ml of:

- |   |              |                           |
|---|--------------|---------------------------|
| A | Chymotrypsin | 1 mg/ml in L <sub>2</sub> |
| B | Lysozyme,    | "                         |
| C | RN-ase       | "                         |
| D | DN-ase       |                           |
| L | control,     | L <sub>2</sub> medium.    |

40' further incubation after addition to enzyme, then

~~0.02 ml~~ 0.1 + 10.0 DW → { 0.1 ml  
0.01 ml on min st B,

30 Protoplast suspensions: same as those used for exp. #405-5  
before diluting 5x.

Plate counts: mm

|                 | A                  | B     | C     | D     | L        |
|-----------------|--------------------|-------|-------|-------|----------|
| 0.1             | too many, $\infty$ | ~     | ~     | ~     | $\infty$ |
| 0.01            | 113                | 43    | 62    | 74    | 93       |
| Galt            | 0/48               | 2/43  | 0/47  | 0/42  | 1/50     |
| Lact            | 7/48               | 5/43  | 5/47  | 1/42  | 6/50     |
| T <sub>1'</sub> | 19/48              | 18/43 | 31/47 | 16/42 | 23/50    |

DATE: 4/18/58-

REF:

1410

1410  
INTERRUPTION AND DIPLOIDS.

2323, 2735, see overnight rotated cultures, mixce in equal amounts, pulse of 8', then diluted 1/200 in warm bath, further incubation: 20', 40', 60' in water bath

After such times:

$$\begin{array}{l} 0.1 \rightarrow \text{min B, } \\ \frac{1}{10} 0.1 \rightarrow " \\ \frac{1}{10} 0.01 \rightarrow " \end{array} \left. \begin{array}{l} \text{Replicated} \\ \text{1: Stac NC!} \\ \text{2: Stac, Stac B, (P21)} \end{array} \right\}$$

B Lac.

N.B. Numbers meaningless on account of smearing and poor scores on lac. plates as can.

|              |         |         |         |
|--------------|---------|---------|---------|
| D(B, ) total | 20' [A] | 40' [B] | 60' [C] |
|              | 288     | 412     | 181     |
| Stac B,      | 9...29  | —       | 52..20  |
| Stac B,      | 34..42  | —       | 38..42  |

$(\frac{1}{10} \cdot 1 \text{ ml})$ .

Lac +

... digital registration

Lac + 15%  
+ checked  
4/22

2/3)

interrupted only by plating.

**DATE:**

REF: 1410

**DATE:**

22/4

REF

1410/2

19 30<sup>th</sup> April 1958.

**REF:**

1410/3

DATE:

4/21/58

REF:

1411

1

2

3

4

5

6

7

8

9

10

COLCHICINE - Effect on mating

Solution of colchicine 1% in distilled water, non sterile, stored at -20°.

# 3060, 3064 overnight rotated cult:

0.1 ml. to 0.8 ml. Penaffay + 0.1 H<sub>2</sub>O (control)\*

" " + 0.1 colchicine, solution 1%.

1<sup>st</sup> rotation -

C<sub>0</sub>: mixed equal amounts of colchicined broths, + 0.1% ml. colchicine / ml.

I: mixed equal amounts of (Control)\* broths, 0.1 ml water added per ml.

C: mixed equal amounts of (Control)\* broth, 0.1 ml water added per ml.

10' incubation in water bath, then dilution 1/100 in broth, incubation 20', then:  $\frac{1}{10}$  DW → 0.05 minst B,

↓  
 $\frac{1}{10}$  DW → same

↓  
 $\frac{1}{100}$  DW → 0.05 Blac.

|                      | CROSSES        |                 | B LAC      |    |
|----------------------|----------------|-----------------|------------|----|
|                      | $\frac{1}{10}$ | $\frac{1}{100}$ | LAC + LAC- | -  |
| <u>C<sub>0</sub></u> | 152            | 6               | 6          | 17 |
| <u>I</u>             | 69             | 7               | 6          | 14 |
| <u>C</u>             | 146            | 14              | 5          | 15 |

Conclusions. There is perhaps a small decrease in

No. of prototyles adding colchicine to the mating mixture (but not adding it in advance to the culture; any enzyme destroying colchicine?)-

April 26 1958

REF: 1401-2.

