Friday 21st Jan '55
(written on Sat).

Cell Wall Prep. (done in Microbiological hut with McSalter.)

Micrococcus hypodictum:

3 litres grow in 3 rotating (aerated) flask.
on their standard medium. (prob. 2 to 3 gms dry weight).

bugs spun down (15 min. in Interrational), + supernatant decanted.
[Did in ~~tar~~ 100 cc tubes, 8 at a time.]

~~Bu~~ Bugs resuspended in 200 ml distilled H_2O.
Broken in Mickle weecherae.

~~2~~ about 10 ml ~~or~~ bugs suspension + 10 ml little glass beads
(2 lots at a time) ~~do~~ vibrated for about 15 mins.
Filtered off glass balls on sintered glass filter.
Centrifuged in high speed angle (Servo) centrifuge.
If a yellow patch in middle of sediment, is showed unbroken bugs. These batches have to be vibrated again [happened to several batches]
Eventually all o.k. ~~Two~~ ~~Per~~ walls resuspended in ~~about~~ ~~a~~ distilled water. [roughly 2 gm is 200 ml]

Washing

i.e. wash, + centrifuge in high-speed angle: first wash. distilled H_2O.
Transferred to our lab. + used our high-speed angle. Did not run at max speed; ≃ 8000 revs
Centrifuged for 10 to 15 minutes: washed twice with ~1 M NaCl. Then 3. times with distilled water. Resuspended in distilled water + 2 drops each of chloroform, + left to stand over weekends in frig.

~1 M NaCl
2 with NaCl
several with H_2O