

11/19/56

(3)

Separation of Tumor Myeloma Protein X5563

Gen'l procedure:

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

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

Dialyze 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 homogenized in Potter hand homogenizer 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. Dialyzed

against $\frac{1}{3}$ sat'd $(NH_4)_2SO_4$ in cold for 5 hrs.

→ Ppt ③ washed x1 \bar{c} $\frac{1}{3}$ sat'd $(NH_4)_2SO_4$ + frozen

Supernatant ③ dialyzed ag. $\frac{1}{2}$ sat'd $(NH_4)_2SO_4$

→ ppt ④ washed \bar{c} $\frac{1}{2}$ sat'd $(NH_4)_2SO_4$ + frozen

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

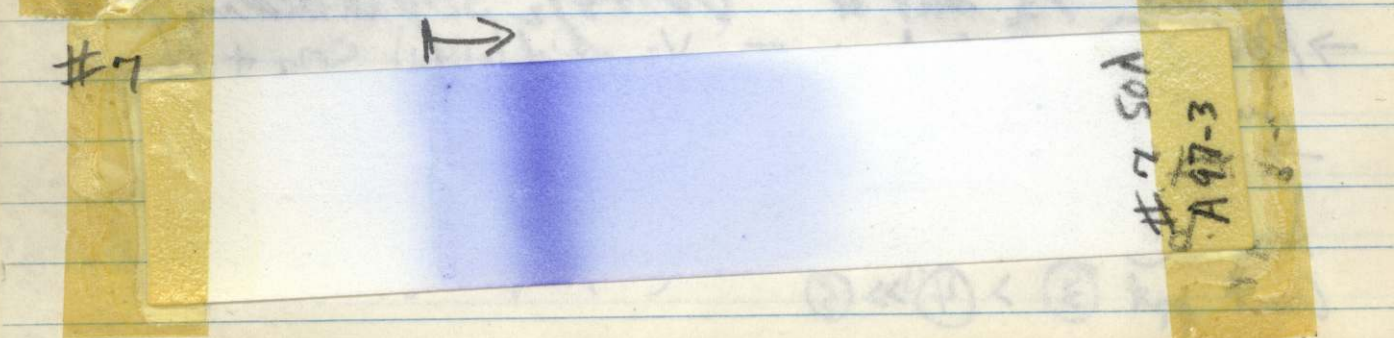
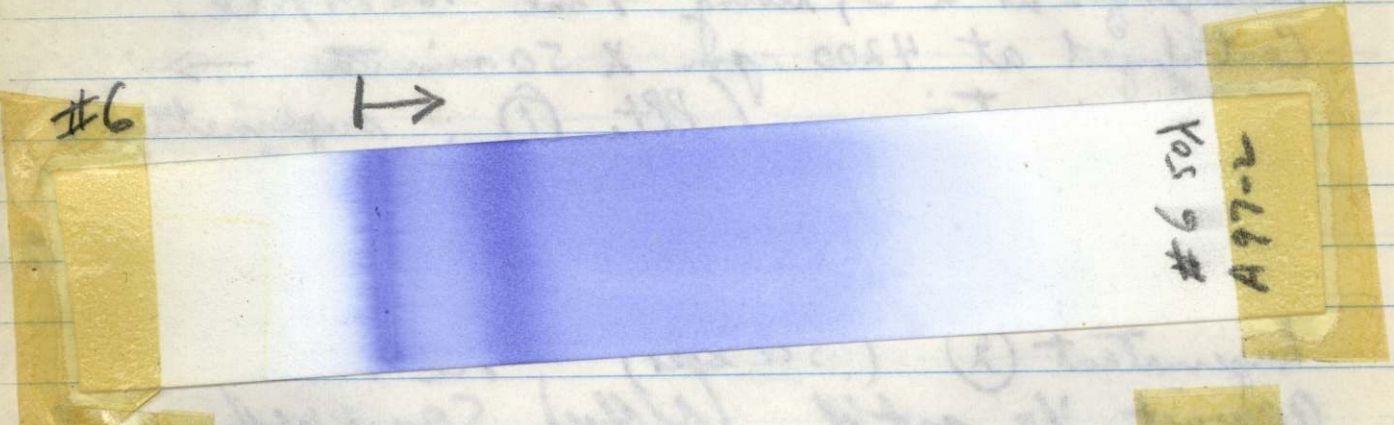
Amnt ppt ③ > ④ >> ⑤