1 ORE = W 3060 overnight { 3X 10ml → 3  
 2 overnight 1 W 30645 rotated { 30X 10ml → 0.3 ml. in fresh <sup>1</sup> <sub>2</sub> <sup>3</sup><sub>4</sub> <sup>5</sup><sub>6</sub> <sup>7</sup><sub>8</sub> <sup>9</sup><sub>10</sub> <sup>11</sup><sub>12</sub> <sup>13</sup><sub>14</sub> <sup>15</sup><sub>16</sub> <sup>17</sup><sub>18</sub> <sup>19</sup><sub>20</sub> <sup>21</sup><sub>22</sub> <sup>23</sup><sub>24</sub> <sup>25</sup><sub>26</sub> <sup>27</sup><sub>28</sub> <sup>29</sup><sub>30</sub> <sup>31</sup><sub>32</sub> <sup>33</sup><sub>34</sub> <sup>35</sup><sub>36</sub> <sup>37</sup><sub>38</sub> <sup>39</sup><sub>40</sub> <sup>41</sup><sub>42</sub> <sup>43</sup><sub>44</sub> <sup>45</sup><sub>46</sub> <sup>47</sup><sub>48</sub> <sup>49</sup><sub>50</sub> <sup>51</sup><sub>52</sub> <sup>53</sup><sub>54</sub> <sup>55</sup><sub>56</sub> <sup>57</sup><sub>58</sub> <sup>59</sup><sub>60</sub> <sup>61</sup><sub>62</sub> <sup>63</sup><sub>64</sub> <sup>65</sup><sub>66</sub> <sup>67</sup><sub>68</sub> <sup>69</sup><sub>70</sub> <sup>71</sup><sub>72</sub> <sup>73</sup><sub>74</sub> <sup>75</sup><sub>76</sub> <sup>77</sup><sub>78</sub> <sup>79</sup><sub>80</sub> <sup>81</sup><sub>82</sub> <sup>83</sup><sub>84</sub> <sup>85</sup><sub>86</sub> <sup>87</sup><sub>88</sub> <sup>89</sup><sub>90</sub> <sup>91</sup><sub>92</sub> <sup>93</sup><sub>94</sub> <sup>95</sup><sub>96</sub> <sup>97</sup><sub>98</sub> <sup>99</sup><sub>100</sub> <sup>101</sup><sub>102</sub> <sup>103</sup><sub>104</sub> <sup>105</sup><sub>106</sub> <sup>107</sup><sub>108</sub> <sup>109</sup><sub>110</sub> <sup>111</sup><sub>112</sub> <sup>113</sup><sub>114</sub> <sup>115</sup><sub>116</sub> <sup>117</sup><sub>118</sub> <sup>119</sup><sub>120</sub> <sup>121</sup><sub>122</sub> <sup>123</sup><sub>124</sub> <sup>125</sup><sub>126</sub> <sup>127</sup><sub>128</sub> <sup>129</sup><sub>130</sub> <sup>131</sup><sub>132</sub> <sup>133</sup><sub>134</sub> <sup>135</sup><sub>136</sub> <sup>137</sup><sub>138</sub> <sup>139</sup><sub>140</sub> <sup>141</sup><sub>142</sub> <sup>143</sup><sub>144</sub> <sup>145</sup><sub>146</sub> <sup>147</sup><sub>148</sub> <sup>149</sup><sub>150</sub> <sup>151</sup><sub>152</sub> <sup>153</sup><sub>154</sub> <sup>155</sup><sub>156</sub> <sup>157</sup><sub>158</sub> <sup>159</sup><sub>160</sub> <sup>161</sup><sub>162</sub> <sup>163</sup><sub>164</sub> <sup>165</sup><sub>166</sub> <sup>167</sup><sub>168</sub> <sup>169</sup><sub>170</sub> <sup>171</sup><sub>172</sub> <sup>173</sup><sub>174</sub> <sup>175</sup><sub>176</sub> <sup>177</sup><sub>178</sub> <sup>179</sup><sub>180</sub> <sup>181</sup><sub>182</sub> <sup>183</sup><sub>184</sub> <sup>185</sup><sub>186</sub> <sup>187</sup><sub>188</sub> <sup>189</sup><sub>190</sub> <sup>191</sup><sub>192</sub> <sup>193</sup><sub>194</sub> <sup>195</sup><sub>196</sub> <sup>197</sup><sub>198</sub> <sup>199</sup><sub>200</sub> <sup