Attempt to detect Transfer of C^{14} from C^{14} labeled "RNA" to Protein — 7/11

Preparation of C^{14} labeled RNA

Trial Run	1	2	3	4	5	6
Cacodylate pH 7 1M	0.02					
.15 M $MgCl_2$	0.01					
ATP 0.01	0.02					
C^{14}AA 4.5×10^7/ml	0.02					
H_2O	.23	.38	.33	.23	.23	.23
pH 4.6 enz 6/25	0.2	0.05	0.10	0.20	0.20	0.20
Time at 30°	0'	10'	10'	10'	15'	20
2' ct	41	4218	1945	15,354	15,662	15,482
1' ct	21	2134	5478	1677	1831	1991
Δ Total	-	4236	1914	15,312	15,620	15,440

Large scale Run

1M cacodylate	0.4
M Cl_2	0.2
ATP	0.4
C^{14} AA	0.4
pH 4.6 enz	10.5

Incub 20' at 30°. Added 1.2 ml 55% PCA. Spun in cold. Washed ppt 2x c̄ 25 ml 1% PCA. Suspended ppt in 5 ml cold H_2O. With stirring in ice added 1M KOH until solution began to clear pH ca 5.5. At pH 6.4 solution was fairly clear. Then added 0.015 ml KOH & pH went up to ca 8.5. Immediately brought back down to ca pH 7 c̄ 1M HCl. Spun down some insol material.

Sup was 5 ml (not quantitative transfer)

plated 2 0.01 ml aliquots
1) 818
2) 786 } 832

∴ 83,200 cpm/ml or total of 416,000

Transfer Exp.

	1	2	3	4	5	6	7	8	9	10	11
Tris M/2 pH 7.3	0.05										
0.1 M $MgCl_2$	0.01										
0.2 M $MnCl_2$	0.01										
ATP 0.1 M	0.02	.02	.02	.02	.02						.02
lyped Protoplast pellet E VI 24 hrs	.05										—
C^{14} labeled RNA	0.4	—	.	.	.	.	.	.	.	.	—>
H_2O						.02	.02	.02	.02	.02	.05

Time at 30' 0' 20' 40' 20' 40' 0' 20' 40' 20' 40' 40'

Rx stopped by adding 0.5 ml 10% cold TCA.

Tubes # 1, 2 3, 6, 7, 8 were washed 2x @ 2.5 ml cold 1% PCA. Dissolved in 1 ml NH_4OH & plated 0.5 ml

Tubes # 4, 5, 9, 10 & 11 were washed 2x @ 10% PCA then extracted @ 90° in 2 ml 5% TCA at 100° for 15'. Spun washed 1x @ 10% TCA & then dissolved & plated as above.

	cold	cold	cold	hot	hot	cold	cold	cold	hot	hot	hot
2' at	22,184	14,985	14,041	232	226	28,900	10,010	4233	231	204	147

Doesn't look like there is much transfer. The radioactivity is ~~cold~~ hot PCA soluble to hot PCA insoluble (protein).