Ppts ③, ④, ⑤ ^{each} taken up in 3 ml ^{cold} .85% NaCl + centrifuged. Almost all of ③ dissolved, ca. 1/2 of ④ + 1/2 of ⑤ dissolved. Ppts were washed x2 in 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 sac, this was cent. off, washed x2 in 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 (5mg)	.200	204
"	.207	
#6 .2ml	.103	13 mg/ml
#7 .2ml	.058	7 mg/ml
#8 .2ml	.018	2.2 "

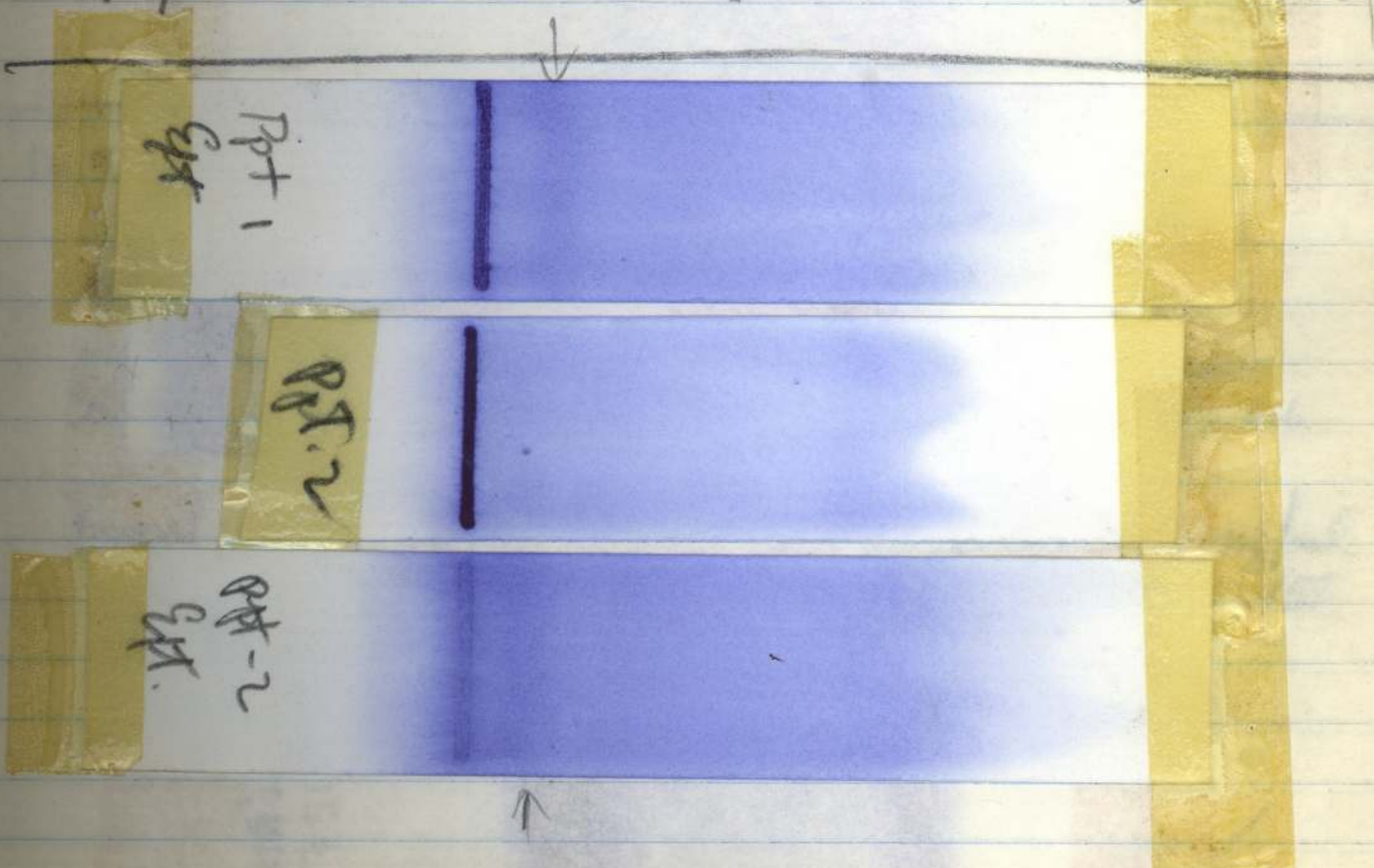


Alb prot not in either #6 or #7

12/1

Buret protein detem

Sample	O.D.	mg/ml	
Alb (.5ml 1%) (= 5mg)	202		
"	197 } 200		
ppt #1 - sal est. .2ml	.062	1.7	50
ppt #2 \bar{p} sal est. "	.175	22.	20
ppt #2 - sal est "	.092	11.5	50
4-P - sal est "	.006	.75	50
6-P " " "	.008	1.	50
7-P " " "	.002	.25	50



6

60-200

4-P
opt.

6-P
opt

7-P
opt.

#8

X5563
human

$$\frac{200}{100} \times 1 = .3 \text{ mg}$$

12/3

1110

7

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. in buret

$\approx 10 \text{ mg} / .2 \text{ ml} = 50 \text{ mg} / \text{ml}$

Mouse
Serum

Saline
Tumor
Extract

12/7 M. Serum protein fr. paper

10 μ serum on each of 4 paper strips

Strips cut + sections bet 3 + 10 mm. fr. origin

put into tube + fluid cent. off
eluted in saline. (1.0 cc)

then paper

inner tube in holes
at bottom

Buret:

1

.02 ml

cent off

O.D.

.012

mg/ml

15

2

.02 ml

eluted