>201</sup><sub>202</sub> <sup>203</sup><sub>204</sub> <sup>205</sup><sub>206</sub> <sup>207</sup><sub>208</sub> <sup>209</sup><sub>210</sub> <sup>211</sup><sub>212</sub> <sup>213</sup><sub>214</sub> <sup>215</sup><sub>216</sub> <sup>217</sup><sub>218</sub> <sup>219</sup><sub>220</sub> <sup>221</sup><sub>222</sub> <sup>223</sup><sub>224</sub> <sup>225</sup><sub>226</sub> <sup>227</sup><sub>228</sub> <sup>229</sup><sub>230</sub> <sup>231</sup><sub>232</sub> <sup>233</sup><sub>234</sub> <sup>235</sup><sub>236</sub> <sup>237</sup><sub>238</sub> <sup>239</sup><sub>240</sub> <sup>241</sup><sub>242</sub> <sup>243</sup><sub>244</sub> <sup>245</sup><sub>246</sub> <sup>247</sup><sub>248</sub> <sup>249</sup><sub>250</sub> <sup>251</sup><sub>252</sub> <sup>253</sup><sub>254</sub> <sup>255</sup><sub>256</sub> <sup>257</sup><sub>258</sub> <sup>259</sup><sub>260</sub> <sup>261</sup><sub>262</sub> <sup>263</sup><sub>264</sub> <sup>265</sup><sub>266</sub> <sup>267</sup><sub>268</sub> <sup>269</sup><sub>270</sub> <sup>271</sup><sub>272</sub> <sup>273</sup><sub>274</sub> <sup>275</sup><sub>276</sub> <sup>277</sup><sub>278</sub> <sup>279</sup><sub>280</sub> <sup>281</sup><sub>282</sub> <sup>283</sup><sub>284</sub> <sup>285</sup><sub>286</sub> <sup>287</sup><sub>288</sub> <sup>289</sup><sub>290</sub> <sup>291</sup><sub>292</sub> <sup>293</sup><sub>294</sub> <sup>295</sup><sub>296</sub> <sup>297</sup><sub>298</sub> <sup>299</sup><sub>300</sub> <sup>301</sup><sub>302</sub> <sup>303</sup><sub>304</sub> <sup>305</sup><sub>306</sub> <sup>307</sup><sub>308</sub> <sup>309</sup><sub>310</sub> <sup>311</sup><sub>312</sub> <sup>313</sup><sub>314</sub> <sup>315</sup><sub>316</sub> <sup>317</sup><sub>318</sub> <sup>319</sup><sub>320</sub> <sup>321</sup><sub>322</sub> <sup>323</sup><sub>324</sub> <sup>325</sup><sub>326</sub> <sup>327</sup><sub>328</sub> <sup>329</sup><sub>330</sub> <sup>331</sup><sub>332</sub> <sup>333</sup><sub>334</sub> <sup>335</sup><sub>336</sub> <sup>337</sup><sub>338</sub> <sup>339</sup><sub>340</sub> <sup>341</sup><sub>342</sub> <sup>343</sup><sub>344</sub> <sup>345</sup><sub>346</sub> <sup>347</sup><sub>348</sub> <sup>349</sup><sub>350</sub> <sup>351</sup><sub>352</sub> <sup>353</sup><sub>354</sub> <sup>355</sup><sub>356</sub> <sup>357</sup><sub>358</sub> <sup>359</sup><sub>360</sub> <sup>361</sup><sub>362</sub> <sup>363</sup><sub>364</sub> <sup>365</sup><sub>366</sub> <sup>367</sup><sub>368</sub> <sup>369</sup><sub>370</sub> <sup>371</sup><sub>372</sub> <sup>373</sup><sub>374</sub> <sup>375</sup><sub>376</sub> <sup>377</sup><sub>378</sub> <sup>379</sup><sub>380</sub> <sup>381</sup><sub>382</sub> <sup>383</sup><sub>384</sub> <sup>385</sup><sub>386</sub> <sup>387</sup><sub>388</sub> <sup>389</sup><sub>390</sub> <sup>391</sup><sub>392</sub> <sup>393</sup><sub>394</sub> <sup>395</sup><sub>396</sub> <sup>397</sup><sub>398</sub> <sup>399</sup><sub>400</sub> <sup>401</sup><sub>402</sub> <sup>403</sup><sub>404</sub> <sup>405</sup><sub>406</sub> <sup>407</sup><sub>408</sub> <sup>409</sup><sub>410</sub> <sup>411</sup><sub>412</sub> <sup>413</sup><sub>414</sub> <sup>415</sup><sub>416</sub> <sup>417</sup><sub>418</sub> <sup>419</sup><sub>420</sub> <sup>421</sup><sub>422</sub> <sup>423</sup><sub>424</sub> <sup>425</sup><sub>426</sub> <sup>427</sup><sub>428</sub> <sup>429</sup><sub>430</sub> <sup>431</sup><sub>432</sub> <sup>433</sup><sub>434</sub> <sup>435</sup><sub>436</sub> <sup>437</sup><sub>438</sub> <sup>439</sup><sub>440</sub> <sup>441</sup><sub>442</sub> <sup>443</sup><sub>444</sub> <sup>445</sup><sub>446</sub> <sup>447</sup><sub>448</sub> <sup>449</sup><sub>450</sub> <sup>451</sup><sub>452</sub> <sup>453</sup><sub>454</sub> <sup>455</sup><sub>456</sub> <sup>457</sup><sub>458</sub> <sup>459</sup><sub>460</sub> <sup>461</sup><sub>462</sub> <sup>463</sup><sub>464</sub> <sup>465</sup><sub>466</sub> <sup>467</sup><sub>468</sub> <sup>469</sup><sub>470</sub> <sup>471</sup><sub>472</sub> <sup>473</sup><sub>474</sub> <sup>475</sup><sub>476</sub> <sup>477</sup><sub>478</sub> <sup>479</sup><sub>480</sub> <sup>481</sup><sub>482</sub> <sup>483</sup><sub>484</sub> <sup>485</sup><sub>486</sub> <sup>487</sup><sub>488</sub> <sup>489</sup><sub>490</sub> <sup>491</sup><sub>492</sub> <sup>493</sup><sub>494</sub> <sup>495</sup><sub>496</sub> <sup>497</sup><sub>498</sub> <sup>499</sup><sub>500</sub> <sup>501</sup><sub>502</sub> <sup>503</sup><sub>504</sub> <sup>505</sup><sub>506</sub> <sup>507</sup><sub>508</sub> <sup>509</sup><sub>510</sub> <sup>511</sup><sub>512</sub> <sup>513</sup><sub>514</sub> <sup>515</sup><sub>516</sub> <sup>517</sup><sub>518</sub> <sup>519</sup><sub>520</sub> <sup>521</sup><sub>522</sub> <sup>523</sup><sub>524</sub> <sup>525</sup><sub>526</sub> <sup>527</sup><sub>528</sub> <sup>529</sup><sub>530</sub> <sup>531</sup><sub>532</sub> <sup>533</sup><sub>534</sub> <sup>535</sup><sub>536</sub> <sup>537</sup><sub>538</sub> <sup>539</sup><sub>540</sub> <sup>541</sup><sub>542</sub> <sup>543</sup><sub>544</sub> <sup>545</sup><sub>546</sub> <sup>547</sup><sub>548</sub> <sup>549</sup><sub>550</sub> <sup>551</sup><sub>552</sub> <sup>553</sup><sub>554</sub> <sup>555</sup><sub>556</sub> <sup>557</sup><sub>558</sub> <sup>559</sup><sub>560</sub> <sup>561</sup><sub>562</sub> <sup>563</sup><sub>564</sub> <sup>565</sup><sub>566</sub> <sup>567</sup><sub>568</sub> <sup>569</sup><sub>570</sub> <sup>571</sup><sub>572</sub> <sup>573</sup><sub>574</sub> <sup>575</sup><sub>576</sub> <sup>577</sup><sub>578</sub> <sup>579</sup><sub>580</sub> <sup>581</sup><sub>582</sub> <sup>583</sup><sub>584</sub> <sup>585</sup><sub>586</sub> <sup>587</sup><sub>588</sub> <sup>589</sup><sub>590</sub> <sup>591</sup><sub>592</sub> <sup>593</sup><sub>594</sub> <sup>595</sup><sub>596</sub> <sup>597</sup><sub>598</sub> <sup>599</sup><sub>600</sub> <