Development of plaque assay for PV on PV-transformed cells.

9/19/67

Used PY-6 - these are 3T3 (mouse cells) transformed with PV by T. Benjamin.

How done by him?

Took 1 large petri plate of "confluent" PY-6 cells - removed the medium and washed plate 1x with 5 ml of trypsin-TD. Add 4 ml of trypsin-TD to cover cells and incubate ~5' at 37° (actually, should watch to see when holes in the layers begin to form). Added 1 ml serum to plate (to stop trypsin action) and suspend cells by putting up and down in pipette.

Cells were diluted 1:10 in Eagles-10% serum and 5 ml of cell suspension put down in each of 6 plates.

Incubate at 37° for 2 days. After second day one could already see cells sitting down on plate and eventually formed cells on 9/21/67.
Media removed from each of plates and then detached with 2.5 ml of Hypein-TD for 5' at 37'. Added 0.5 ml serum per plate in cells, more than removed from plate.

Total volume cell suspension ~ 16 ml.

Above suspension distributed 2-ergot 3 small petri plates ~ 5 ml each and X-rayed.

Distance 26.5 cm.

1. 30" ~ 300 sec
2. 90" 1500-1500 R
3. 240" ~ 3000-3000 R

Cells were then centrifuged for 5' at 13 g cent in desk top centrifuge. Resuspended in 1 ml of medium (EAGLE's + 20% CS) 1/10 drop 1/10 to cell count.

~ 10 cells per large square ~ 2500 cells 2 cm di

= 4 x 10^6 cells/lit plate

Took 1.8 ml of each suspension (1.6 x 10^6 cells) and diluted to 5 ml (~3 x 10^5 cells/lit). Put down 5 ml on each of 5 plates.

1.6 x 10^6 cells/lit. Incubate at 37'. About 6-7 hours later looked like cells were attaching to plates.
On 9/22 visual inspection of plates (cells) looked like they were growing. In every of 9/22 cells, placed at 35°C.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Color of medium</th>
<th>State of cells</th>
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</thead>
<tbody>
<tr>
<td>C</td>
<td>yellow</td>
<td>Confluent - some mitotic figures</td>
</tr>
<tr>
<td>E</td>
<td>yellow - pink</td>
<td>Nearly confluent</td>
</tr>
<tr>
<td>S</td>
<td>pink</td>
<td>not confluent - more mitotic figures but there were not entirely new</td>
</tr>
</tbody>
</table>

Cells were placed at 35°C.

Changed medium on all cells and kept at 35°C until 9/25.

4/25

Removed media - washed plates with 5x of Tyrode buffer.

Used two virus strains for infection:

- LP 1407 (large plaque): 10⁴ plaques/ml 10⁻⁴ and 10⁻³
- P 16 (small plaque): 10⁸ plaque-forming units (pfu) dil. 10⁻⁵ and 10⁻⁶

Infected as follows:

- Two plates from each X-ray dose received 0.1 ml of LP 1407 (lo) or 0.1 ml 10³ dil of LP 1407 (hi)
- Total 8 plates

- Two plates from each X-ray dose received 0.1 ml 10⁻⁵ dil of P 16 (hi)
- Total 8 plates

The virus suspension was spread over surface by tipping plates and then incubated at 37°C for 1-15 hours.
Preparation of agar overlay.

- Add 2 ml of 1.5% agar and equal volume of 2x Eagle's medium + 3.5% horse serum to a 25 cm² flask.
- Put a little of this overlay over the cells, taking care not to disturb monolayer.
- Add 2 ml of bicarbonate to agar overlay.
- Incubate at 37°C after 2 days.

Plates with 35° or 40° show distinct yellow to yellow-pink, but 4° plates still looked good.

Overlayed the 30° and 40° plates with 2 ml of agar medium with extra bicarbonate and put back in incubator.

The 4° plate was not overlaid.

10/2

Make up stain mixture

1 flask neutral red agar mixed with flask of 2x Eagle's medium added last serum to 3.5%. Should add about 1 g bicarbonate wherever overlaid too weakly (but did not do this now).

Overlay all plates.