000

0

MOUSE
SERUM

8)

($\approx 3 \text{ mg prot}$)

0.2 cc of cent. fluid DNP-lyd + hydroal \bar{c}
5.7 N HCl for 16 hrs (refluxed)

Ether sol ($\frac{1}{2}$ lost) \rightarrow tiny amounts ^{DNP} glut +
asp acids + DNP-OH

Water sol dried + taken up in acetic
anhydride \rightarrow several spots, mostly E-DNP-lys.
which was well separated. Estimate about

.1 or .2 MM E-DNP-lys.

(9)
L-Lysine decarboxylase prep.

Ref: JBC 156, 401, 1944 Zittle + Eldred

B. cadaveris (But # 6578) - acetone dried prep used.

Buffer: 0.2 M pH 6.0 phosphate

KH_2PO_4 3.72 gm

$\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$ 11.4 gm

} \rightarrow 100 ml = 1.1 M

L-lysine standard sol'n:

1.6402

- 1.4176

0.2226 gm \rightarrow 25 ml = .0487 M

Radioactive lysine 12/20/56

(uniformly labelled)

2 cc water added to vial →

50 $\mu\text{C}/\text{cc}$ - frozen

.1 ml of this → 3.5 cc \bar{c} normal saline
 $= \frac{50}{35} \mu\text{C} = \frac{10}{7} = 1.4 \mu\text{C}/\text{cc}$

Preliminary Mouse Study

X 5563 - no Rx

0.5 cc lyp* IV in tail vein = 0.7 μC

#	lyp 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, liver, 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
 \bar{c} 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 \rightarrow ppt.
Ppt redissolved in saline (small amt undissolved).