sup>601</sup><sub>602</sub> <sup>603</sup><sub>604</sub> <sup>605</sup><sub>606</sub> <sup>607</sup><sub>608</sub> <sup>609</sup><sub>610</sub> <sup>611</sup><sub>612</sub> <sup>613</sup><sub>614</sub> <sup>615</sup><sub>616</sub> <sup>617</sup><sub>618</sub> <sup>619</sup><sub>620</sub> <sup>621</sup><sub>622</sub> <sup>623</sup><sub>624</sub> <sup>625</sup><sub>626</sub> <sup>627</sup><sub>628</sub> <sup>629</sup><sub>630</sub> <sup>631</sup><sub>632</sub> <sup>633</sup><sub>634</sub> <sup>635</sup><sub>636</sub> <sup>637</sup><sub>638</sub> <sup>639</sup><sub>640</sub> <sup>641</sup><sub>642</sub> <sup>643</sup><sub>644</sub> <sup>645</sup><sub>646</sub> <sup>647</sup><sub>648</sub> <sup>649</sup><sub>650</sub> <sup>651</sup><sub>652</sub> <sup>653</sup><sub>654</sub> <sup>655</sup><sub>656</sub> <sup>657</sup><sub>658</sub> <sup>659</sup><sub>660</sub> <sup>661</sup><sub>662</sub> <sup>663</sup><sub>664</sub> <sup>665</sup><sub>666</sub> <sup>667</sup><sub>668</sub> <sup>669</sup><sub>670</sub> <sup>671</sup><sub>672</sub> <sup>673</sup><sub>674</sub> <sup>675</sup><sub>676</sub> <sup>677</sup><sub>678</sub> <sup>679</sup><sub>680</sub> <sup>681</sup><sub>682</sub> <sup>683</sup><sub>684</sub> <sup>685</sup><sub>686</sub> <sup>687</sup><sub>688</sub> <sup>689</sup><sub>690</sub> <sup>691</sup><sub>692</sub> <sup>693</sup><sub>694</sub> <sup>695</sup><sub>696</sub> <sup>697</sup><sub>698</sub> <sup>699</sup><sub>700</sub> <sup>701</sup><sub>702</sub> <sup>703</sup><sub>704</sub> <sup>705</sup><sub>706</sub> <sup>707</sup><sub>708</sub> <sup>709</sup><sub>710</sub> <sup>711</sup><sub>712</sub> <sup>713</sup><sub>714</sub> <sup>715</sup><sub>716</sub> <sup>717</sup><sub>718</sub> <sup>719</sup><sub>720</sub> <sup>721</sup><sub>722</sub> <sup>723</sup><sub>724</sub> <sup>725</sup><sub>726</sub> <sup>727</sup><sub>728</sub> <sup>729</sup><sub>730</sub> <sup>731</sup><sub>732</sub> <sup>733</sup><sub>734</sub> <sup>735</sup><sub>736</sub> <sup>737</sup><sub>738</sub> <sup>739</sup><sub>7310</sub> <sup>7311</sup><sub>7312</sub> <sup>7313</sup><sub>7314</sub> <sup>7315</sup><sub>7316</sub> <sup>7317</sup><sub>7318</sub> <sup>7319</sup><sub>7320</sub> <sup>7321</sup><sub>7322</sub> <sup>7323</sup><sub>7324</sub> <sup>7325</sup><sub>7326</sub> <sup>7327</sup><sub>7328</sub> <sup>7329</sup><sub>7330</sub> <sup>7331</sup><sub>7332</sub> <sup>7333</sup><sub>7334</sub> <sup>7335</sup><sub>7336</sub> <sup>7337</sup><sub>7338</sub> <sup>7339</sup><sub>73310</sub> <sup>73311</sup><sub>73312</sub> <sup>73313</sup><sub>73314</sub> <sup>73315</sup><sub>73316</sub> <sup>73317</sup><sub>73318</sub> <sup>73319</sup><sub>73320</sub> <sup>73321</sup><sub>73322</sub> <sup>73323</sup><sub>73324</sub> <sup>73325</sup><sub>73326</sub> <sup>73327</sup><sub>73328</sub> <sup>73329</sup><sub>73330</sub> <sup>73331</sup><sub>73332</sub> <sup>73333</sup><sub>73334</sub> <sup>73