11/19/56 (3)

Separation of Tumor Myeloma Protein X5563

Gen'l: procedure:

Homogenize tissue in Potter hand hemogenizer, using .05 M KCl, at 2°C.

Centrifuge at hi speed in cold (2-4°C).

Dialyse supernatant against 30, 50, 100% sat'd. $(NH_4)_2SO_4$ successively, isolating each ppt. Test for γ-glob-mobility-protein in each fraction by paper electrophoresis.

11/19

Tissue: Pooled mouse tumor tissue, frozen for months. X5563

Wt = 10.4 gm (wet) (39.70 − 29.30).

1st ground frozen in mortar + pestle using dry ice. Then hemogenized in Potter hand hemogenizer at 2°C, using 9 ml .05 M KCl. Centrifuged at 4200 rpm x 50 minutes → poor separation. (PPt. (1); supernatant (1))

Recent. using hi speed head (42 rpm x 125) (ppt (2) super (2)) → sl turbid supernate.

Supernatant (2) (5cc lost) = 7cc. Dialysed against 1/3 sat'd $(NH_4)_2SO_4$ in cold rm for 5 hrs. → Ppt (3) washed x1 c̄ 1/3 sat'd $(NH_4)_2SO_4$ + frozen.

Supernatant (3) dialysed ag. 1/2 sat'd $(NH_4)_2SO_4$ → ppt (4) washed c̄ 1/2 sat'd $(NH_4)_2SO_4$ + frozen

Super (4) dial ag sat'd $(NH_4)_2SO_4$ → ppt (5) - frozen

Amt ppt (3) > (4) >> (5)

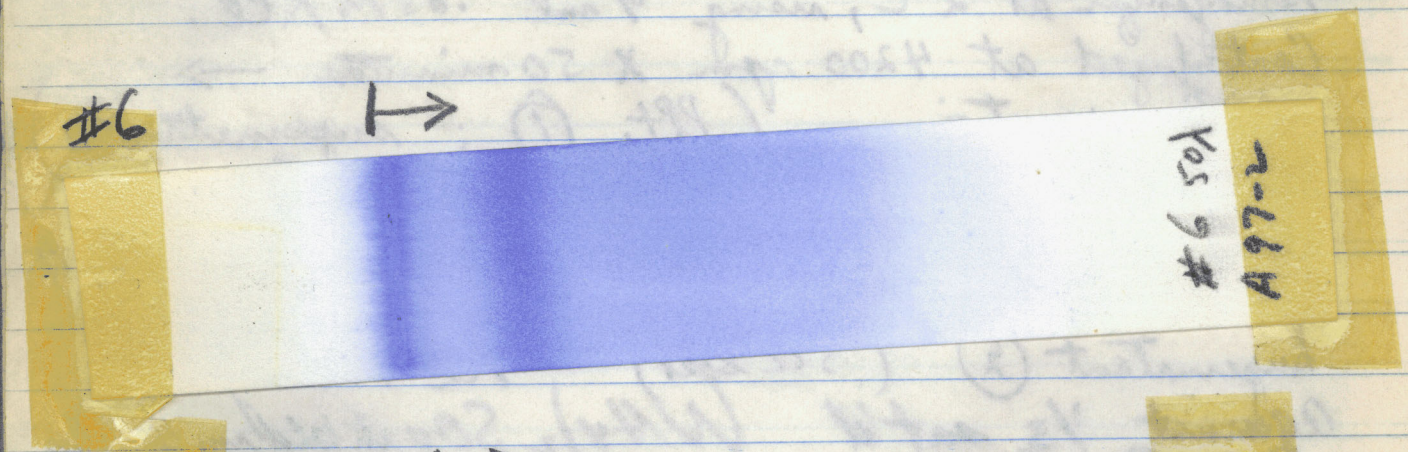
Ppts 3, 4, 5 each taken up in 3 ml cold .85% NaCl + centrifuged. Almost all of 3 dissolved, ca. ½ of 4 + ½ of 5 dissolved. Ppts were washed x2 c̄ cold saline + frozen (labelled 3-P, 4-P + 5-P). Saline-soluble material dialyzed against .85% NaCl for 24 hrs in cold rm. Ppt formed in each sacs. This was cent. off, washed x2 c̄ saline + frozen (6-P, 7-P, 8-P). Supernatants (6, 7, 8) run for total prot. + frozen.

