9/19/56

## Purification of Polymerase -

### Preparation of Strep Ppt

To 300 ml of 10' sonorate (20 mg protein/ml - Bovine serum albumin as standard) add with stirring 300 ml of M/20 Tris, 7.5, add slowly with mechanical stirring 45. ml of 5% Strep $SO_4$, stand 10' in ice, centrifuge in #6 Servall tubes, 5' at #85, collect ppt. (Save 5.0 ml of supernatant for assay).
To each tube add 10 ml of M/20 $KPO_4$, 7.4, disperse ppt. with plastic homogenizer attached to stirring motor.
Note: Ppt. suspended well once, then resuspended twice more, whole operation required about 25'.
Pool suspension from all tubes, wash tubes + make up to 310 ml with M/20 $KPO_4$. Mix.

### Spinco Treatment -

~280 ml of
Transfer suspension to Spinco Tubes (38 ml), centrifuge 120' at 30000 RPM in Spinco in Rotor 30 - Collect supernatant.
Note: There are two deviations from normal procedure here.
1.) Usually after suspension of Strep. ppt., it is centrifuged in Servall before Spinoing.
2.) Spinco centrifugation usually at 40000 RPM in Rotor 40 which gives higher force field.

One tube broken in centrifuge - Vol. of supernatant 237. ml.

### Nuclease Treatment:

Remove 2.0 ml for Assay + Protein Det., + UV.
To remainder ~ 235 ml make following additions

3.7 ml of .3M Mg Cl2
.23 ml of DNAase, 100 γ/ml
.023 ml of RNAase, 1 mg/ml - Worthington

2hr 5 hrs at 37°

UV of Spinco Sup = .02 → 1.0 at 260 mμ = .278 = 13.9 OU/ml.

Nuclease Treated Spinco Sup: 0.5 ml + 0.5 ml of 7% PCA (cold) stand 5' in ice, centri. UV of .05 → 1.0 at 260 mμ = .530 = 21.2 OU/ml = ~50% Hydrol

(see over)

Dialysis:

Dialyze at 2° vs 4 liters of M/100 Tris, 7.5 for 16 hrs. Moderate ppt. forms which was removed by centrifugation. ~~Concen~~ Shall freeze & concentrate by lyophilization to volume less than 30 ml. Thaw, Transfer to cylinder & dilute to total volume of 38.0 ml with ice-water. This is "Lyophilized "P" Enzyme"