335</sup><sub>73336</sub> <sup>73337</sup><sub>73338</sub> <sup>73339</sup><sub>73340</sub> <sup>73341</sup><sub>73342</sub> <sup>73343</sup><sub>73344</sub> <sup>73345</sup><sub>73346</sub> <sup>73347</sup><sub>73348</sub> <sup>73349</sup><sub>73350</sub> <sup>73351</sup><sub>73352</sub> <sup>73353</sup><sub>73354</sub> <sup>73355</sup><sub>73356</sub> <sup>73357</sup><sub>73358</sub> <sup>73359</sup><sub>73360</sub> <sup>73361</sup><sub>73362</sub> <sup>73363</sup><sub>73364</sub> <sup>73365</sup><sub>73366</sub> <sup>73367</sup><sub>73368</sub> <sup>73369</sup><sub>73370</sub> <sup>73371</sup><sub>73372</sub> <sup>73373</sup><sub>73374</sub> <sup>73375</sup><sub>73376</sub> <sup>73377</sup><sub>73378</sub> <sup>73379</sup><sub>73380</sub> <sup>73381</sup><sub>73382</sub> <sup>73383</sup><sub>73384</sub> <sup>73385</sup><sub>73386</sub> <sup>73387</sup><sub>73388</sub> <sup>73389</sup><sub>73390</sub> <sup>73391</sup><sub>73392</sub> <sup>73393</sup><sub>73394</sub> <sup>73395</sup><sub>73396</sub> <sup>73397</sup><sub>73398</sub> <sup>73399</sup><sub>733100</sub> <sup>733101</sup><sub>733102</sub> <sup>733103</sup><sub>733104</sub> <sup>733105</sup><sub>733106</sub> <sup>733107</sup><sub>733108</sub> <sup>733109</sup><sub>733110</sub> <sup>733111</sup><sub>733112</sub> <sup>733113</sup><sub>733114</sub> <sup>733115</sup><sub>733116</sub> <sup>733117</sup><sub>733118</sub> <sup>733119</sup><sub>733120</sub> <sup>733121</sup><sub>733122</sub> <sup>733123</sup><sub>733124</sub> <sup>733125</sup><sub>733126</sub> <sup>733127</sup><sub>733128</sub> <sup>733129</sup><sub>733130</sub> <sup>733131</sup><sub>733132</sub> <sup>733133</sup><sub>733134</sub> <sup>733135</sup><sub>733136</sub> <sup>733137</sup><sub>733138</sub> <sup>733139</sup><sub>733140</sub> <sup>733141</sup><sub>733142</sub> <sup>733143</sup><sub>733144</sub> <sup>733145</sup><sub>733146</sub> <sup>733147</sup><sub>733148</sub> <sup>733149</sup><sub>733150</sub> <sup>733151</sup><sub>733152</sub> <sup>733153</sup><sub>733154</sub> <sup>733155</sup><sub>733156</sub> <sup>733157</sup><sub>733158</sub> <sup>733159</sup><sub>733160</sub> <sup>733161</sup><sub>733162</sub> <sup>733163</sup><sub>733164</sub> <sup>733165</sup><sub>733166</sub> <sup>733167</sup><sub>733168</sub> <sup>733169</sup><sub>733170</sub> <sup>733171</sup><sub>733172</sub> <sup>733173</sup><sub>733174</sub> <sup>733175</sup><sub>733176</sub> <sup>733177</sup><sub>733178</sub> <sup>733179</sup><sub>733180</sub> <sup>733181</sup><sub>733182</sub> <sup>733183</sup><sub>733184</sub> <sup>733185</sup><sub>733186</sub> <sup>733187</sup><sub>733188</sub> <sup>733189</sup><sub>733190</sub> <sup>733191</sup><sub>733192</sub> <sup>733193</sup><sub>733194</sub> <sup>733195</sup><sub>733196</sub> <sup>733197</sup><sub>733198</sub> <sup>733199</sup><sub>733200</sub> <sup>733201</sup><sub>733202</sub> <sup>733203</sup><sub>733204</