11/22

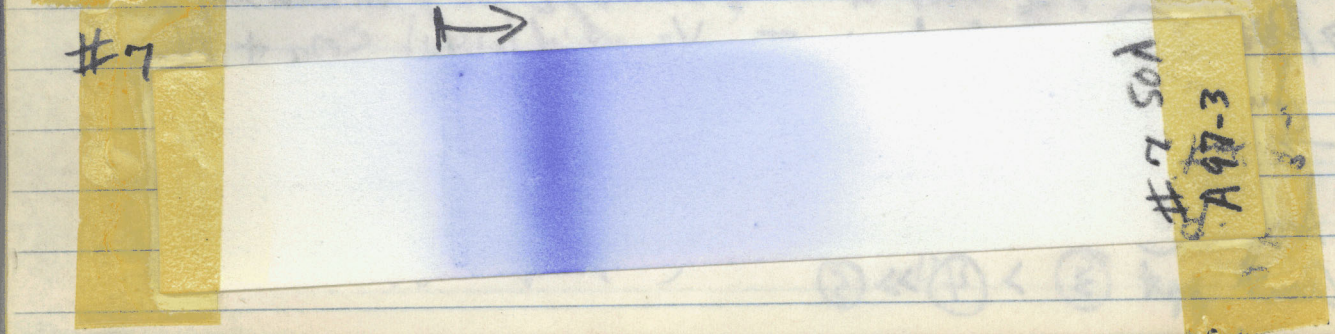
Deter prot in 6, 7, 8 by biuret method:

Sample	O.D.	Prot mg/ml
.5 ml 1% bovine alb (5 mg)	.200 } 204	
"	.207	
#6 .2 ml	.103	13 mg/ml
#7 .2 ml	.058	7 mg/ml
#8 .2 ml	.018	2.2 "

