

Murdoch's lab book

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These pages were kindly provided by Professor Sergio Moreno.

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Dear Sergio,

Here are parts of my experimental notebook, as promised. In the end, I thought it better to send you copies which you would not need to return. In most cases they are as good as the originals except that they don't register that we often used red as well as black ink, for instance to separate multiple curves or points on the same graph. There are 15 pages describing four experiments on nucleoside diphosphokinase in 1988 (in the end never published). The experiments were written up by Jim Creanor but at intervals I made Summaries such as the one on p.3075. This is how I worked with Jim who started with me as a Technician and then spent more than 20 years until he reached the status and pay of a tenured Senior Lecturer. He was very able at the technical level and very careful. I wrote up the papers and for the last ten years or so and I put his name first. I was lucky to have him, as this kind of permanent research post is very rare in our Universities. Obviously it was different with other collaborators. I wrote up papers with Bela but on the basis of finished graphs etc that he had made.

The details of what is in our experiments are fairly straightforward. Among other things they should include strain, medium, temperature and date (including year which is important) and page number which is the main reference. My page numbers go from 1 to 3300 since 1964. Make sure the axes of graphs are labelled.

Some people prefer bound volumes but I find it essential to have a loose-leaf notebook because of the different types of graph paper that have to be inserted. I have used binders that have three rings (paper with three holes, as in these copies) because they hold the sheets much more firmly than the commoner two ring binders. This is standard in the U.S. but can be got in Britain. I also have an American 3-hole paper punch for the graph paper. I don't know what is available in Spain. These 3-hole sheets last well over the years, but the plastic ring reinforcements do not since their rubber adhesive breaks down. If you can still get them, ones with gum like postage stamps are better.

Indexing is very important. At the start of each binder, I have an INDEX sheet with a brief description of c.40 experiments (a copy of one is enclosed). My binders will usually include two of these index sheets. Then I have a much smaller loose-leaf INDEX BOOK with topics and page numbers (a copy enclosed). The topics are in alphabetical order and they are listed in a third index at the start of this book. The supplier of this book is printed in light type on the left of the page. I can assure you that all these indices are essential if you are trying to recollect an experiment of ten years earlier!

Yours sincerely

Meredith.



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3072.	NDKK test. Conc. surface of freeze dried & freeze thawed.
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3075.	<u>cdc 2</u> . Induction synchrony. NDKK <u>SUMMARY</u>
3076	NDKK test. Effect of media on plates.
3077	<u>cdc 2</u> . Induction synchrony. NDKK ✓
3078	972. Induction synchrony control. 29° → 35° → 29°
3079	972. SC. NDKK. ?
3080	" " " " continuous. No steps
3081	" " " " No steps.
3082	" " " " Various assay methods. Steps only in some assays.
3083	NDKK test. Conc. surface.
3084 } 3085 }	<u>cdc 10 NBC41</u> . Induction synchrony. Varying time at 35°C (but 3.5-4 h)
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3090	" " " " " " No steps.
3091	" " " " " " Steps.
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(2-33)

100 ml ENN-3 culture growing at 29°C: at 5:45 a.m. culture shifted to 35°C.
 OD 9.42 = 0.196. Culture shifted back to 29°C at 9:45.

Every 15' throughout the experiment 0.1 ml added to 10 ml saline & counted.

At the same time 1.5 ml sample spun down in a weighed tube, most of the supernatant was removed & the cells resuspended in exactly 200 µl hepes. 4 x 10 µl samples placed in Eppendorf tubes & frozen at -20°C.

ASSAY 1. Two sets removed from deep freeze immediately after the experiment & assayed. 450 µl substrate containing 96 µl 10⁻¹M ATP, 60 µl 10⁻¹M TDP, 294 µl hepes, 23 µl IAA & 3 µl ³²P-ATP. 10 µl of this added to each sample & incubated 20' at 30°C. 5 µl spotted then plates washed & run as usual.

ASSAY 2. Two sets assayed the following day: substrate made at twice normal conc. 192 µl 10⁻¹M ATP, 120 µl 10⁻¹M TDP, 130 µl hepes, 23 µl IAA, µl ³²P-ATP. 10 µl of this added to each sample & incubated 'at °C. 5 µl spotted then plates washed & run as usual.

Assay 1 blank 3138 ATP 131172

Time	Cell No.	SAMPLE	Time	cpm-blank
15'	1.01 x 10 ⁶	1	15'	21343 15123
30'	0.99	2	30'	17148 14279
45'	1.01	3	45'	17700 16449
1hr	1.03	4	1hr	15162 17603
1:15	1.08	5	1:15	17884 21086
1:30	1.51	6	1:30	11993 12763
1:45	1.64	7	1:45	12492 15109
2hr	1.70	8	2hr	24399 15465
2:15	1.75	9	2:15	21652 16223
2:30	1.75	10	2:30	16320 9743
2:45	2.11	11	2:45	10041 12310
3hr	2.72	12	3hr	23791 14645
3:15	3.21	13	3:15	17379 8247
3:30	3.30	14	3:30	14132 17509
3:45	3.35	15	3:45	13211 17476
4hr	3.53	16	4hr	5688 10912
4:15	3.84	17	4:15	17511 10532
4:30	4.41	18	4:30	21522 18334
4:45	5.09	19	4:45	18812 5576
5hr	5.54	20	5hr	13263 6529

NOPK in Induced Synchronous Culture of cdc 2-33

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