sub> <sup>733205</sup><sub>733206</sub> <sup>733207</sup><sub>733208</sub> <sup>733209</sup><sub>733210</sub> <sup>733211</sup><sub>733212</sub> <sup>733213</sup><sub>733214</sub> <sup>733215</sup><sub>733216</sub> <sup>733217</sup><sub>733218</sub> <sup>733219</sup><sub>733220</sub> <sup>733221</sup><sub>733222</sub> <sup>733223</sup><sub>733224</sub> <sup>733225</sup><sub>733226</sub> <sup>733227</sup><sub>733228</sub> <sup>733229</sup><sub>733230</sub> <sup>733231</sup><sub>733232</sub> <sup>733233</sup><sub>733234</sub> <sup>733235</sup><sub>733236</sub> <sup>733237</sup><sub>733238</sub> <sup>733239</sup><sub>733240</sub> <sup>733241</sup><sub>733242</sub> <sup>733243</sup><sub>733244</sub> <sup>733245</sup><sub>733246</sub> <sup>733247</sup><sub>733248</sub> <sup>733249</sup><sub>733250</sub> <sup>733251</sup><sub>733252</sub> <sup>733253</sup><sub>733254</sub> <sup>733255</sup><sub>733256</sub> <sup>733257</sup><sub>733258</sub> <sup>733259</sup><sub>733260</sub> <sup>733261</sup><sub>733262</sub> <sup>733263</sup><sub>733264</sub> <sup>733265</sup><sub>733266</sub> <sup>733267</sup><sub>733268</sub> <sup>733269</sup><sub>733270</sub> <sup>733271</sup><sub>733272</sub> <sup>733273</sup><sub>733274</sub> <sup>733275</sup><sub>733276</sub> <sup>733277</sup><sub>733278</sub> 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<sup>733335</sup><sub>733336</sub> <sup>733337</sup><sub>733338</sub> <sup>733339</sup><sub>733340</sub> <sup>733341</sup><sub>733342</sub> <sup>733343</sup><sub>733344</sub> <sup>733345</sup><sub>733346</sub> <sup>733347</sup><sub>733348</sub> <sup>733349</sup><sub>733350</sub> <sup>733351</sup><sub>733352</sub> <sup>733353</sup><sub>733354</sub> <sup>733355</sup><sub>733356</sub> <sup>733357</sup><sub>733358</sub> <sup>733359</sup><sub>733360</sub> <sup>733361</sup><sub>733362</sub> <sup>733363</sup><sub>733364</sub> <sup>733365</sup><sub>733366</sub> <sup>733367</sup><sub>733368</sub> <sup>733369</sup><sub>733370</sub> <sup>733371</sup><sub>733372</sub> <sup>733373</sup><sub>733374</sub> <sup>733375</sup><sub>733376</sub> <sup>733377</sup><sub>733378</sub> <sup>733379</sup><sub>733380</sub> <sup>733381</sup><sub>733382</sub> <sup>733383</sup><sub>733384</sub> <sup>733385</sup><sub>733386</sub> <sup>733387</sup><sub>733388</sub> <sup>733389</sup><sub>733390</sub> <sup>733391</sup><sub>733392</sub> <sup>733393</sup><sub>733394</sub> <sup>733395</sup><sub>733396</sub> <sup>733397</sup><sub>733398</sub> <sup>733399</sup><sub>733400</sub> <sup>733401</sup><sub>733402</sub> <sup>733403</sup><sub>733404</sub> <sup>733405</sup><sub>733406</sub> <sup>733407</sup><sub>733408</sub> <sup>733409</sup><sub>733410</sub> <sup>733411</sup><sub>733412</sub> <sup>733413</sup><sub>733414</sub> <sup>733415</sup><sub>733416</sub> <sup>733417</sup><sub>733418</sub> <sup>733419</sup><sub>733420</sub> <sup>733421</sup><sub>733422</sub> <sup