#6 →



#7 →

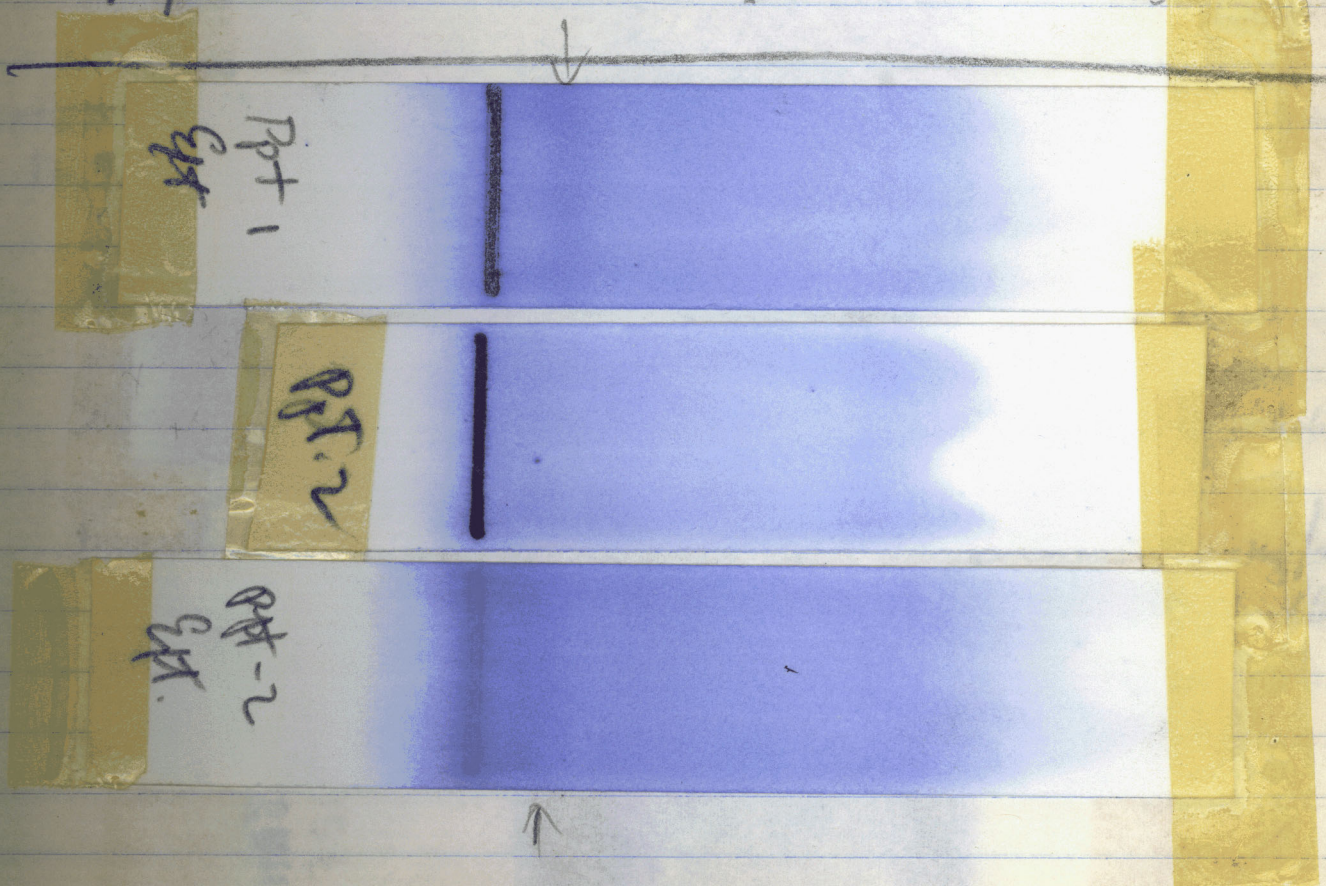


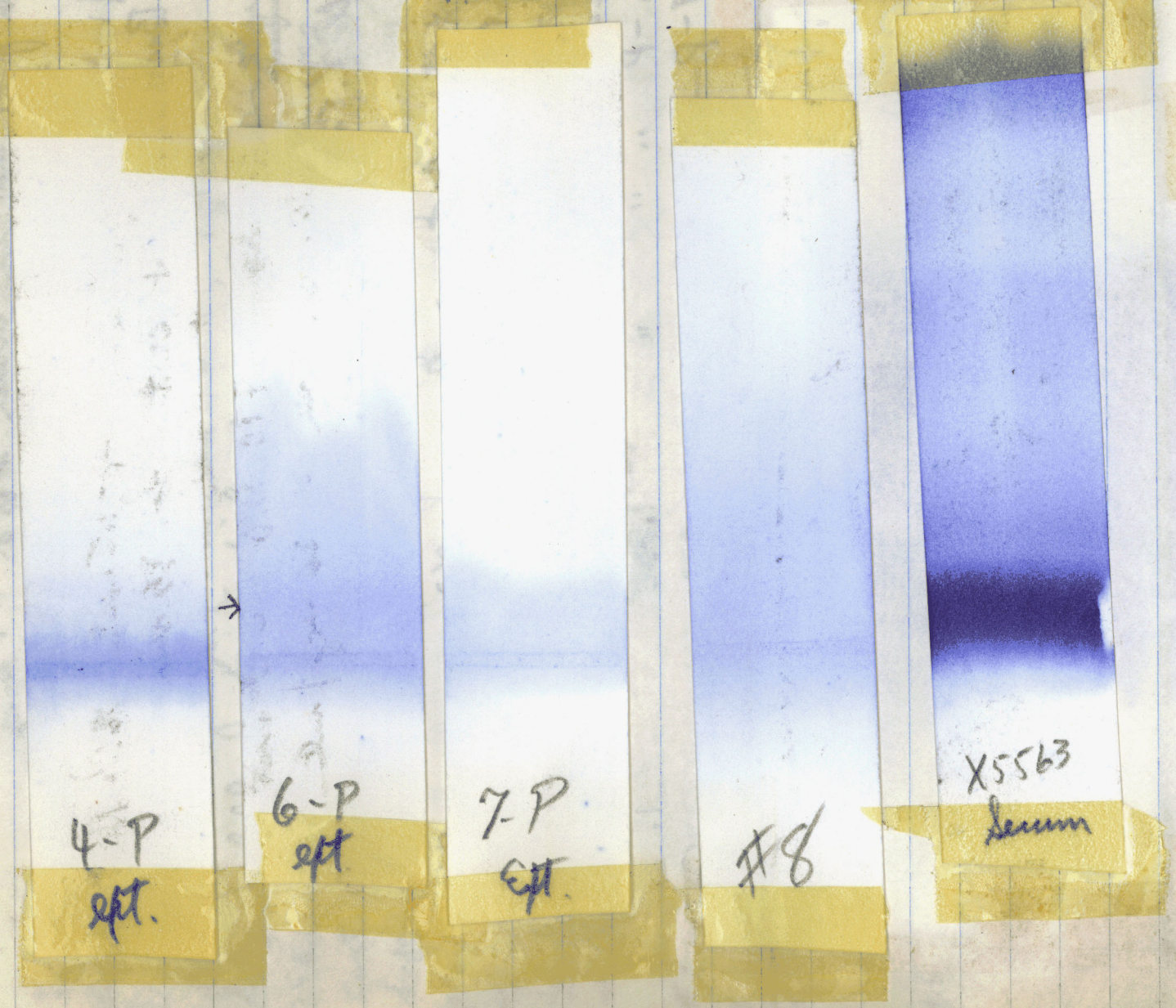
Alb prot not in either #6 or #7

12/1

Buret protein deter.

Sample		O.D.	mg/ml	λ
Alb (.5 ml 1%) (= 5 mg)		.202		
"		.197	200	
ppt #1 - sal ept.	.2 ml	.062	7.7	50
ppt #2 p̄ sal ept.	"	.175	22.	50
ppt #2 - sal ept	"	.092	11.5	50
4 - P - sal ept	"	.006	.75	50
6 - P " "	"	.008	1.	50
7 - P " "	"	.002	.25	50