Water insoluble:

B8-4



Water soluble:

B8-5

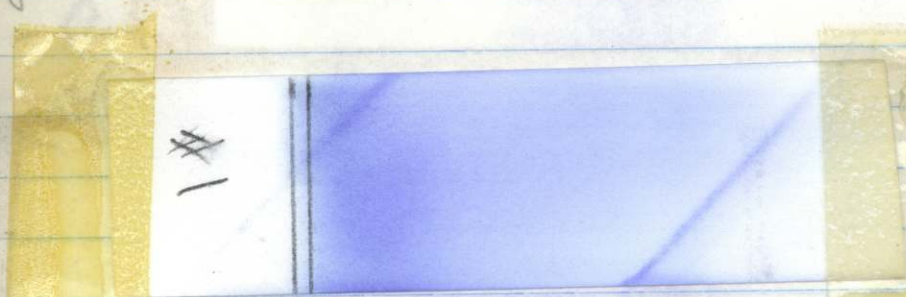


12/24 Water soluble (labelled "B") thawed \rightarrow more ppt which was cent. off + washed \pm water (ppt B-1) Supernatant re-dialys. against water \rightarrow sl ppt (B-2) (super = B-3).

12)

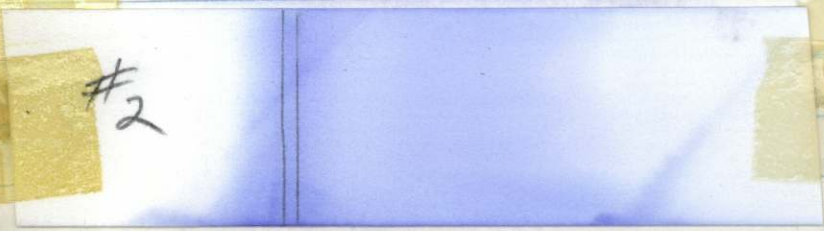
12/25 Electrophoresis

- #1 B-3
- 2 Sal. ext. tumor $\frac{1}{3}$ $(NH_4)_2SO_4$ sat'd - sol (A-1) p14
- 3 " " water sol. (A-3) "
- 4 " " $\frac{1}{3}$ $NH_4_2SO_4$ - insol (in saline) (A-3) "
- 5 B-1 taken up in saline
- 6 Repeat water insol p. 11
- 7 Water insol (p. 3) A-4 p 14
- 8 Water insol B-2



#1

water-sol



#2

$\frac{1}{3}$ $(NH_4)_2SO_4$ soluble



#3

water-sol.



#4

$\frac{1}{3}$ $(NH_4)_2SO_4$ insol.

→ 50 ml
 blue sol'n of L-tyrosine HCl (as carrier)

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. ag water → water-sol (A-3)

→ water insoluble (A-4)

1 pt. dial ag $\frac{1}{3}$ sat'd $(NH_4)_2SO_4$ → insol (A-2)

& soluble (A-1). [see pp 12 + 13 for electrophoresis]

12/29/56

(15)

X 5563 Tumor Extract - "C"

7 gm tumor + 75 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 \bar{c} cold saline (ppt = C-1) + washings added to supernatant (\rightarrow total vol 30cc).

Supernatant = C-2. 6cc of this ppt in deep freeze. Remainder dialyzed against cold dist. water. (12³⁰/_P 12/29)

After this dialysis small amt ppt formed + cent. off (= C-3). ^{Tubex broke in cent. cup.} Supernatant dial. ^{running tap} ag. water for 40 hours more \rightarrow ppt C-4. Supernate = C-5.

C-2 frozen \rightarrow ppt (= "C-2 ppt.")

To C-3 + C-4 14.5 cc saline added, resp \rightarrow saline sol. part + sal. insol. Part of C-4 saved.

2/4/56

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