3060 x 3064.

1/4/2

5 1 ml samples:

1 ml.

A

Pulse 1 minute.

1:20 dilution:

= 20 ml.

1 ml. sample.

incubate

1:100 dilution

→ 15 minutes → 1 ml. B

dilute 1:10

→ 1 ml. C

blend

dilute 1:10

→ 1 ml. D

count

incubate 45 m.

→ 1 ml. E

count

→ 1 ml. F

count

A - F. interruption by pulsing as TL count.

B - F. interruption by bac ratio.

C - D. viability of zygotes.

C - E. interruption by bac ratio

B, C, E vs D, F viability of zygotes as TL count.

C, E vs D, F viability of input.

storage; methods of thawing

# FREEZE

DATE: 4/26/58,

REF: 1412

3060 & r.c. conc. 3 x batch 0.2 ml each in 50 ml flask same  
 3064 30 x 0.2 ml each

After 1' pulse add 19.6 ml batch from test tube. (1/5 dil)  
 → sample for further dilution (0.1 ml + 9.9 prewarmed batch)

(1/5000)

(A<sub>1</sub>)  
glyceral  
freeze

trough: 0.2 + 0.6 glyceral 20%

15' incubation

chill  
in icebath  
2 ml

incubate  
further 45'

(B)  
glyceral  
freeze  
0.2 + 0.6

Blend

1+9 H<sub>2</sub>O

(E)  
glyceral  
freeze  
0.2 + 0.6

(F)  
plate

(C)  
glyceral  
freeze  
0.2 + 0.6

(D)  
plate

1+9 H<sub>2</sub>O

40 Immediate pictures: D, F on bin #8, 0.05 & 0.1

Counts:

0.1      0.05

D      12, 13      7, 9

F      45, 30      16, 25

**DATE:**

REF: 1412

1      2      3      4      5      6      7      8      9      10

C, E plated on 4/28/58 after thawing in water bath  
without further dilution (they are 2.5 x more concentrated  
than wmpaste stock, 0, F). Plate on minst B<sub>1</sub>, 3/4 o, and  
0.05 ml.

10 B thawed in water bath on 4/28/58, divided into  $B_1$  &  $B_2$   
 $B_1$ : incubated 45'; plated 0.1 & 0.05 on minst B.  
 $B_2$ : plated at once      4      1

## Plate counts :

|      | $B_1$  | $B_2$  | $C$    | $E$    |
|------|--------|--------|--------|--------|
| 0.1  | 19, 15 | 2, 11* | 26, 27 | 30, 47 |
| 0.05 | 6, 8   | 4, 2   | 8, 9   | 31, 12 |

30 Comparison between C & D : C, total 40 col.  $\times 2.5 = 28$

D, total 41

$$\text{Survival} \quad \frac{28}{41} = 67\%$$

$$E \text{ & } F : \quad E_{\text{total}} \text{ 120 vol } \% 2.5 = 48$$

F 115 col

$$\text{Survival } \frac{48}{116} = 40\%.$$

B, & C B, 48 vol.

C 70 col.

DATE: 4/29/58

REF: 1412

1      2      3      4      5      6      7      8      9      10

$A_1$  and  $A_2$  tested for ability to resume mating.

**A<sub>1</sub>** thawed → **A<sub>2</sub>** thawed

**0'**                  **0'**

↓                  ↓

1/10 in prewarmed broth      1/40 in 0.1 + 3.9%  
0.2 + 1.8%  
broth

immediate (after 1')      in prewarmed broth

15' incub.      15' incub.

↓                  ↓

0.5 + 4.5 DW      0

platings after dilution in water 1/10      platings after 1/10 dilution in water and blending

45' incub.      45' incub.

↓                  ↓

(A)      (B)      (C)

platings after 1/10 water dilution

C made on second independent tube because water bath went haywire meanwhile.

Platings: .1 + .05 ml on minifit B.

Plate recombination controls -

Frozen parents (see exp 1412/2):

# 3060 [0.1 ml + 9.9 ml broth]  $\rightarrow$  [0.5 + 9.5 ml]  $\rightarrow$  1/10 DW  
30 x conc. from expon. culture, considered 20% of saturation.

# 3064 [0.1 ml + 9.9 ml broth]  $\rightarrow$  [0.5 + 9.5 ml]  $\rightarrow$  1/10 DW  
30 x conc. saturated culture.

From dilutions in water: platings with 0.05 + 0.05  
0.01 + 0.01