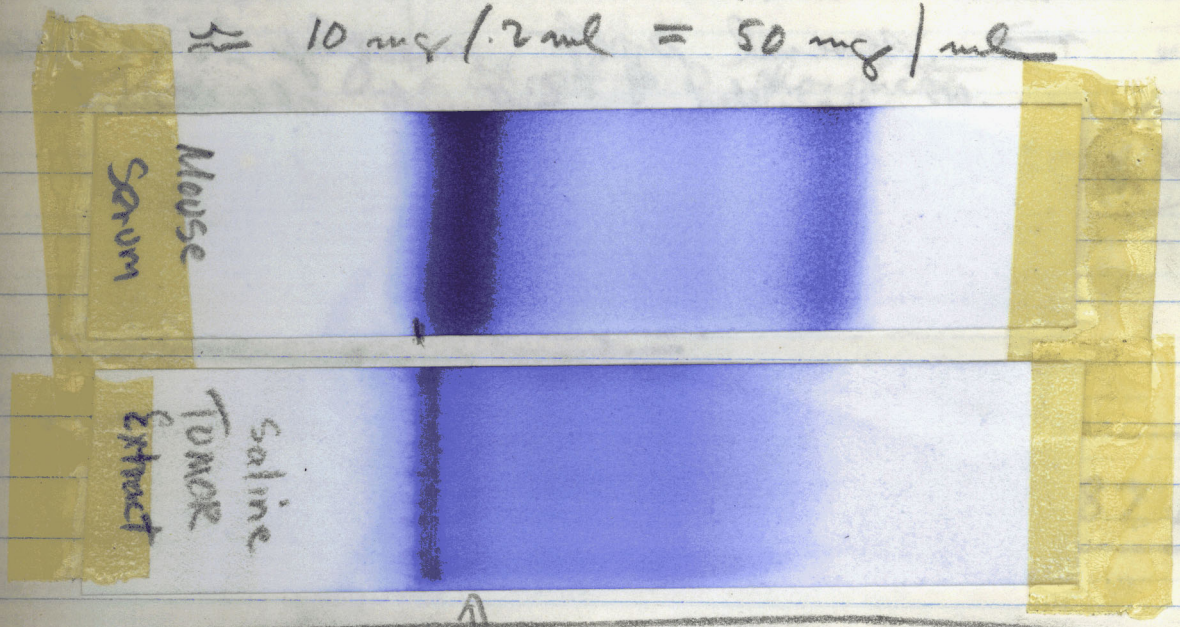


(6)

12/3 1/10

Saline extract of whole tumor

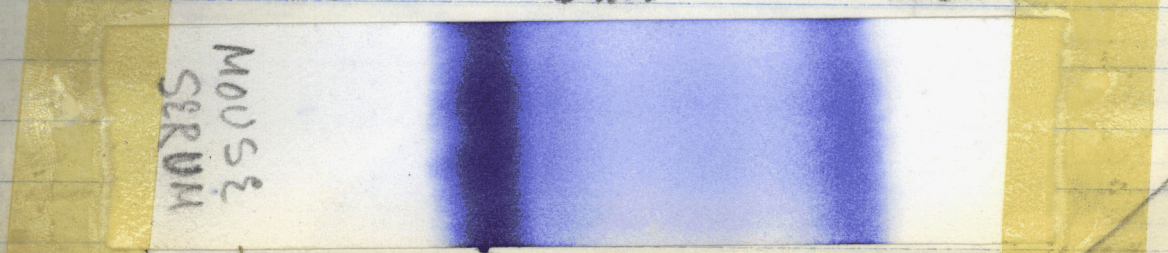
Approx 2 gm tumor + 1.5 ml .85% NaCl
homogenized + cent for 30 min.
Cloudy supernate taken
.2 ml → 410 O.D. c̄ biuret
≈ 10 mg/.2 ml = 50 mg/ml



12/7 M. Serum protein fr. paper.
10 λ serum on each of 4 paper strips
Strips cut + sections bet 3 + 10 mm. fr. origin
put into tube + fluid cent. off → Then paper
eluted c̄ saline. (1.0 cc) → Inner tube c̄ holes at bottom

Biuret:

	Amt		O.D.	mg/ml
1	.02 ml	cent off	.012	15
2	.02 ml	eluted	000	0



8)

(≅ 3 mg prot)

0.2cc of cent. fluid DNP-rxd + hydrol c̄
5.7 N HCl for 16 hrs (refluxed).
Ether sol (½ lost) → tiny amts DNP glut +
asp acids + DNP-OH
Water sol dried + taken up in acidic
acetone → several spots, mostly ε-DNP-lys.
which was well separated. Estimate about
.1 or .2 μM ε-DNP-lys.

L-Lysine decarboxylase prep

Ref: JBC 156, 401, 1944 Zittle + Eldred

B. cadaveris (Brit # 6578) — acetone dried prep used.

Buffer: 0.2 M pH 6.0 phosphate

KH_2PO_4 3.72 gm
$K_2HPO_4 \cdot 3H_2O$ 11.4 gm } → 100 ml = 1.M

L-lysine standard sol'n:

1.6402
-1.4176
.2226 gm → 25 ml = .0487 M

Radioactive lysine 12/20/56
(uniformly labelled)
2 cc water added to vial →
50 μc/cc — frozen
.1 ml of this → 3.5 cc c̄ normal saline
= 50/35 μc = 10/7 = 1.4 μc/cc

Preliminary Mouse Study
X 5563 — no Rx
0.5 cc lys* IV via tail vein = 0.7 μc

#	lys given	Bled		Tumor frozen
1	12:27 PM	12:58 PM	31'	1:03
IP 2	12:35 (IP)	1:12		1:20
3	12:10	2:15	125'	2:23
4	12:26	4:30		4:35
5	12:23 (12/20)	8:40 AM 12/21		8:45

Tumors, livers, serum frozen. Carcasses of 4 + 5 frozen
Tubes labelled #-T, #-L, #-S

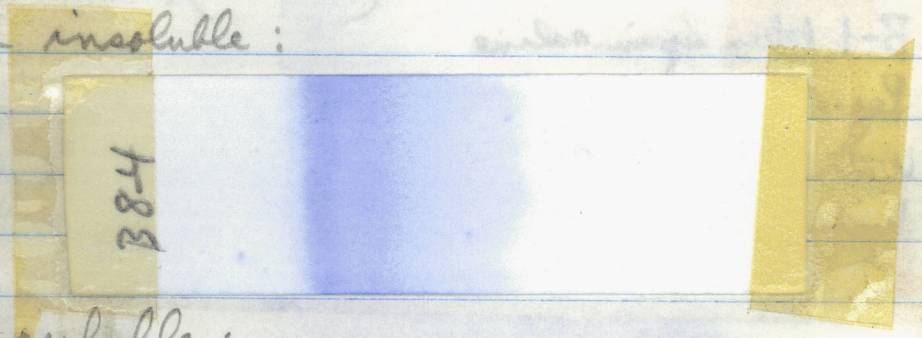
1/23/57
Standard radioactive lysine I
.1 ml of sol'n injected above → 50 ml
c̄ 11 m Molar sol'n. of L-lysine HCl (as carrier).

12/14

Isolation Tissue Protein

Saline extract from p.7 dialysed against tap water overnight → ppt.
ppt redissolved in saline (small amt undissolved).

Water insoluble:



Water soluble:

12/24 Water soluble (labelled "B") thawed → more ppt which was cent. off + washed ≈ water (ppt B-1) Supernatant re-dialys. against water → sl ppt (B-2) (super = B-3).

(12)

12/25 Electrophoresis

#1 B-3

2 Sal. ext. tumor $\frac{1}{3}(NH_4)_2SO_4$ sat'd - sol. (A-1) p 14

3 " " water sol. (A-3) "

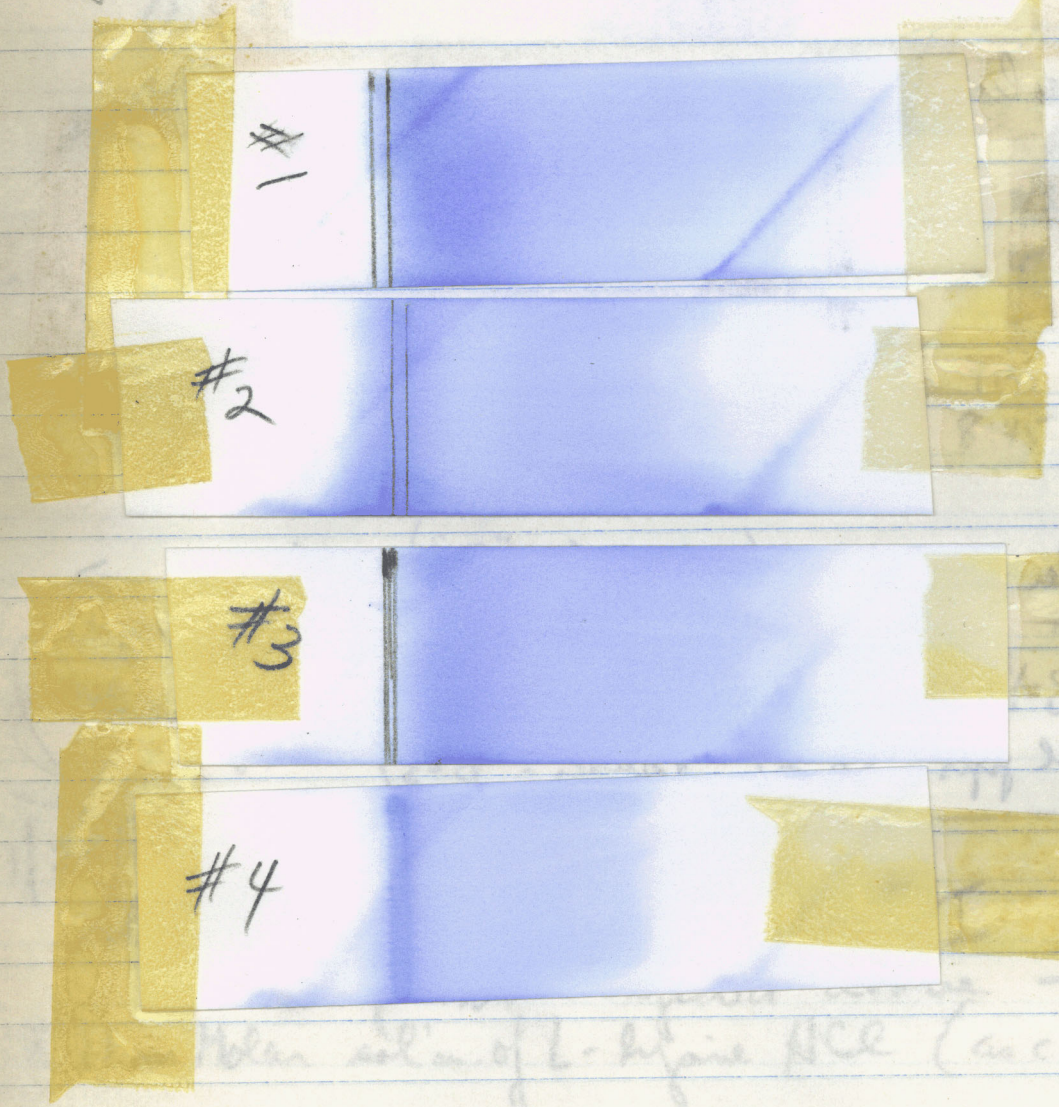
4 " " $\frac{1}{3}$ NH_4 SO_4 - insol (in saline) (A-2) "

5 B-1 taken up in saline

6 Repeat water insol p. 11

7 Water insol (fr 3) A-4 p 14

8 Water insol B-2



water-insol.

water-insol.

water-insol.

water-insol.

14) 12/20/56

X5563 Tumor extract
" Liver extract

3 gm tumor + 3 cc saline
1 gm liver + 1 cc saline
Both tumor & liver first washed in saline to remove blood. Homogenized in P-E homogenizer & power stirrer.

Tumor extract:

Liver extract:

Tumor extract divided into 2 equal pts.
1 pt. dial. aq water → water-sol (A-3)
→ water insoluble (A-4)
1 pt. dial aq 1/3 sat'd $(NH_4)_2SO_4$ → insol (A-2)
+ soluble (A-1). (see pp 12 + 13 for electrophoresis)

12/29/56 (15

X5563 Tumor Extract - "C"

7 gm tumor + 15 cc saline.
Homogenized in P-E homogenizer using power stirrer for 10 minutes in cold. (done in 3 portions)
Centrifuged at 4000 rpm x 45 minutes.
Ppt washed x 2 c̄ cold saline (ppt = C-1) + washings added to supernatant (→ total vol 30cc).
Supernatant = C-2. 6cc of this put in deep freeze. Remainder dialysed against cold dist. water. (12 30 P 12/29)

After this dialysis small amt ppt formed + cent. off (= C-3). Tube broke in cent. cup. Supernatant dial. ag. running tap water for 40 hours more → ppt C-4. Supernate = C-5.
C-2 frozen → ppt (= "C-2 ppt.")
To C-3 + C-4 14.5 cc saline added, resp → saline sol. part + sal. insol. Part of C-4 saved.

2/4/56

C-5 thawed + ppt centrifuged off (= C-5 ppt).
Supernate ~~dialysed~~ ultrafiltered down to ca 1/3 original volume (= C-6) saline added + sl. amt ppt centrifuged off.